Saudi Dental Journal (2020) 32, 181–186

King Saud University

Saudi Dental Journal

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ORIGINAL ARTICLE

Guidelines for dental care provision during the COVID-19 pandemic

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Received 29 March 2020; revised 31 March 2020; accepted 1 April 2020
Available online 7 April 2020

KEYWORDS

Coronavirus;
SARS-CoV-2;
COVID-19;
Dental Care;
Guideline Development;
Pandemic

Abstract Since the coronavirus disease 2019 (COVID-19) outbreak was declared a pandemic on 11 March 2020. Several dental care facilities in affected countries have been completely closed or have been only providing minimal treatment for emergency cases. However, several facilities in some affected countries are still providing regular dental treatment. This can in part be a result of the lack of universal protocol or guidelines regulating the dental care provision during such a pandemic. This lack of guidelines can on one hand increase the nosocomial COVID-19 spread through dental health care facilities, and on the other hand deprive patients' in need of the required urgent dental care. Moreover, ceasing dental care provision during such a period will incense the burden on hospitals emergency departments already struggle with the pandemic.

This work aimed to develop guidelines for dental patients' management during and after the COVID-19 pandemic.

Guidelines for dental care provision during the COVID-19 pandemic were developed after considering the nature of COVID-19 pandemic, and were based on grouping the patients according to condition and need, and considering the procedures according to risk and benefit.

It is hoped that the guidelines proposed in this work will help in the management of dental care around the world during and after this COVID-19 pandemic.
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1. Introduction

1.1. Background

At a media briefing on 11 March 2020, the Director-General of the World Health Organization (WHO) declared coronavirus disease 2019 (COVID-19) outbreak a pandemic (WHO, 2020a). COVID-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Originating in

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sdentj.2020.04.001
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Wuhan, China, the first COVID-19 case was reported to the WHO country office in China on 31 December 2019. As of 29 March 2020, COVID-19 has been recognized in over 200 countries, areas and territories, with a total of over 575,000 confirmed cases and over 26,000 deaths (WHO, 2020b).

Similar to SARS-CoV and the Middle East Respiratory Syndrome (MERS-CoV) virus, SARS-CoV-2 is zoonotic virus. Zoonotic viruses can spread from non-human animals to humans. In this case, Chinese horseshoe bats (Rhinolophus sinicus) are the most probable origin and pangolin (Manis javanica) as an intermediate host (Li et al., 2020).

The asymptomatic incubation period of the virus is estimated to be between 2 and 12 days; however, up to 24 days incubation period was reported in some studies (C.C. Lai et al., 2020). The most common symptoms of coronavirus disease are fever, tiredness, dry cough and shortness of breath. More than 80% of cases are mild and recover from the disease without needing special treatment. However, around 15% of cases are categorised as severely ill and the remaining 5% are categorised as critically ill. In severe and critical cases, acute respiratory disease can lead to pneumonia, kidney failure, and even death.

Although it is still early to determine the case fatality ratio (CFR); today it is estimated to be over 4.5% (WHO, 2020b).

While the mild COVID-19 cases do not require specific care, and usually symptomatic treatment and home isolation are enough. Oxygen therapy is the major intervention for patients with severe cases. Critical cases management on the other hand is case dependant and will usually need intensive care (Chughtai and Malik, 2020; Guan et al., 2020; WHO, 2020c). Although not completely understood at this stage, human-to-human transmission is now believed to be mainly via saliva associated respiratory droplets and contact transmission. However, fecal-oral transmission is possible as SARS-CoV-2 was identified in the stool of patients (Holshue et al., 2020).

Vertical transmission (from mothers to their new-borns) however, is not yet confirmed (H. Chen et al., 2020). Moreover, aerosol and fomite transmission of SARS-CoV-2 is also plausible as the virus can remain viable and infectious in aerosols for at least three hours and on surfaces for days (van Doremalen et al., 2020). Transmission from asymptomatic COVID-19 carriers possibility was also reported (Bai et al., 2020; C.-C. Lai et al., 2020).

To date, real-time reverse transcription polymerase chain reaction (rRT-PCR) test is utilised for the qualitative detection of nucleic acid from SARS-CoV-2 in upper and lower respiratory specimens obtained through nasopharyngeal and/or oropharyngeal swabs. Viral RNA has been also isolated from the plasma of some patients (Huang et al., 2020).

