SARS-CoV-2 Transmission Risk and Oral Decontamination: Scarce Evidence Albeit Promising Future*

Riesgo de transmisión del SARS-CoV-2 y descontaminación oral: escasa evidencia aunque prometedor futuro

Risco de transmissão de SARS-CoV-2 e descontaminação oral: evidências escassas embora futuro promissor

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*Integrative Review of Literature

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ABSTRACT

Background: Oral decontamination recommendations/guidelines have exploded during the COVID-19 pandemic for the contemporary dental practice, due to SARS-CoV-2 relative high presence in saliva and the possibility of risk contagion through its aerosolization. However, such guidelines are mostly based on research carried out for other diseases caused by different viruses and/or bacteria, low-level evidence publications, and/or anecdotal information. ***Purpose:*** To review the biological basis for the use of oral antiseptics to decrease viral load in saliva as a

plausible mechanism for reducing SARS-CoV-2 transmission risk, including other aspects such as pathogenesis, angiotensin converting enzyme 2 expression in the oral cavity, aerosolization, and oral antiseptics potential mechanistic virucidal properties. ***Results:*** Our group could only identify a limited number of reports evaluating specific direct effects of commonly used oral antiseptics (Hydrogen Peroxide, Povidone-Iodine and Chlorhexidine) on SARS-CoV-2, however, these reports are limited to surface disinfection, *in vitro* activity, or preliminary *in vivo* observations. ***Conclusion:*** Although we conclude that there is no direct evidence of clinical effectiveness of the use of mouth rinses prior to dental procedures with antiseptic solutions for SARS-CoV-2 specifically to date, we here present recommendations that could aid in reducing the risk of transmission in the dental office.

Keywords

cetylpyridinium; chlorhexidine; dentistry; hydrogen peroxide; hypochlorous acid; mouth, oral decontamination; oral microbiology; povidone-iodine; prevention; SARS-CoV-2

RESUMEN

Antecedentes: En la práctica dental contemporánea las recomendaciones o pautas de descontaminación oral se dispararon durante la pandemia de COVID-19, debido a la presencia relativamente alta de SARS-CoV-2 en la saliva y la posibilidad de riesgo de contagio a través de su aerosolización. Sin embargo, dichas pautas se basan principalmente en investigaciones

realizadas para otras enfermedades causadas por diferentes virus o bacterias, publicaciones con evidencia de bajo nivel o información anecdótica. ***Objetivo:*** Revisar la base biológica del uso de antisépticos orales para disminuir la carga viral en la saliva como un mecanismo plausible para reducir el riesgo de transmisión de SARS-CoV-2, incluyendo otros aspectos como la patogénesis, la expresión de la enzima convertidora de angiotensina 2 en la cavidad oral, la aerosolización y los antisépticos orales con propiedades virucidas potenciales. ***Resultados:*** Nuestro grupo solo pudo identificar un número limitado de informes que evalúan los efectos directos específicos de los antisépticos orales de uso común (peróxido de hidrógeno, povidona yodada y clorhexidina) sobre SARS-CoV-2; sin embargo, estos informes se limitan a la desinfección de superficies, la actividad *in vitro* u observaciones preliminares *in vivo*. ***Conclusión:*** Aunque llegamos a la conclusión de que no existe evidencia directa de la efectividad clínica del uso de enjuagues bucales antes de procedimientos dentales con soluciones antisépticas para SARS-CoV-2 específicamente hasta la fecha, aquí presentamos recomendaciones que podrían ayudar a reducir el riesgo de transmisión en el consultorio odontológico.

Palabras clave

acido hipocloroso; cavidad oral; cetilpiridinio; clorhexidina; descontaminación oral; microbiología oral; odontología; peróxido de hidrógeno; prevención; SARS-CoV-2; yodopovidona

RESUMO

Antecedentes: As recomendações/diretrizes de descontaminação oral explodiram durante a pandemia de COVID-19 para a prática odontológica contemporânea, devido à presença relativa elevada do SARS-CoV-2 na saliva e à possibilidade de risco de contágio por meio de sua aerossolização. No entanto, essas diretrizes baseiam-se principalmente em pesquisas realizadas para outras doenças causadas por diferentes vírus e/ou bactérias, publicações de evidências de baixo nível e/ou informações anedóticas. ***Objetivo:*** Revisar a base biológica para o uso de anti-sépticos orais para diminuir a carga viral na saliva como um mecanismo plausível para reduzir o risco de transmissão de SARS-CoV-2, incluindo outros aspectos, como patogênese, expressão da enzima conversora de angiotensina 2 na cavidade oral, aerossolização, e anti-sépticos orais potenciais propriedades virucidas mecanísticas. ***Resultados:*** Nosso grupo só conseguiu identificar um número limitado de relatórios avaliando os efeitos diretos específicos de antissépticos orais comumente usados (peróxido de hidrogênio, povidona-iodo e clorexidina) no SARS-CoV-2, no entanto, esses relatórios são limitados à desinfecção de superfície, *in vitro* atividade, ou observações preliminares *in vivo*. ***Conclusão:*** Embora possamos concluir que não há evidência direta de eficácia clínica do uso de enxaguatórios bucais antes de procedimentos odontológicos com soluções anti-sépticas para SARS-CoV-2 especificamente até o momento, apresentamos aqui recomendações que podem auxiliar na redução do risco de transmissão no consultório odontológico.

Palavras-chave

ácido hipocloroso; boca, descontaminação oral; cetilpiridínio; clorexidina; microbiologia oral; odontologia; peróxido de hidrogênio; povidona-iodo; prevenção; SARS-CoV-2

INTRODUCTION

The relevance of COVID-19 in the spectrum of infectious-contagious diseases is extremely high for the dental profession. Although the factual SARS-Cov-2 infectious potential is currently unknown, some important factors related to dental practice make it an important and sensitive issue: 1) The virus is apparently found in saliva at all stages of the disease (1-4). 2) The probability of continued presence of SARS-CoV-2 in the oral cavity is very high due to its tropism to upper and lower respiratory tracts (5, 6). 3) The contagion routes include drops and possibly aerosols as routes for their dissemination (7, 8). 4) The epithelia of the oral cavity, the tongue and the ducts of the salivary glands highly express angiotensin converting enzyme "ACE2" receptors (although the receptor role in infection is not completely elucidated) (9-12). 5) There is a large percentage of asymptomatic cases that could become a focus of transmission (13-15). Taken together, these factors have justifiably led the dental community to strongly consider preventive efforts in reducing risk to protect both clinicians and patients in the dental office environment.

The protocols published to date for dental care during the COVID-19 pandemic mostly include recommendations for the use of antiseptic solutions in rinses as an important strategy to lower the viral load in the oral cavity. However, when analyzing the scientific basis that supports the recommended solutions, one can find that they range from anecdotal recommendations to those based on studies carried out on viruses with either similarities to other coronaviruses (CoVs) or very different characteristics from SARS-CoV-2 such as non-enveloped viruses (16). Only some direct evidence on SARS-CoV-2 is available from *in vitro* studies (17) and some *in vivo* preliminary results. In this integrative review we aim for a two-fold objective: 1) to discuss the

plausible biological basis for the use of oral antiseptics to decrease the viral load in saliva as a mechanism for preventing or reducing SARS-CoV-2 transmission risk and 2) to analyze current recommendations from the biological basis and then based on the chemical composition and antiviral activity of the different antiseptics available on the market. We also aim to establish criteria that allows the dentist to give adequate clinical instructions to their patients that could help decrease the virus transmission risk. We hope from here researchers could generate questions that serve as a starting point for new research that better supports clinical decision-making and worldwide preventive recommendations.

MATERIALS AND METHODS

A comprehensive review of the scientific literature was conducted in the Medline (PubMed), Web of Science, SciELO, Scopus, and Google Scholar databases until June 25, 2020. The MeSH keywords used included: "COVID-19," "SARS-CoV-2," "ACE2 (angiotensin converting enzyme 2)," "Anti-Infective Agents," "Anti-Bacterial Agents," "Antiviral Agents," "Dentistry," "Aerosols," "Mouthwashes," "CPC (Cetylpyridinium chloride)," "Chlorhexidine," "Hydrogen peroxide," "Povidone-Iodine," and "Hypochlorous acid," which were combined with Boolean operators "AND" and "OR." All articles, short communications, letters to the editor, alerts, opinions of scientific societies, and institutional protocols that were accessed, in English or Spanish, in animals or humans, were included without restrictions or time limits of publication, type of study or research design. Non-specific SARS-CoV-2 literature was also considered as this allowed obtaining relevant information for the analysis and potential extrapolation to the new CoV strain. We also included a comprehensive search strategy for unconventional interventions: we included "nasal wash," "nasal rinse," "nasal irrigation," "nasal spray," "nasal lavage,"

"hypertonic," "isotonic," "saline," "gargling," "steam inhalation," and "stomach saline wash" terms. Study population was defined as cases of "rhinitis," "rhinosinusitis," "sinusitis," "common cold," "upper respiratory tract infection," "coronavirus," and "rhinovirus." Irrelevant articles were excluded through limiting our search to the title and abstract.