1.2. SARS-CoV-2 transmission and dental treatment

Given the novelty of the disease, no cases of SARS-CoV-2 transmission in a dental setting are identified yet. However, given the high transmissibility of the disease and considering that routine dental procedures usually generate aerosols; during the course of this pandemic, alterations to dental treatment should be considered to maintain a healthy environment for the patients and the dental team.

SARS-CoV-2 has been isolated from the saliva of COVID-19 patients (To et al., 2020). Moreover, salivary gland epithelial cells can potentially be infected by SARS-CoV and become a major source of the virus in saliva (Liu et al., 2011). Even after patient recovery, recusancy during the convalescence period was reported (D. Chen et al., 2020). This is plausible since the presence of some virus strains in saliva for as long as 29 days have been reported in the literature (Barzon et al., 2016; Zuanazzi et al., 2017).

In addition to blood and salivary contamination, the majority of routine rental treatments generate significant amounts of droplets and aerosols. This is usually related to the utilisation of devices and equipment such ultrasonic scalers, air-water syringes and air turbine handpieces.

1.3. Dental treatment during the pandemic

Despite the large-scale community transmission of COVID-19 in China during the epidemic; demand for urgent dental treatment decreased by only 38% (Guo et al., 2020). This shows that the public need for urgent dental care even during this pandemic will always be essential. To date, it has been two weeks since COVID-19 outbreak was declared as a pandemic; yet several dental institutes, regulatory and advisory bodies still do not have a clear vision about the worldwide impact this pandemic can have on dental services. Dental associations responses and actions around the world varied from advising practitioners to close their practices in California, USA (CDA, 2020); to reducing the number of routine check-ups in the UK (Scottish Government, 2020); to no advice at all from several dental associations around the world.

Such unclarity is expected due to the varying degree of COVID-19 outbreak in different countries and due to the fact that the previous global pandemic was influenza about 100 years ago (Mills et al., 2004).

However, according to the US Government COVID-19 response plan published by the US Department of Health and Human Services (HHS) on 13 March 2020, this COVID-19 pandemic could last over 18 months (HHS, 2020).

Closing dental practices during the pandemic can reduce the number of affected individuals, but will increase the suffering of the individuals in need of urgent dental care. It will also incense the burden on hospitals emergency departments.

This calls for the creation of standard guidelines for dental care provision during the worldwide spread of the pandemic and/or local epidemic outbreaks.

1.4. Aim

It is the purpose of this work to develop a guideline for dental patients' management during and after the COVID-19 pandemic.

2. Methods

To develop guidelines for dental care provision during the pandemic, the following factors should be considered:

1. The incubation period of the virus is believed to be up to 14 days; and transmission from asymptomatic COVID-19 carriers is possible (Bai et al., 2020; Guan et al., 2020; C.-C. Lai et al., 2020).

2. Aerosol and fomite transmission of SARS-CoV-2 is plausible (van Doremalen et al., 2020).
3. It is unclear yet, but COVID-19 recusancy might be possible and some virus strains can be present in saliva for as long as 29 days (Barzon et al., 2016; D. Chen et al., 2020; Zuanazzi et al., 2017).
4. Some confirmed COVID-19 carriers might need urgent dental care at some point.

That necessitates:

1. Screening every asymptomatic patient meticulously.
2. Considering every patient as a potential asymptomatic COVID-19 carrier.
3. Considering recently recovered patients as potential virus carriers for at least 30 days after the recovery confirmation by a laboratory test
4. Identifying the urgent need of the patient and focusing on managing it with minimally invasive procedures.
5. Categorising dental treatment according to the urgency of the required treatment and the risk and benefit associated with each treatment.
6. Identifying the required dental treatment for each patient and the risks and benefits associated with that treatment.
7. Using contact, and airborne precautions including proper aerosol-generating procedures personal protective equipment (PPE) for every procedure.

From there, patients in need of dental care should be categorised according to the probability of them being COVID-19 affected or carriers (Fig. 1).

The dental treatment should also be classified according to the severity of the case and the degree of procedure invasiveness and risk (Table 1).

2.1. Patients screening and categorisation

Whenever possible, tele-screening of the patients is strongly advised, and at the first point of contact, patients should be screened for any COVID-19 symptoms and any recent contact with confirmed COVID-19 patients and/or recent travel to recent disease epicentres. For active and recently recovered confirmed cases, dental treatment should only be considered after coordination with primary physician. Disease history, and current stage should be meticulously evaluated. Any suspected or confirmed COVID-19 patients' treatment should be postponed if possible or performed in an airborne infection isolation rooms (AIIRs) or negative pressure rooms ideally at a hospital setting.