RESULTS AND DISCUSSION

COVID-19: Knowns and Unknowns

Since the first report of COVID-19 made in December 2019 in Wuhan, China (15,18), the disease has spread throughout the world in such a way that the World Health Organization declared it pandemic on March 11, 2020, at which time more than 110,000 people were affected worldwide (19). The most common reported manifestations encompass fever, fatigue, and dry cough. Other reported symptoms include myalgia, chest tightness, dyspnea, nausea, vomiting, and diarrhea. The most common laboratory findings are lymphopenia or leukopenia. CT scans of the chest show typical images of viral pneumonia with multiple bilateral ground glass image opacities (20-22). About 26 % of patients can present with anosmia and 22 % ageusia with a duration of about one week (23), in addition to other neurotropic signs such as headache, vomiting, and confusion. About 20 % of cases require hospitalization (24). People with comorbidities prior to the development of obstructive pulmonary disease or who are complicated by secondary bacterial pneumonia are those who present a more serious clinical picture; some of the associated comorbidities are high blood pressure, diabetes, obesity, and chronic lung diseases, active smokers, and the elderly are also more susceptible (25). The so-called "cytokine storm," a systemic inflammatory syndrome that occurs due to hyperactivation of immune cells specifically leukocytes and endothelial cells that has been

described in other diseases and is not exclusive to COVID-19, occurs more frequently in these comorbid patients, being one of the most important factors for higher mortality rates. Other mechanisms associated with mortality are multi-organ failure, acute respiratory disturbance, and disseminated intravascular coagulation (26).

SARS-CoV-2 infection has a high degree of infectivity during the incubation period, with rapid transmission even in young patients, in whom the disease develops rapidly and with manifestations that may be atypical, but with milder signs than in older patients. The median incubation time is 2 days with a range between 1-4 days in these patients (20). Additionally, a higher SARS-CoV-2 load is found in older adults, which could be associated with impaired immunity or with an increased expression of ACE2 (27). Noteworthy, a large number of asymptomatic undiagnosed cases likely end up affecting the real COVID-19 pandemic potential due to their particular epidemiological characteristics. Such cases are usually patients with mild or even non-existent symptoms, which is why a large number of people may be exposed to contagion through them (15). The actual frequency of asymptomatic cases as well as the time during which these cases can be reservoirs of the virus are currently unknown (28). The evidence that asymptomatic patients can transmit the disease is rapidly increasing (29,30). It is estimated that an asymptomatic patient could infect a number close to 100 individuals (13) and that transmission through them is responsible for 50-80 % of COVID-19 cases (14,15). It is also not known whether patients in the recovery phase might be possible transmitters (31).

COVID-19 Pathogenesis

SARS-CoV-2 biology. SARS-CoV-2 is not the first emerging virus to cause a pandemic situation. To date, 4 circulating endemic CoV strains in different populations are known (229E, HKU1, NL63, OC43). If the progression of CoV infections follows the same pattern as 2009 H1N1 influenza, it is believed that it could become the fifth endemic CoV for humans (15). One of the main microscopic characteristics of CoVs is their corona appearance, which is produced by the presence of spicular protein projections.

CoVs can infect the respiratory, gastrointestinal, liver, and central nervous systems of humans, livestock, birds, bats, mice, and many other wild animals (32). The current classification of CoVs recognizes 39 species in 27 subgenres, five genera, and two subfamilies that belong to the family Coronaviridae, suborder Cornidovirineae, the order Nidovirales and kingdom Riboviria (33). The CoV subfamily is divided into four genera: alphacoronavirus, betacoronavirus, gammacoronavirus, and deltacoronavirus, among which α- and β-CoVs can infect mammals, while the other two genera can infect both birds and mammals (24,34). SARS-CoV-2 is the seventh member of the CoV family that infects humans and is subsequent to the MERS-CoV and SARS-CoV viruses belonging to the same group. It is a betacoronavirus from group 2B and shows a similarity in its genetic sequence with SARS-CoV above 70 % (35). The CoV genome is a single stranded positive sense RNA (+ ssRNA) (~ 30 kb) with a 5'-cap structure and a 3'-poly-A tail. Genomic RNA is used as a template to directly translate polyprotein 1a / 1ab (pp1a / pp1ab), which encodes non-structural proteins (NSPS) to form the replication-transcription complex (RTC) in double-membrane vesicles (DMV) (36).

The replication mutation rates of RNA viruses are higher than those of DNA viruses and the genomes of RNA viruses are generally less than 10 kb; however, the CoV genome is larger, at 30 kb approximately (37,38). The SARS-CoV-2 envelope is composed of a lipid bilayer that comes from the host cell membrane, in which four structural proteins (N, M, E and S) are coupled together with a set of nonstructural proteins. Protein N forms the nucleocapsid and its main function is the binding to the CoV genome, it participates in viral RNA replication and in host responses against infection. The M protein works in the virus assembly as it activates cell membranes to produce new viral particles. In several CoVs the M protein is in the vicinity of the Golgi apparatus. E is the smallest protein, it is widely expressed within the cell during the replication cycle, but only a small portion of it is incorporated into the virion envelope. Due to its location in the endoplasmic reticulum, Golgi apparatus and intermediate compartments, which are sites of high intracellular traffic, it is thought to be related to assembly and budding. Furthermore, it has been described that E may be involved in the pathogenesis of the virus (39) (figure 1).

FIGURE 1
SARS-COV-2 STRUCTURE

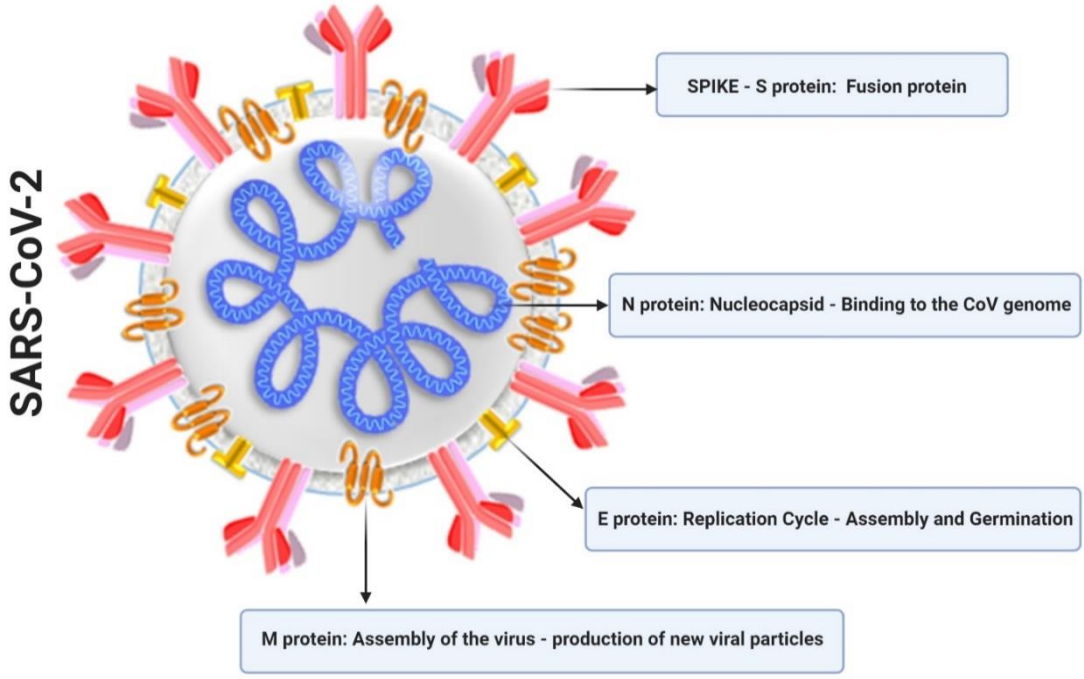

Figure 1. The genome of SARS-CoV-2 is a single stranded positive sense RNA composed by 30 kb approximately. The envelope is a lipid bilayer to which four proteins are coupled: 1- Protein N, forms the nucleocapsid and its main function is the binding to the CoV genome, 2- Protein M, works in the virus assembly activating cell membranes to produce new viral particles, 3- Protein E, is widely expressed within the cell during the replication cycle and 4- Protein S (Spike), a class I fusion trimeric glycoprotein with different functional domains; the S2 subunit of Spike mediates the fusion of the virus and the cell membrane, while the S1 subunit is associated with receptor binding functions.

The Spyke (S) protein is a class I fusion trimeric glycoprotein, which undergoes a substantial structural rearrangement in its process of binding the viral membrane with the host cell membrane (40). Protein S in SARS-Cov-2 is different from that of other CoVs and the rapid spread of COVID-19 is possibly attributed to this (41). S has different functional domains, close to the amino terminus, S1, and the carboxy terminus, S2. The S2 subunit is a transmembrane protein that mediates the fusion of the virus and the cell membrane, while the S1 subunit is peripheral and is associated with receptor binding functions (39). After efficiently binding to the cell surface, the virus enters the cytosol using acid-dependent proteases, which disrupt protein S, followed by

fusion of the virus cell membranes and the host cell. Spike is cleaved at 2 positions in the S2 domain of the protein; the first cleavage separates the 2 domains (RBD receptor-binding domains and the fusion domain) and the second, exposes the fusion peptides; the breaking leads to the detachment of S1 and to the re-bending (Refolding) of S2 (40) (figure 1)**.** The fusion process occurs in endosomes. Once this happens, there are structural changes that allow the membranes to fuse, and the viral genome is released into the cytosol of the host cell. This occurs in the same way in SARS-CoV and SARS-CoV-2. The cleavage / fusion process is mediated by a protein called furin. Furin clears the SARS-CoV-2 S protein at the S1 / S2 site, which is required for efficient cell / cell fusion (42).

It should be emphasized that the proteases expressed by the host cells decide the efficiency of the entry of the virus and thus its pathogenicity. In SARS-CoV-2 the S protein has 12 nucleotides extras that form sequences equal to the furin-like cleavage site, rich in arginine (42), which facilitates the priming of protein S and therefore the efficiency of the spread of SARS-CoV-2, compared to other CoVs (41). Additionally, changes in the S protein are responsible for variations in tissue tropism and the pathogenesis of the virus. On the other hand, S is the main target of neutralizing antibodies against SARS-CoV-2 (39,43). Studies have also shown that the pattern of expression of proteases in host cells may be an additional determinant in the tropism of SARS-CoV-2 for the cells it infects (43).

SARS-CoV-2 receptors. SARS-CoV-2 recognizes angiotensin-converting enzyme 2 (ACE2) and it is through this enzyme that it binds to host cells, by a binding process dependent on serine proteases (TMPRSS2 and TMPRSS11D7) (12). ACE2 is a homologue of the angiotensin-

converting enzyme (CE) in the renin-angiotensin-aldosterone system (RAAS), involved in the regulation of blood pressure and electrolytic homeostasis (44). Specific inhibitors of this enzyme are the most prescribed class of drugs in cardiology for the treatment of hypertension and heart failure. Some genetic variations in the receptor expression may be related to the existence of resistant populations (10). SARS-CoV-2 has a binding affinity for ACE2 10-20 times greater than that of SARS-CoV (40), which could partially explain the apparently easier transmissibility of the new CoV, further increasing the susceptibility of the host cells to its entry. Recently, three (3) additional SARS-CoV-2 receptors have been reported: 1) CD147, also known as Basigin, which is mainly expressed in erythrocytes, tumoral tissues, inflamed tissues, and pathogen-infected cells (45). 2) CD26, also known as DPP4, also found in epithelia and hematopoietic cell lines (46) that could explain cellular cross-reactivity with normal cells or facilitate virus entry in immature immune cells (47). 3) Neuroplin-1 (NRP1) a receptor involved in the development of the cardiovascular system and angiogenesis among others that enhances the infectivity of SARS-CoV-2 by binding substrates cleaved by furin (48).

SARS-CoV-2 via ACE2 entry into the body. There is a widespread expression of ACE2 in healthy human organs. Receptor expression has been searched for by various methods, some of which only indicate the presence of transcripts or the DNA that encodes it and others the expression of the protein and its location in tissues, which could be more useful when explaining entry routes. Through the expression of mRNA for ACE2 it has been found in cardiovascular tissues, kidney, and testicles, as well as in tissues of the gastrointestinal system such as ileum, duodenum, jejunum, cecum, and colon. Furthermore, the expression of mRNA for ACE2, although limited in central nervous tissue, is positive in neurons and glial cells of the brain, so it could be thought that there is neuro invasion

by the new CoV, which explains the neurotropic signs of COVID-19 (49). High ACE2 expression was found in endothelial cells of veins and arteries in all the tissues studied, as well as in associated smooth muscle cells, which would allow the virus to spread to multiple organs by this route. However, since the virus has not been found in several organs, it could be assumed that vascular abnormalities and inflammatory changes could be related to the systemic cytotoxic effects of immune reactions caused by CoV infection. Similarly, this could indicate that as with HIV, CoVs (SARS) need a co-receptor molecule to enter cells (9). Considering that the pulmonary alveoli and the small intestine are organs that are related to the external environment, the expression of ACE2 in the epithelial cells of these 2 tissue types postulates them as potential entry routes for SARS-CoV-2. SARS-CoV-2 then could initially enter through the mucous membrane of the upper respiratory tract, especially the nasal or pharyngeal epithelium, or directly into the lower respiratory tract and infect the bronchial and alveolar epithelial cells from there. Once this phase has started, the virus can pass from the lung potentially into the blood and initiate a viremia, reaching other organs that express ACE2. The intestine could also be reached orally (10) (figure 2).

FIGURE 2
SARS-COV-2 / ACE2 INTERACTION IN INFECTION. POTENTIAL ENTRY ROUTES

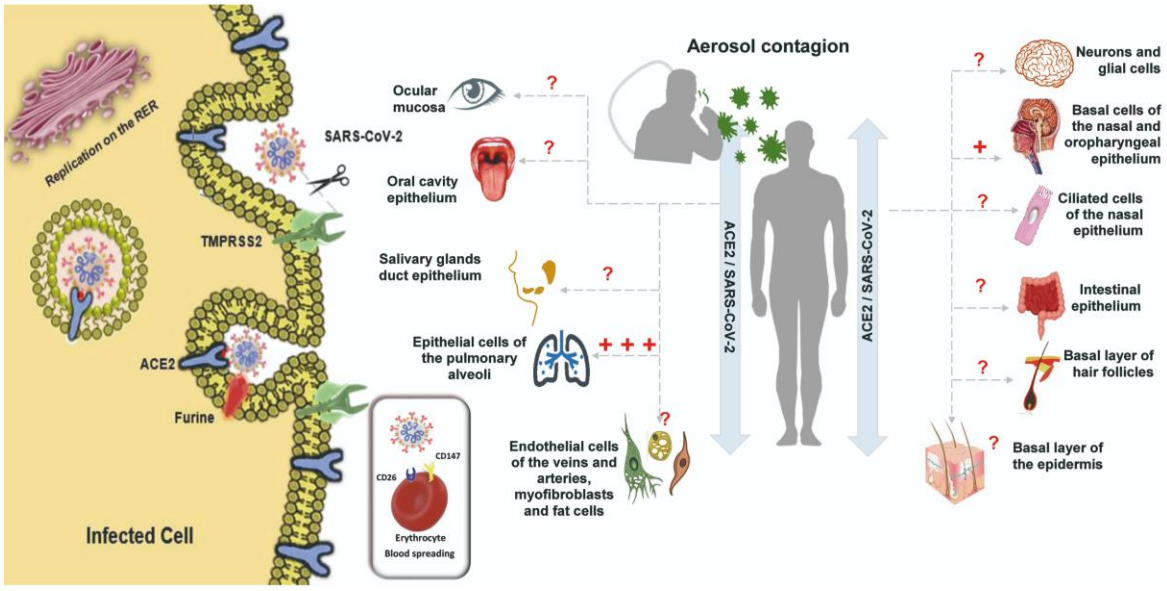

Figure 2. The entry of SARS-CoV-2 into the human body depends on the interaction with receptors and enzymes on the surface of host cells. The virus primarily binds to ACE2 in the presence of serine proteases (TMPRSS2). The cleavage process of the S protein and its fusion with the host cell is mediated by the Furin protein, whose role seems to be important in the greater spread efficiency of SARS-CoV-2 in relation to other CoVs. The expression of CD147 and CD26 on the erythrocyte mediates virus infection as well as its possible entry into immature immune cells. ACE2 is expressed mainly in the epithelium of the pulmonary alveoli. Despite their expression in multiple tissues, it is not certain whether all of them represent routes of infection. For example, in the epithelium of the oral and nasal cavity as well as in the epidermis, ACE2 is expressed in the basal layer and not in the superficial layers, so its relevance in the initial infection is debatable, especially through aerosols.

ACE2 in the Oral Cavity

Using analysis of databases of RNA sequences from normal tissues and of tumor origin, it was determined in the oral cavity that 95.86 % of ACE2-positive cells are found in the tongue, above the oral mucosa and gingival tissues (11). In *Rhesus macaque*, high expression has been demonstrated in cells morphologically compatible with epithelial cells from the ducts of the salivary glands (50). Such high expression of ACE2 mRNA has also been reported for minor salivary glands, being even higher than in the lung (51) as well as the expression of marginal levels of TMPRSS2 mRNA in cells. Epithelial of the salivary glands can lead us to think that the salivary glands could be a site of entry for SARS-CoV-2 (12).

Presence and Viral Load of SARS-CoV-2 in Saliva

Several factors can be associated with viral load, especially the time after infection and the stages of the disease. The average duration of viral spread has been estimated at 24.5 days and appears to be independent of the clinical manifestations of the patient, but the duration is longer in symptomatic patients (25.2 days) vs. asymptomatic patients (22.6 days), especially those with chest pain and sputum production (23). Saliva may be a source of COVID-19 transmission among humans, but the study of the viral load in saliva is not only related to the routes of transmission, but also to the development of less invasive diagnostic tests that are easier to perform under safer conditions (2). In saliva, the virus can also be studied in its active replicative state, which is the

most possible form of transmission. Given the accessibility of saliva, the role of the molecular characteristics of the virus and the potential for genetic changes that are related to high transmissibility and that may be possible therapeutic targets could be studied (1).

Three routes are proposed by which the virus could reach saliva: 1) The virus arrives from the respiratory tract by fluids that are exchanged with these organs. 2) From the blood the virus reaches the gingival crevicular fluid. 3) Due to infection of major and minor salivary glands it is released into saliva through their ducts (52). The primary evidence of saliva as a transmission route has been obtained from studies carried out with other CoVs given their similarities. One of the most cited is the one performed on Rhesus Macaque monkeys, in which the virus was found in the oral swabs of 4/4 animals vs. 2/4 with infection in the lung, suggesting an increase in viral reproduction in the upper respiratory tract, including salivary gland ducts, in early stages of the disease. This study also suggests that the lining epithelial cells of the salivary gland ducts are a source of virions found in saliva droplets in these early stages. Similarly, viral mRNA has been found in saliva taken directly from the opening hole of the salivary gland ducts, confirming the possibility that the salivary glands are a site of replication and that the virus is released directly into saliva (21). However, it is important to note that there is a possibility that virions found in saliva come from other sources, such as respiratory secretions, as viral load in sputum is significantly greater than in the oropharynx and nasopharynx especially in late stages of infection (50,53).