For these guidelines' development, after the screening, patients are proposed to be divided into five groups (Fig. 1):

A. Asymptomatic and unsuspected, unconfirmed COVID-19 case.
B. Symptomatic and/or suspected, unconfirmed COVID-19 case.
C. Stable confirmed COVID-19 case.
D. Unstable confirmed COVID-19 case.
E. Recovered confirmed COVID-19 case.

Current COVID-19 patients are considered stable if the case is mild and no hospitalisation or oxygen therapy is

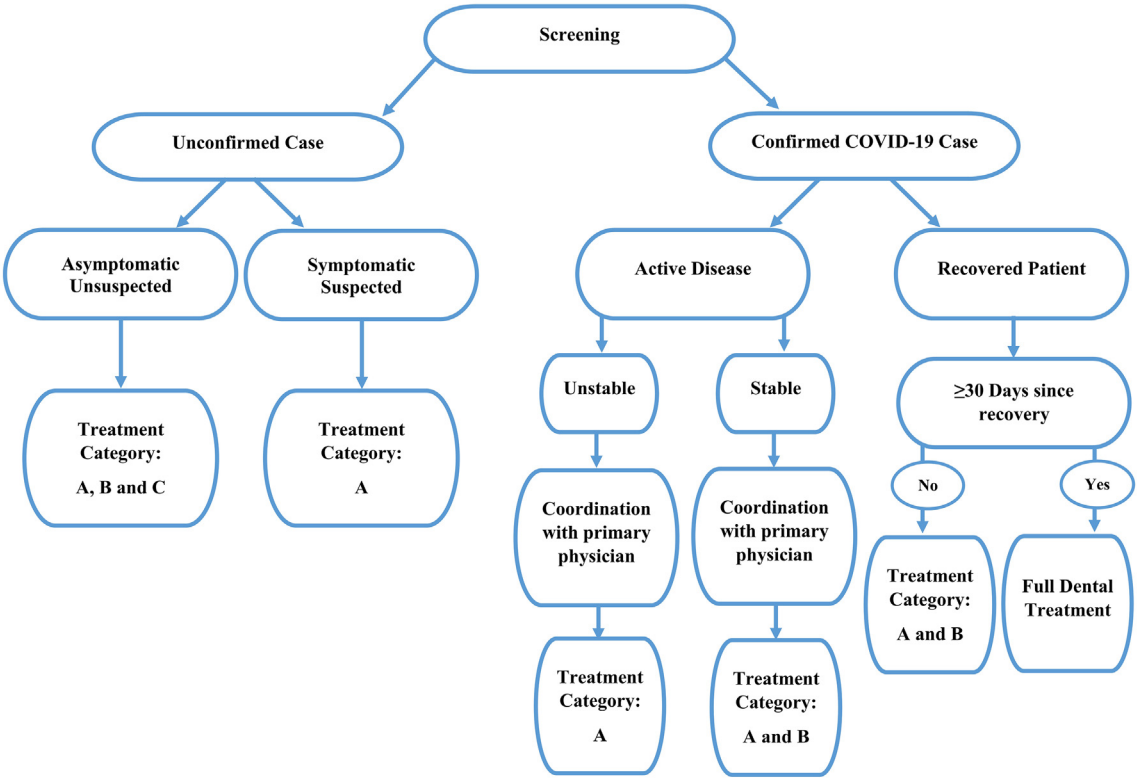


Fig. 1 Flowchart showing the dental patients screening and categorisation method during the COVID-19 pandemic as well as the categorisation method of the affected patients after the pandemic.

Table 1 A guidance table showing the categories of dental treatments and the variety of treatments that can be provided for the patient during the COVID-19 pandemic.