Greater viral loads have been reported in the nose than in the throat; however, in both sites it has found to be equally high immediately after symptoms onset. Viral load can also be similar in symptomatic and asymptomatic and indicates greater potential for transmission in days after

infection. Nevertheless, it is important to note there is no correlation between virus positive qPCR and presence of cultivable virus; the latter could be cases where there may be mild or non-existent symptoms with very modest levels of detectable viral RNA in the oropharynx for at least 5 days (6).

The correlation of the detection of SARS-CoV-2 in serial samples of saliva with samples of nasopharyngeal swaps is high. As recently reported, the sensitivity of saliva tests can reach 87 % (95 % CI, 65-97 %) (1). In serial samples of expectorated saliva from 12 patients diagnosed with COVID-19 (qPCR and culture), virus detection in saliva was achieved in 11/12 specimens (91.7 %). Conversely, patients with negative nasopharyngeal isolates showed saliva to also be negative. Therefore, saliva samples have been recommended for monitoring as they are easier to take (54). A recent meta-analysis reports a sensitivity of these tests of 91 % vs. 98 % of the tests with nasopharyngeal isolate samples in patients previously confirmed with COVID-19 (55).

Despite the apparent utility of saliva as a sample for the detection of SARS-CoV-2 and the diagnosis of COVID-19 there are still many gaps. The forms of saliva collection, its handling and pre-test processing, and other variables with reference to its manipulation in the laboratory must be standardized before making an accurate interpretation of the biomarkers in saliva related to the infection by SARS-CoV-2. It is necessary to determine the viral load in saliva of asymptomatic and pre-symptomatic patients. The great advantage is that in saliva analytes that go beyond the presence of the virus can be identified (56).

To date, data on the transmission of SARS-CoV-2 by blood or serum are scarce. Although the presence of virus mRNA in blood has been demonstrated in percentages as low as 1 % of samples

(57), there are no data on the viral load in these fluids or lymphocytes in the different periods of infection, specifically during the incubation period. Thus, it cannot be ensured that there is a risk of contagion by blood either by contact or by transfusions (58). There is also no published evidence of the presence of SARS-CoV-2 in crevicular fluid, although it could be a possibility that is hypothetical and speculative at this time (59). Given the low percentages of viremia in the blood, speculation that the virus may pass from the blood into the crevicular fluid and from there into saliva seems rather unlikely.

Transmission Routes in the Dental Practice

Given the low amount of evidence, it is essential that all possible routes of transmission are considered so that all prevention fronts are covered. Transmission routes can include upper and lower airway secretions and saliva (both airborne through aerosols and by contact with contaminated surfaces) as well as fecal-oral transmission (27), which has also been considered a plausible route. Our review will focus on aerosol transmission.

Coughing and sneezing produce ejections that are multiphase turbulent clouds containing hot humid air, and suspended droplets of mucosalivar fluid, which may contain pathogens (60). The contamination range of the droplets is largely determined by its size. The physical mechanism of droplet formation at the outlet of the mouth after sneezing, analyzed by high-speed imaging, reflects that the breakdown of the droplet fluid continues outside the respiratory tract during violent exhalations. This includes a complex cascade of events ranging from leaf formation, to bag bursts, to ligaments, which eventually break into drops. The viscoelasticity of the mucosalivar fluid plays an important role in the delay of the fragmentation causing the combination of the precursors of the

drops that form along stretched ligaments, which affects the final size distribution of the drops (61). Historically, the study of aerosol generation in dental practice has been a priority topic. These studies have generated evidence of the presence of viruses such as HIV, hepatitis B, influenza, and herpes viruses in small aerosolized particles (62). During respiration, it has been found that drops containing microorganisms and viruses such as influenza can also be produced, hence it is thought that SARS-CoV-2 can be transmitted by coughing, sneezing, breathing and even when speaking (63).

In SARS-CoV-2 positive hospitalized patients, medical procedures such as bag valve-mask ventilation, non-invasive ventilation, and intubation can create localized aerosols that could allow this type of transmission to those involved in the maneuver. Given the low knowledge in this regard, the Public Health Agency of Canada guidelines requests that suspected or confirmed patients be placed in airborne isolation (negative pressure and constant air change) and, if not available, in a room alone behind closed doors (64). Large droplets can infect nearby subjects and small droplets containing viral particles suspended in the air, could be transmitted over larger distances (52). The real potential for large droplets to deposit on surfaces or impact the face or eyes of a subject is not very clear; it has been estimated that drops with a diameter greater than or equal to 100 μm tend to fall rapidly and travel short distances (not more than 2 meters) before evaporating, while the smallest of less than 20 μm evaporate and become "droplet nuclei," remaining in the air for much longer being able to travel distances greater than 2 meters (65,66). The drops that are emitted when speaking are smaller than those emitted when coughing or sneezing, but they are large enough to carry pathogens. Furthermore, it has been shown that talking can produce more drops than coughing (14). Talking hard emits thousands of drops of oral fluid per second, which if they are in a stagnant microenvironment have a decay time ranging from 8-

14 min, which would correspond to a nuclei droplet of ca 4 μm in diameter, or 12-21 μm before dehydration (67). Those speaking drops generated by asymptomatic SARS-CoV-2 carriers could be considered possible routes of transmission, since they can generate airborne virus transmission in closed environments.

Despite the controversies over the potential for transmission by aerosols, today it is accepted that SARS-CoV-2 remains with the potential of infection for hours in aerosol drops, although it is clarified that it is necessary to know the viability of the viral particles present there (8). The physical characteristics of the droplets have dominated the discussion about their transmission in air, but the chemical characteristics of the droplets have usually been neglected, despite the fact that this microenvironment in the droplet is an important determinant of their stability. For example, the viability of the influenza virus is inversely correlated with the concentration of salt in evaporated drops that contain few proteins (68). When the drops are expelled from the respiratory system, they undergo changes due to environmental conditions. Except in very humid environments, the water in the drops evaporates quickly, so the concentration of salts, proteins and other components increases. The pH also influences the change of the drops. These changes have important implications for the viability of any pathogen that is inside them and therefore can affect the efficiency of the transmission of infectious diseases by drops and aerosols (69).

Can Oral Decontamination Reduce SARS-CoV-2 Transmission Risk?

Concerns about aerosol production-dependent contamination in dental practice has existed for a long time (70) and significantly regained in the face of the new pandemic. Even though to date no documented transmission of SARS-CoV-2 by aerosols in dental practice has been reported yet,

aerosols generated in the dental practice can create a highly polluted environment conducive to transmission (31). Aerosolized saliva exposure peak for dentists/assistants has been estimated to range from 0.014-0.12 µL at 15 minutes (71). Furthermore, aerosolized microorganisms in dental offices can remain in suspension for up to 4 hours after dental procedures, so staff can be exposed at the time protective equipment is removed. Therefore, reducing the burden of microorganisms in the oral cavity seeking aerosolization reduction and cross contamination reduction becomes instrumental (72).

Dentists are classified by the Occupational Safety and Health Act (OSHA) as high risk exposure workers to known or suspected COVID-19 positive patients (73). According to OSHA recommendations in the context of the global pandemic, dental procedures involving the generation of aerosols should be fully avoided. Due to the impact of the pandemic on humanity and the ease of transmission and dissemination of COVID-19, panic has been generated among the population. Dentists around the world, who despite having extensive biosafety knowledge/experience in their professional practice, are in a state of anxiety and fear while working in patients; this has led to modifying their services in accordance with the guidelines or treatment protocols for patients who attend dental consultation during the COVID-19 pandemic (74). Dentists then ought to implement prevention strategies to reduce SARS-CoV-2 transmission risk by focusing on disinfection of the workplace, exhaustive hand hygiene, proper personal protective equipment (PPE) use, and importantly aiming to reduce virus aerosolization. Oral decontamination then becomes a key strategy in pursuing the latter.

Nevertheless, the biggest limitation with oral decontamination protocols is the lack of proper evidence documenting a real virucidal activity the clinician can count on. To date, there is no

evidence of clinical effectiveness of the use of mouth rinses prior to dental procedures with antiseptic solutions for SARS-CoV-2 specifically (75). As the dental profession adapts to the new challenges, and more applicable research is procured, here we will review the composition and function of the most common oral decontamination products aiming to reduce transmission risk. The most common oral antiseptics aiming for COVID-19 inactivation are listed in Table 1.

TABLE 1
ANTISEPTICS POTENTIALLY EFFECTIVE IN REDUCING THE VIRAL LOAD OF SARS-COV-2 IN SALIVA

Antiseptic	Mechanism of action	Microbicidal action	Action on enveloped viruses	Direct evidence in SARS-CoV-2	Recommendations in the literature
Hydrogen peroxide (H_2O_2)	Oxidizing agent	Gram positive Gram negative Sporulated bacteria Virus Yeasts	MERS, SARS, SARS-CoV-2	***In vitro****:* H_2O_2 at 3 % and 1.5 % concentrations. Minimal virucidal activity after 15 and 30 seconds of contact time on SARS-COV-2 USA-WA1/2020 strain (76). ***In vitro***: H_2O_2 at 1.5 % (in a commercial product). Minimal reduction with 30 s exposure time on 4 isolates of SARS-CoV-2, under conditions mimicking nasopharyngeal secretions (77). ***In vitro*** **- Infectivity assay**. H_2O_2 0.05 % v/v appeared to have potent anti-viral activities; however, disruption of cell morphology was apparent (79).	H_2O_2 3 % for nasal (nebulization 2 times a day) and oral (3 times a day) rinsing in patients who present the first symptoms of SARS-CoV-2 infection (78).
Povidone iodine (PVP-I) ($C_6H_9I_2NO$)	Oxidizing agent	Gram-positive Gram-negative Sporulated bacteria Fungi Protozoa Virus	H5N1, H5N3, H7N7, H9N2, Influenza A, Enterovirus, Coxsackie, Ankara, Ebola, SARS-CoV, MERS-CoV, SARS-CoV-2	***In vitro*** **on surfaces**: PVP-I at 0,5 % 1 % and 1,5 % completely inactivate SARS-CoV-2 USA-WA1/2020 strain within 15 seconds of contact (80). ***In vitro*** **suspension assays:** Antiseptic solution (PVP-I 10 %), skin cleanser (PVP-I 7.5 %), gargle and mouth wash (PVP-I 1 %), and throat spray (PVP-I 0.45 %). Virucidal activity ≥ 99.99 % against SARS-CoV-2, within 30 s of contact (81). PVP-I at concentrations of 0.5 %, 1.25 % and 1.5 %. Completely inactivation of SARS-CoV-2 USA-WA1/2020 strain (76) PVP-I 1 % (in a commercial product) on 4 isolates of SARS-CoV-2, under conditions mimicking nasopharyngeal secretions. 30 s exposure time reduced viral infectivity to up to three orders of magnitude to background levels (80).	Routine administration of PVP-I indicated mainly in symptomatic patients infected by SARS-CoV-2, especially during the first week after the onset of symptoms when viral loads in saliva are higher (82). **In dental care:** -Pre-operative rinse with PVP-I between 0.2 % and 1 % (83). -Pre-operative rinse with PVP-I between 0.5-1.5 % for 15-30 seconds (76).

				In vitro - Infectivity assay: 5 % (v/v) povidone-iodine blocked viral infectivity associated with cytotoxicity in the infection assay (79). ***In vivo:*** Four COVID-19+ patients. PVP-I 1 %, 15 ml decrease load viral for up to 3 hours (82). PVP-I 0.5 % w/v (in a commercial product). Significant increase in the Ct value fold change at 6 h. Sustained effect in reducing viral load in saliva (84).	
Hypochlorous acid (HClO)	Oxidizing agent	Gram-positive Gram-negative Virus	HSV-1, Respiratory Syncytial virus, Influenza A, Human CoV 229E	**Non-existent**	It was not recommended in any of the reviewed protocols
Cetyl pyridinium chloride (CPC) ($C_{21}H_{38}ClN$)	Cations displacement and -COO neutralization of membrane proteins	Gram-positive Gram-negative Fungi Virus	Influenza Virus, HVB SARS-CoV-2	***In vivo:*** CPC 0.075 % (in a commercial product) significant increase in the Ct value fold change at 5 min and 6 h post-rinsing with 20 ml (84). ***Unpublished Results – Product news:*** CPC 0.075 % (in a commercial product) effect of 99 % in neutralizing the SARS-CoV-2 virus in saliva (85).	In dental care: Pre-operative rinsing with Cetylpyridinium chloride 0.05 % to 0.1 % (83).
Chlorhexidine ($C_{22}H_{30}Cl_2N_{10}$)	Displacement of anions present in membrane proteins	Gram-positive Gram-negative Fungi Yeasts Enveloped virus	VHS, Cytomegalovirus, Influenza A, Parainfluenza, VHB, VIH-1, VHS-1, SARS-CoV-2	***In vitro:*** Chlorhexidine 0.2 % in a non-alcoholic base (in a commercial product) on 4 isolates of SARS-CoV-2, under conditions mimicking nasopharyngeal secretions. Virucidal activity could be observed with 30 s exposure time (77). ***In vitro*** **- Infectivity assay**: 50 % v/v CHX inactivated the virus associated with residual mouth rinse-induced cell cytotoxicity (79). ***In Vivo:*** Two COVID-19^{+} patients hospitalized. Contradictory results (86). CHX at 0,2 % w/v. 6 patients COVID-19+. Varied effect on Ct values after 5 min rinsing, with a tendency to maintain reduced viral loads at 3 h and 6 h post rinsing (84)	-Gargling before, during and after induction and stabilization of hospitalized patients (87).

Hydrogen Peroxide (H_20_2)

Oxygenated agents such as hydrogen peroxide (H_2O_2), buffered sodium peroxyborate, and peroxycarbonate, are recommended for short-term use as disinfectants. H_2O_2 in particular, can be seen as a natural disinfectant because after use it rapidly breaks down into non-toxic products (water and oxygen). It was first synthesized in 1818 by Louis Jacques Thénard, by reaction between nitric acid and barium peroxide, but currently it is produced from anthra-hydroquinone when it reacts with oxygen under pressure, which generates hydrogen peroxide and anthraquinone. Hydrogen peroxide is a light blue liquid that when diluted appears colorless; it is soluble in water and is composed of hydrogen and oxygen (88). H_2O_2 oxidizes when in contact with organic matter, metals and alkaline solutions by producing hydroxyl free radicals that react with lipids and proteins. H_2O_2 has also been found to cause DNA damage by oxidation, induced by reactive oxygen species (ROS) that are released when hydrogen peroxide is degraded. Oxygen free radicals are molecules with unpaired electrons that contain oxygen. Hydrogen peroxide is normally reduced to water (H_2O), but some metal ions such as iron, copper and titanium can contribute to the production of highly reactive hydroxyl free radicals (OH-) which can damage tissues (89) (figure 3A). An important characteristic of hydrogen peroxide is its high instability, as it can be degraded by catalysis, exposure to light, movement and temperature.

In terms of antiseptic properties, H_20_2 is considered broad-spectrum for gram-positive, gram-negative, sporulated bacteria, yeasts and viruses. The variable levels of catalase and other peroxidases in the microorganisms explain the various degrees of tolerance that can be presented to this agent, especially at low concentrations (3 % to 6 %) (90). Therefore, the concentration at which H_20_2 gets used determines its real antiseptic effect, being bactericidal between 3 and 6 %.

However, at this concentration H_20_2 has limited activity on spores and requires longer contact times. In contrast, high concentration solutions (10 % to 30 %) have shown rapid in vitro effect on spores (91).

Viruses such as HCoV, MERS, and SARS have been found to persist on inanimate metal, glass, and plastic surfaces for more than 9 days, and can be effectively inactivated by disinfection procedures with agents such as ethanol between 62 % and 71 %, sodium hypochlorite, and 0.5 % hydrogen peroxide, among others, it is considered that its use may be key in preventing the spread of SARS-CoV-2, especially considering that since it is a new infection no specific therapy is available (92). Some studies have found that H_20_2 is effective for the inactivation of CoVs such as the CCV strain I-71, HCoV strain 229E and SARS-VOC isolation FFM-1 on inanimate surfaces, when tested at concentrations that are between 0.001 % and 1 %, in a dose-dependent manner, with exposure times between 1 and 10 minutes, where the lowest concentrations are those that require the longest exposure time (93-95). The inactivation of SARSCoV-2 on inanimate surfaces with the use of hydrogen peroxide has led to increased use during the pandemic in efforts to reduce hospitalization times (78). The latter report proposes that not only H_20_2 may be beneficial due to its oxidation properties, but also indirectly through innate immune antiviral responses via TLR3 overexpression (96) potentially reducing the progression of infection to the lower respiratory tract. Consequently, the use of 3 % H_2O_2 has been recommended for performing nasal and oral lavages in patients who present the first symptoms of SARSCoV-2 infection, in whom the diagnosis has already been made. Also, for patients who already have frank disease symptoms who are quarantined at home and/or hospitalized patients who do not need intensive care (78). The suggested protocol for mouthwash is to be carried out 3 times a day, while for the nasal mucosa

exposure should be carried out by nebulization 2 times a day only because of its irritant effects. This therapeutic scheme still needs further evidence for adoption.

An *in vitro* study determined the antiviral efficacy of eight commercially available oral rinses based on different active compounds (exact formulations for these oral rinses were not publicly available due to patent-related restrictions), were tested on 4 isolates of SARS-CoV-2, under conditions mimicking nasopharyngeal secretions. The virucidal activity was determined with a quantitative suspension test for 30s exposure time. For 1.5 % stabilized hydrogen peroxide, a log reduction factor between 0,33 % - 0,78 % for 3 strains was demonstrated, achieving a reduction which was not better than other tested compounds (77).

Another *in vitro* study made on SARS-CoV-2 USA-WA1/2020 strain cultures testing a H_2O_2 solution at 3 % and 1.5 % to represent clinically recommended concentrations (15s/30s exposure time) showed minimal virucidal activity for both contact times (76). Recently, a commercial product containing 1.5 % H_2O_2 was evaluated *in vitro* for virucidal effect and cytotoxicity in HeLa-hACE2 cells and in oral epithelial cells, reporting potent anti-viral activities as the consequence of rinse-mediated cellular damage. A 5 % (v/v) dilution of the product completely blocked viral infectivity (79).