Dental Treatments Categories				
A Emergency	B Urgent conditions that can be managed with minimally invasive procedures and without aerosol generation	C Urgent conditions that need to be managed with invasive and/or aerosol-generating procedures	D Non-urgent	E Elective
Unstable maxillofacial fractures that can compromises the patient's airway.*	Severe dental pain (7≤) from pulpal inflammation that requires tooth extraction.**	Severe dental pain (7≤) from pulpal inflammation that need to be managed with aerosol generating procedures.**	Removable dentures adjustments or repairs.	Initial or periodic oral examinations and recall visits.
Diffuse soft tissue bacterial infection with intraoral or extraoral swelling that can compromises the patient's airway.*	Severe dental pain (7≤) from fractured vital tooth that can be managed without aerosol generation.**	Severe dental pain (7≤) from fractured vital tooth that need to be managed with aerosol generating procedures.**	Asymptomatic fractured or defective restoration.	Aesthetic dental procedures.
Uncontrolled postoperative bleeding.*	Dental trauma with avulsion/luxation that can be minimally managed without aerosol generation.	Dental trauma with avulsion/luxation that need invasive/Aerosol Generating Procedures	Asymptomatic fractured or defective fixed prosthesis.	Restorative treatment of asymptomatic teeth.
	Surgical postoperative osteitis or dry socket that can be managed without aerosol generation.*	Deboned fixed prosthesis cleaning and temporary cementation.	Asymptomatic fractured or defective orthodontic appliance.	Extraction of asymptomatic teeth.
	Pericoronitis or third-molar pain that can be managed without aerosol generation.	Removable dentures adjustments for radiation/oncology patients.	Chronic periodontal disease.	Orthodontic procedures other than those in category B/C.
	Stable maxillofacial fractures that requires no intervention.*	Fractured or defective fixed prosthesis causing soft tissue injury.		Routine dental cleaning and preventive therapies.
	Localised dental/periodontal abscess that can be managed without aerosol generation	Acute periodontal disease.		Replacement of missing tooth/teeth with fixed or removable prosthesis.
	Fractured or defective fixed orthodontic appliance causing soft tissue laceration.			Dental implant surgery.

* Usually managed by oral and maxillofacial surgeons.
** Pain assessment is carried out using the Universal Pain Assessment Tool (UPA).

required. Sever and critical cases are classified as unstable. Confirmed recovery is considered if the patient has been asymptomatic for at least 30 days after the last negative laboratory test.

2.2. Treatment categorisation

For these guidelines' development, dental procedures are proposed to be divided into five categories (Table 1):

A. Emergency management of life-threatening conditions.
B. Urgent conditions that can be managed with minimally invasive procedures and without aerosol generation.
C. Urgent conditions that need to be managed with invasive and/or aerosol-generating procedures.
D. Non-urgent procedures.
E. Elective procedures.

2.3. Treatment considerations

1. Intraoral imaging should be restricted and extraoral radiographs should be utilised to reduce the excessive salivation and gag reflex associated with intraoral radiographs.
2. Using 0.23% povidone-iodine mouthwash for at least 15 s before the procedure can reduce the viral load in the patient's saliva (Eggers et al., 2018).
3. Disposable and single-use instruments and devices should be used whenever possible to reduce the cross-infection risks.

4. Rubber dam should be used whenever possible as this will significantly reduce the spread of microorganisms (Cochran et al., 1989).
5. The dental treatment should be as minimally invasive as possible.
6. Aerosol-generating procedures should be avoided whenever possible.
7. Whenever pharmacologic management of pain is required, Ibuprofen should be avoided in suspected and confirmed COVID-19 cases (Day, 2020).

3. Discussion

To date, no universal protocol or guideline is available for dental care provision to active or suspected COVID-19 cases. In fact, no universal guidelines are available for dental care provision during the times of any epidemic, pandemic, national or global disaster. Due to that lack of a standard, dental care provision has completely stopped or significantly decreased in several affected countries. In addition to increasing the affected populations suffering, this will also incense the burden on hospitals emergency departments already struggle with the pandemic. This lack of guidelines can also increase the nosocomial COVID-19 spread through dental health care facilities.

Due to the possibility of COVID-19 recusancy and the fact that some viruses can be present in saliva for as long as 29 days after recovery of the patient 16–18. The management of recently recovered COVID-19 patients was also considered in these guidelines. This can help in reducing and preventing new outbreaks.

The guidelines developed in this work are general guidelines and the final decision will always be provided through the practitioner's judgment. For instance, if the required treatment cannot be provided for the patient due to his/her patient category; the practitioner's judgment and evaluation of the case can provide for other alternative methods of management. Otherwise, the treatment should be postponed and pharmacologic management of the pain and/or infection should be considered. Moreover, the dental treatments categories in Table 1, covers most but not all of the dental cases. Therefore, case and treatment categorisation should always be considered by the practitioner.