Iodopovidone

Iodopovidone is a mixture of iodine with the water-soluble polymer polyvinylpyrrolidone (PVP-I). The antiseptic form consists of a complex of polyvinylpyrrolidone, hydrogen iodide and free iodine. The antimicrobial action of PVP-I occurs after free iodine (I2) dissociates from the polymer

complex and penetrates microorganisms through the formation of pores that generate solid-liquid interfaces in the lipid membrane, causing loss of cytosol leading to the death of the microorganism. In addition to direct killing action on bacteria, PVP-I also inhibits the release of virulence factors such as exotoxins, endotoxins, and tissue proteases (97). Additionally, it oxidizes nucleic acids and proteins, which produces enzymatic denaturation and block of the metabolic pathways of microorganisms (98) (figure 3A).

The formulations of PVP-I show antiviral properties by inhibition of essential enzymes such as neuraminidase, which blocks the release of the virus from the host cell, preventing further spread to uninfected cells. It also inhibits viral hemagglutinin which results in blocking the binding of the virus to the receptor on the host cell. It has action on essential surface proteins for the dissemination of encapsulated viruses (99). Whether some types of viruses are sensitive or resistant to PVP-I ultimately depends on whether they are encapsulated or not. This suggests that there are specific mechanisms against certain types of viruses (100-103). Interestingly, there are no reports of resistance of microorganisms developed in response to treatments with PVP-I, possibly due to this wide variety of mechanisms of action and multiple targets in pathogens (104).

PVP-I virucidal activity has been well documented in vitro, however no clinical in vivo studies could be found in this regard. For example, PVP-I available in a gargling form inactivated a panel of viruses including adenoviruses, mumps, rotavirus, polio, coxsackie, rhinovirus, herpes simplex, rubella measles influenza and HIV (105). PVP-I products that included gargling and throat spray have demonstrated rapid antiviral activity against a highly pathogenic (H5N1) and low pathogenic strains (H5N3, H7N7, and H9N2) of avian influenza A virus with only 10 seconds incubation (106). PVP-

I 1.56 mg/mL inhibited infection in MDCK cells of human and avian influenza A virus strains: H1N1, H3N2, H5N3 and H9N2, which may be useful in preventing infection and preventing the spread of avian and human influenza (99). Similarly, other in vitro studies have shown that PVP-I is effective in hand wash against murine norovirus, enteroviruses and coxsackievirus (98) and that its use in different presentations as 4 % PVP-I as a skin cleaner, 7.5 % in surgical cleansers and 1 % in rinse can be effective against Ankara modified vaccine virus (used as a reference to test activity against encapsulated viruses) as well as MERS-CoV within 15 seconds of application. Both forms of skin use have been effective against the Ebola virus (100, 103).

Some studies conducted during epidemic outbreaks of SARS-CoV and MERS-CoV have shown the effectiveness of antiseptic products based on PVP-I. Treatment of SARS-CoV with 0.47 % PVP-I in gargle form, reduces the virus ability to infect Vero cells in vitro when treatment is performed for 2 minutes (107). Due to the pandemic, in vitro tests have been carried out on SARS-CoV-2. Different PVP-I forms have been developed and tested using topical gel formation technology in nasal spray and eye drops. Both products rapidly inactivate SARS-CoV-2 in a dose and time dependent manner and inhibit viral infection of VERO cells. No toxicity was observed for the formulations used. Also, a significant inactivation was noted with virus preincubation with these formulations at the lowest concentration used; however, no clinical studies have been conducted yet (108). Similarly, a new iodine-based product called CupriDyne® designed for use on interior and exterior surfaces, was shown to be effective in inactivating the virus in a time-dependent manner by reducing the viral titer to 99 % after 30 minutes and below the detection limit after 60 min. (17). Other PVP-I solutions for oral use in concentrations of 0.5 %, 1 %, and 1.5 % completely inactivate the USA-WA1 / 2020 strain of SARS-CoV-2 within 15 seconds of

contact. This important finding could justify the use of oral rinses with PVP-I before dental procedures for patients and health personnel during the COVID-19 pandemic (80).

It was indirectly shown that gargling with 30 mL of PVP-I (70mg / ml) diluted 1:30, can be used for the prevention of the common cold and influenza as it decreased the percentage of absence from middle school compared to those who did not use it. Additionally, it is more tolerable in terms of taste, feel and odor after use than other antiseptics (109). These results suggest that PVP-I can provide protective exposure at the level of the oropharynx for individuals who are at high risk of exposure to oral and respiratory pathogens. However, the exact duration of virucidal action of PVP-I once applied to the mucosa and the time for recovery of viral load to pretreatment levels after application is unknown. Taking into account that SARS-CoV-2 has been shown to be vulnerable to oxidation in vitro, the use of 0.2 % PVP-I has been recommended in order to reduce the viral load in saliva (110).

Recently, the virucidal activity of PVP-I against SARS-CoV-2 was assessed for 4 products: antiseptic solution (PVP-I 10 %), skin cleanser (PVP-I 7.5 %), gargle/mouth wash (PVP-I 1 %) and throat spray (PVP-I 0.45 %). Results show that all four products achieved ≥ 99.99 % virucidal activity against SARS-CoV-2, corresponding to ≥ 4 $\log_{10}$ reduction of virus titer, within 30 s of contact, providing *in vitro* evidence of a rapid virucidal activity that supports hand hygiene and oral decontamination recommendations for use (81).

In vitro SARS-CoV-2 USA-WA1/2020 strain cultures were treated with a solution of PVP-I at different concentrations (0.5 %, 1.25 %, and 1.5 %) to represent clinically recommended

concentrations (15s/30s exposure time). All PVP-I oral antiseptic rinse concentrations completely inactivated SARS-CoV-2 at both exposure times. Therefore, preprocedural rinsing with diluted PVP-I in the range of 0.5 % to 1.5 % seems to be a good election for preprocedural rinsing to decrease the risk of SARS-Cov-2 infection (76).

The study by Meister *et al.* (77) measured the antiviral efficacy of a compound containing PVP-I 1.0 % and compared it against other seven commercially available oral rinses containing different active compounds. SARS-CoV-2 clinical isolates were tested (under conditions mimicking nasopharyngeal secretions) and the virucidal activity was determined with a quantitative suspension test with 30 s exposure time. For 1 % PVP-I reduced viral infectivity to up to three orders of increased magnitude was reported.

Also, a potent *in vitro* anti-viral effect of 0.1 % (v/v) PVP-I has been reported associated with cytotoxicity. Briefly, HeLa-hACE2 cells were treated with 2-fold serial dilutions in the medium of povidone-iodine, (or other commercial products containing CPC, CHX, and essential oils) for 20 sec, and cell viability was determined. All 50 % (v/v) dilutions of mouth rinses were highly toxic to HeLa-hACE2 and oral epithelial cells 0.5 % (v/v) dilutions of povidone-iodine were highly toxic to cells. They also determined the effect of 2h exposure of mouth rinses on cell viability for comparison with the duration of viral attachment in the infection assay and found that 0.1 % (v/v) diluted povidone-iodine significantly affected cell viability after 2 h exposure. After the compounds were highly diluted (non-cytotoxic dilutions) to measure on replication-competent SARS-CoV-2 viruses added to Vero cells. Cell morphology was monitored as a crude measure of cytopathic effects as well as fluorescence intensity from SARS-CoV-2 infection. Diluted

povidone-iodine (0.1 % v/v) appeared to have potent anti-viral activities; however, disruption of cell morphology was apparent indicating that the putative anti-viral effect of these two agents was likely a consequence of cytotoxicity. Although this study found that commercially available mouthwashes may be cytotoxic to oral tissue cells, many of the formulations used in *in vitro* studies should be considered as well tolerated in clinical use (79).

The impact *in vivo* of a mouthwash with PVP-I 1 % for 1 minute on the salivary viral load of SARS-CoV-2 was evaluated in 4 patient COVID-19+.The results show that in 2 of the 4 participants the PVP-I resulted in a significant drop in viral load, which remained for at least 3 h (82). Recently, a randomized control trial, using 0.5 % w/v of a commercially available mouthwash containing PVP-I (4 patients for PVP-I group), found an increase in Ct value fold change at 5 min, 3h, and 6h post-rinsing, compared to the water group patients, (with statistically significant difference only at 6 h). The study concludes that PVP-I formulated commercial mouth-rinses may have a sustained effect on reducing the salivary SARS-CoV-2 level in COVID-19 patients (84).

Thus, Preventive iodine therapy should be done within safety limits and taking into account contraindications for its use. Nevertheless, this intervention is a cost effective approach, with low risk, and potentially easy to develop and adopt on a global scale.

Hypochlorous Acid / Saline (HClO)

Hypochlorous acid (HClO) is also known as chloric acid (l), chlorinol, hydrogen chlorate, hydrogen hypochlorite, chlorine hydroxide, electrolyzed water, electrolyzed water in oxidation, and electro-activated water. Physiologically, HClO is naturally produced on cells of innate

immunity by a chain of oxygen-dependent reactions known as respiratory burst, the purpose of which is to kill invading pathogens and control infection. The most abundant anion in humans is chloride (Cl^-), which is necessary for the function of the innate immune system cells. Resting polymorphonuclear cells have been reported to have a high concentration of Cl^-, which is mobilized through the hydrophobic lipid membrane of cells, and whose exchange by enzymatic interactions, mediate the formation of HClO inside the phagosomes (111). Phagocytes can also destroy ingested microorganisms by ROS production during the respiratory burst including HClO, via myeloperoxidase. An activated neutrophil produces around 1.6×10^6 HClO molecules / second and in phagosomes between 28 % and 72 % of the consumed oxygen is converted to HClO; therefore, a continuous supply of chlorine is required for HClO to be produced (112).

The highly destructive power of HClO as a non-selective oxidizing agent has been attributed to its ability to oxidize nucleotides, activate latent enzymes as well as the electron transport system, breaking of both cell membranes and basement membranes and fragment proteins (113). Therefore, it is described as an oxygen-dependent, highly unstable, reactive and oxidizing non-dissociated ion from chlorine, and therefore directly responsible for the rapid, chemotactic and broad-spectrum bactericidal action of chlorine-derived compounds (114) (figure 3A).

HClO solutions are not very stable due to various environmental factors, especially the presence of organic compounds and inorganic ions that result in a rapid consumption of it by oxidation reactions. Industrially, HClO results from the union of the acidic chlorine oxide with water and therefore the producer must use pure water preferably from a water purification system, with the lowest possible content of inorganic compounds and ions (115). It can be generated by electrolysis

of sodium chloride (NaCl) and water (H_2O); at pH 5-6, the chlorine species are almost 100 % HClO, but when the pH drops below 5, it begins to convert to Cl2 (chlorine gas). Above a pH level of 6, it begins to convert to a hypochlorite ion (OCl-).

HClO has a well-established antibacterial and antiviral effect, with greater potency than hydrogen peroxide (116). This effect has been demonstrated in encapsulated and non-encapsulated viruses, in DNA viruses such as herpes simplex virus-1, and RNA viruses such as respiratory syncytial virus, influenza A virus, and human CoV 229E (117). Interestingly, the mechanism for HClO production from a continued supply of NaCl constitutes the basis for the recommendation to use nasal washes and gargles with hypertonic saline in 1.5 % and 3 %; the latter under the assumption that the cells of the Nasal, pharyngeal and oral mucosa can convert NaCl to HClO ergo exerting an antiviral effect (118). In fact, with the use of saline solution, clinical improvements have been evidenced in patients who initiate viral diseases such as influenza (119), reflected in less need for drug consumption, fewer days of disease duration, lower rates of transmission, less viral elimination and less fever development (120,121). However, the evidence needs improvement and therefore the development of randomized controlled clinical trials is expected.

Cetylpyridinium Chloride (CPC)

CPC is a cationic quaternary ammonium compound; this amphoteric surfactant generally contains a quaternary nitrogen, associated with at least one hydrophobic substituent. Their cationic nature allows them to interact with the cell wall and membrane, displacing the bivalent cations Mg2 + and $Ca2^{+}$ (122), altering the lipid bilayer, which generates the exit of the cytoplasmic components, killing the cell by solubilization of the membranes (123). The stabilization process by bivalent

cations occurs in gram-positive and gram-negative bacteria, which also gives it a broad antibacterial spectrum. In addition to its direct microbicidal capacity, CPC has been reported to modulate signaling events associated with cellular transcription of nuclear factor kappa-B (NF-kB), a mechanism used by viruses to increase gene transcription and therefore viral replication (124) (figure 3B).

The virucidal action of CPC is very likely to be just as effective on enveloped and non-enveloped viruses; its ability to kill enveloped viruses gives it a wide spectrum of activity. It was found to rapidly damage virus membranes within minutes after challenge, inhibiting its ability to infect. CPC formulations reduce influenza-associated morbidity and mortality in vivo in mice (125). Topical application of the dual-action formulation known as ARMS-I (Halo™) containing CPC, glycerin, and xanthan gum, used for the prevention and treatment of influenza, has been associated with a trend toward reduced severity and the duration of cough and throat discomfort (126). CPC can also inhibit HBV assembly by interacting with the viral nucleocapsid dimeric viral protein. Additionally, it produces a significant decrease of HVB in cell cultures and an inhibition of its replication in mouse hydrodynamic modeling systems. It has also been shown to be active against poliovirus I (127).

Quaternary ammonium compounds have been used on several occasions for the treatment of several CoVs. Its action in the deactivation of the enveloped virus lipid cover suggests that it may be active against SARS-CoV-2. The real question is whether compounds with well-documented in vitro antiviral activity behave similarly *in vivo* (e.g., destroying the virus capsid and accumulating in lysosomes or endosomes blocking viral entry). If so, CPC is a good option to use

as it is cheap, easy to obtain in hospitals and readily accessible for the consumer. Products such as shampoo and soaps containing CPC could aid in potentially helping to reduce viral spread (128).

In vivo, the randomized controlled trial by Seneviratne *et al*. in 2020 (n = 4 for the CPC group) showed a statistically significant increase in Ct value fold change at 5 min and 6 h post-rinsing with 20 ml of a 0.075 % CPC commercially available mouth-rinse, compared to the water group patients (similar results to PVP-I group). The effect was sustained for 3-6h. Taking into account the small size of the study groups, the authors postulate that CPC mouth-rinse decreased the salivary SARS-CoV-2 levels within 5 min of use, compared to water rinsing (84).

Recently, companies that produce and commercialize antiseptic rinses based on CPC have published press releases (product news), reporting effectiveness above 99 % in neutralizing the SARS-CoV-2 virus in saliva. However, at the time of this publication the results of the studies that support such data have not been published in indexed scientific literature (85).

Chlorhexidine (CHX)

Bisbiguanide salts were first described by a British group in 1954 (129) with various pharmacological properties such as antimalarial, hypoglycemic, antiseptic and antiprotozoal. The chemical structure of chlorhexidine is made up of 2 4-chlorophenyl rings linked to 2 biguanide groups by means of a central hexamethylene chain which, in addition to the positive charge, gives it a high alkalinity (130). The mechanism of action of CHX relies on the strong association of the biguanide group with exposed anions in the membrane/cell wall, particularly acid phospholipids and proteins. At low concentrations, CHX bind to pairs of adjacent phospholipid heads, each bound

to half biguanide displacing associated bivalent cations. This decreases the fluidity of the membrane and alters the osmotic regulation (exit of potassium ions and protons) and the metabolic capacity of the membrane and the enzymes contained by the cell (inhibition of respiration and solute transport). At higher concentrations the interactions are more severe and cause the membrane to acquire a crystalline fluid state, lose its structural stability and allow catastrophic filtration of cellular material (122) (figure 3B).

Chlorhexidine is probably the gold standard for antiseptic oral decontamination. Its most widely used form is digluconate chlorhexidine. Concentrations range from 0.003 % in some oral rinses to 4 % in surgical soaps. It has been incorporated into multiple hygiene products, especially at the hospital level. It is found in hand and skin cleansing formulations, for cleaning infected wounds, in surgical cements, topical antiseptics, oral rinses and gels and attached to slow release vehicles. Its use as an antiplaque agent and therefore with effects on gingival inflammation has been extensively documented (131). As an oral antiseptic, CHX has a greater substantivity than other products. Once a rinse is performed, approximately 30 % of the compound is retained in the oral cavity through electrostatic interactions with the acidic groups on the macromolecules of the mucous secretions that cover the oral surfaces. The sequential displacement of chlorhexidine bound to the oral mucosa caused by bivalent cations such as calcium from saliva is suggestive of the slow release mechanism, up to 8-12 hours (132).

A limited viral inactivation potential/virucidal effect have been described with the use of chlorhexidine and other phenolic disinfectants (133). Based on this observation, multiple protocols for the treatment of SARS-CoV-2 mostly rule out CHX as a useful active ingredient for

disinfection. To date, no publication has been found to prove or disprove its effectiveness; however, there is available data on other viruses with similar characteristics, specifically with the presence of viral envelopes. For example, the in vitro antiviral efficacy of 0.12 % CHX has been tested on Herpes simplex virus (HSV), Cytomegalovirus (CMV), Influenza A, Parainfluenza, Polio, and Hepatitis B (HBV). The results found that 0.12 % CHX was active against all viruses except Polio and that such effect increased with exposure time. Variations in the responses to the antiseptic were undoubtedly observed depending on the exposure time, possibly due to differences in the physical-chemical properties of the virus envelope. It was then proposed that CHX exerted its antiviral effect on virus envelopes and that the absence of envelope on poliovirus was responsible for its ineffectiveness (134). In fact, CHX ineffectiveness on non-enveloped virus (murine norovirus - MNV) has been documented elsewhere (102). More recently, an in vitro investigation was carried out compare the antiviral effects of 2 different CHX formulations (0.12 % and 0.2 %) on HIV-1 and HSV-1, which are high transmission risk in the dental practice. Commercial undiluted formulations completely inhibited HSV-1 as well as the 1:4 dilutions inhibited HIV-1. However, authors acknowledge the limitations of their in vitro experiment to predict in vivo efficacy behavior (135).

Based on the principle of potential efficacy of CHX in enveloped viruses, a preoperative protocol has been proposed for optimizing infection control in the operating room during the COVID-19 pandemic. It includes the use of chlorhexidine in both wipes and gargles for hospitalized patients (87).

The *in vitro* study by Meister *et al.* (77) on the efficacy of various commercial products on SARS-CoV-2 determined a Log reduction factor between 0.5 % and 1.17 % for the virucidal activity after

30 s exposure time for 2 different chlorhexidine formulations (0.2 % in a non-alcoholic base). This reduction was less than the one obtained for PVP-I compound.

The study by Xu *et al.* (79), after an infectivity *in vitro* test and a cytotoxicity assay, reported that 1.5 % (v/v) diluted CHX did not impact cell viability; all 50 % (v/v) dilutions of the 4 mouth rinses used, including CHX, were highly toxic to HeLa-hACE2 and oral epithelial cells. CHX was within the less cytotoxic compounds. They found that CHX reduced infection of Vero cells by 70 %, without apparent impacts on cell morphology; also, 1.5 % or 3 % (v/v) CHX suppressed viral infection by 88 % and 97 %, respectively without an impact on cell viability.

Recently, an uncontrolled *in vivo* study was published in 2 hospitalized COVID-19^{+} patients, who rinsed with 15 ml 0.12 % CHX for 30 seconds; SARS-CoV-2 viral load was measured in saliva samples for 1-4 hours. A significant decrease in viral load was reported with the use of CHX for up to 2 h, after which the viral counts in saliva recovered baseline values (86). A similar study that included 6 patients in the group with CHX 0.2 % w / v (Pearly White Chlor-Rinse) showed a varied effect among saliva Ct values after 5 min rinsing, with a tendency to maintain reduced viral loads at 3 h and 6 h post mouthwashes (84). Although promising, the results of the present study must be carefully evaluated due to sample size limitations and study design.

FIGURE 3

MECHANISM OF ACTION OF THE MOST RECOMMENDED ANTISEPTICS FOR ORAL DECONTAMINATION AS A STRATEGY TO REDUCE THE RISK OF SARS-COV-2 SPREAD

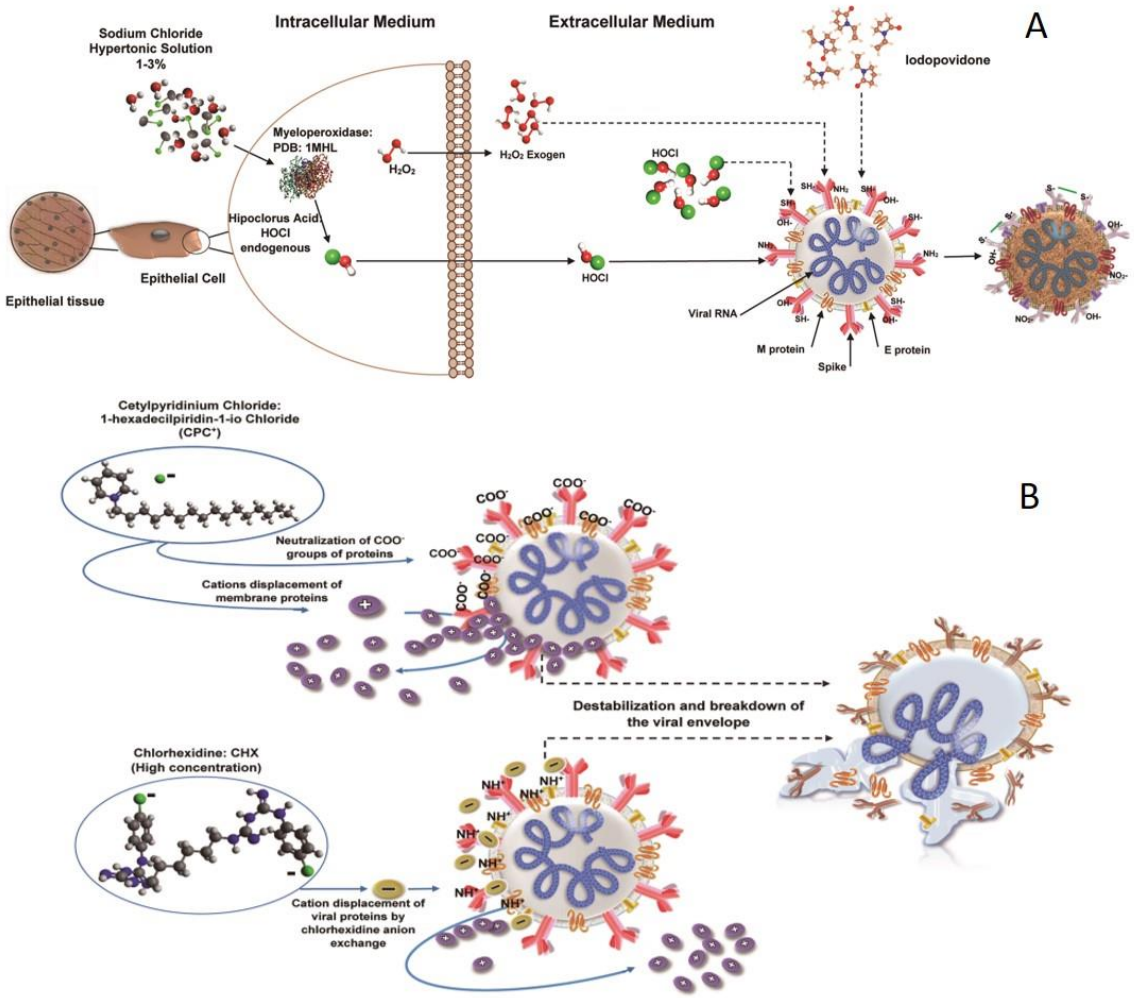

Figure 3. **A.** The –R groups of the amino acids of viral proteins: -SH (thiol), -OH (hydroxyl) and -NH2, -NRH or -NR2 (amino), which are not involved in the covalent bonds of the primary structure, are susceptible to oxidation by agents such as H_2O_2 (hydrogen peroxide), HClO (hypochlorous acid) and $C_6H_9I_2NO$ (povidone iodine). Neighboring -SH groups oxidize to -S-S- and -NH2 to -NO2. This leads to changes in the tertiary and quaternary structures of proteins, causing rupture of the membrane and alteration of function. **B.** $C_{21}H_{38}ClN$ (Cetylpyridinium chloride) and $C_{22}H_{30}Cl_2N_{10}$ (chlorhexidine), due to their cationic and anionic nature, produce positive ($C_{21}H_{38}ClN$) or negative ($C_{22}H_{30}Cl_2N_{10}$) charges displacement present in the viral membrane. In the case of $C_{21}H_{38}ClN$, in addition to the displacement of cations, the negative charges of $–COO^-$ groups of the proteins are neutralized. In both cases, the alteration of the viral membrane leads to its rupture.

CONCLUSIONS

Dentists have been placed in a historically important situation with the current pandemic and its transmission risks, and as such dentists need to be informed with the best available evidence for

the sake of their patients as well as their occupational health and safety. This narrative review has covered general aspects of COVID-19 physiopathology including SARS-CoV-2 characteristics, human receptors mediating cellular entry, disease evolution and viral loads in the oral cavity. As saliva emerges as a very plausible transmission route in asymptomatic patients who are not aware of their infectious potential, strategies to pursue transmission risk reduction ought to be pursued.

When considering the different pathways from which SARS-CoV-2 can reach the oral cavity and colonize the saliva, it is important to note that other respiratory fluids (sputum expectoration) can also saturate the oral pharyngeal region; therefore, gargling emerges as a better strategy when compared to only oral decontamination via mouth washing. Saline washes of the nasal passage, mouth, and throat could probably reduce viral load in the body mechanically (at least) in the initial stage of pathogenesis. This could be similar to hand washing to contain the spread of the infection. Therefore, antiseptic gargles and nasal wash may work in preventing the disease and may also be useful in reducing nasopharyngeal viral load to provide symptomatic relief. Furthermore, it may reduce viral shedding and reduce the transmission of the illness. This may break the chain of infection. COVID-19 disease is mild in eighty percent of patients and resolves spontaneously. Therefore, nasopharyngeal wash may be useful, especially in high risk populations such as subjects with comorbid conditions and above 60 years of age.

This narrative review has also covered the best available evidence from the most common oral decontamination agents readily available for the clinician. We can conclude that there is no high-quality direct evidence of clinical effectiveness of the use of mouth rinses prior to dental procedures with antiseptic solutions for SARS-CoV-2 specifically to date. However, absence of

direct evidence should not mean that nothing works on the virus, rather it should just mean that there is a lack of evidence for the moment. When indirect evidence is considered, particularly scarce on SARS-CoV-2 effects, there are multiple reasons to justify oral decontamination protocols with antiseptic agents. Although there is not sufficient evidence to support the use of one specific agent over the other, the antiviral capacity of all agents here reviewed is sufficiently suggestive of its potential SARS-CoV-2 virucidal capacity. Ergo, oral decontamination should be promoted in our opinion.

In sum, the possibility of completely eradicating SARS-CoV-2 is practically impossible. It is therefore necessary to learn how to establish a coexistence with this virus from different areas, being the dental one of those that requires an important degree of analysis. Current clinical practice is adapted through gradual changes that respond to the level of knowledge available in a dynamic process, in which protocols for clinical care, issued by academic and government organizations, have become very important. However, the dental profession has to quickly adopt such recommendations; surprisingly, a recent survey report from 669 dentists (30 different countries) found that 74 % of dentists (from which 76 % are in hospital settings) did not ask patients to rinse the mouth with antibacterial mouthwash before dental treatment (74). Our well-known biosafety practices in the dental office could have a greater impact in patient behavior and transmission risk reduction.

Finally, although it is clear that oral antiseptics could decrease viral load, the infectivity of the SARS-CoV 2 virus from saliva should be investigated both before and after rinsing with antiseptics, since these compounds act by superficial contact with the virus, but replication within

cells is not affected; therefore, it is crucial to determine how long it would take for the virus to regain infectious potential in saliva. As of today, we can affirm that it is feasible to reduce the viral load in saliva with this type of strategy, but not that said decrease in viral load is an accurate preventive strategy in controlling the spread of the infection and even less use it as a therapeutic strategy. The fact that SARS-CoV-2 is fatal in a significant percentage of cases makes infectivity studies in vivo ethically impossible. In addition, preventive strategies should extend to trying to block the different viral proteins to prevent the virus from entering the cells in a way that combats viral replication, directly avoiding infection.

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