It is hoped that the guidelines proposed in this work will help in the management of dental care around the world during this COVID-19 pandemic, and provide a solid base for further healthcare guidelines development.

CRediT authorship contribution statement

Ali Alharbi: Conceptualization, Methodology, Writing - original draft, Writing - review & editing. **Saad Alharbi:** Methodology, Writing - original draft. **Shahad Alqaidi:** Methodology, Writing - original draft.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

References

Bai, Y., Yao, L., Wei, T., Tian, F., Jin, D.Y., Chen, L., Wang, M., 2020a. Presumed asymptomatic carrier transmission of COVID-19. JAMA – J. Am. Med. Assoc. https://doi.org/10.1001/jama.2020.2565.

Barzon, L., Pacenti, M., Berto, A., Sinigaglia, A., Franchin, E., Lavezzo, E., Brugnaro, P., Palù, G., 2016. Isolation of infectious zika virus from saliva and prolonged viral RNA shedding in a traveller returning from the dominican republic to Italy, January 2016. Eurosurveillance. https://doi.org/10.2807/1560-7917.

CDA, 2020. Dental Office Closure Due to COVID-19 [WWW Document]. https://www.cda.org/Portals/0/cda-covid19-dental-office-closure.pdf.

Chen, D., Xu, W., Lei, Z., Huang, Z., Liu, J., Gao, Z., Peng, L., 2020a. Recurrence of positive SARS-CoV-2 RNA in COVID-19: a case report. J. Infect. Dis. Int. https://doi.org/10.1016/j.ijid.2020.03.003.

Chen, H., Guo, J., Wang, C., Luo, F., Yu, X., Zhang, W., Li, J., Zhao, D., Xu, D., Gong, Q., Liao, J., Yang, H., Hou, W., Zhang, Y., 2020b. Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records. Lancet 395, 809–815. https://doi.org/10.1016/S0140-6736(20)30360-3.

Chughtai, A., Malik, A., 2020. Is Coronavirus disease (COVID-19) case fatality ratio underestimated?. J. Glob. Biosecurity, 1.

Cochran, M.A., Miller, C.H., Sheldrake, M.A., 1989. The efficacy of the rubber dam as a barrier to the spread of microorganisms during dental treatment. J. Am. Dent. Assoc. 119, 141–144. https://doi.org/10.14219/jada.archive.1989.0131.

Day, M., 2020. Covid-19: ibuprofen should not be used for managing symptoms, say doctors and scientists. BMJ 368, m1086. https://doi.org/10.1136/bmj.m1086.

Eggers, M., Koburger-Janssen, T., Eickmann, M., Zorn, J., 2018. In vitro bactericidal and virucidal efficacy of povidone-iodine gargle/mouthwash against respiratory and oral tract pathogens. Infect. Dis. Ther. 7, 249–259. https://doi.org/10.1007/s40121-018-0200-7.

Guan, W., Ni, Z., Hu, Y., Liang, W., Ou, C., He, J., Liu, L., Shan, H., Lei, C., Hui, D.S., Du, B., Li, L., Zeng, G., Yuen, K.-Y., Chen, R., Tang, C., Wang, T., Chen, P., Xiang, J., Li, S., Wang, J., Liang, Z., Peng, Y., Wei, L., Liu, Y., Peng, P., Liu, J., Chen, Z., Li, G., Zheng, Z., Qiu, S., Luo, J., Ye, C., Zhu, S., Zhong, N., 2020. Clinical characteristics of 2019 novel coronavirus infection in China. N. Engl. J. Med. https://doi.org/10.1101/2020.02.06.20020974.

Guo, H., Zhou, Y., Liu, X., Tan, J., 2020. The impact of the COVID-19 epidemic on the utilization of emergency dental services. J. Dent. Sci., 0–3 https://doi.org/10.1016/j.jds.2020.02.002.

HHS, 2020. U.S. Government COVID-19 Response Plan [WWW Document]. US Dep. Heal. Hum. Serv. (accessed 3.29.20) https://int.nyt.com/data/documenthelper/6819-covid-19-response-plan/d367f758bec47cad361f/optimized/full.pdf.

Holshue, M.L., DeBolt, C., Lindquist, S., Lofy, K.H., Wiesman, J., Bruce, H., Spitters, C., Ericson, K., Wilkerson, S., Tural, A., Diaz, G., Cohn, A., Fox, L.A., Patel, A., Gerber, S.I., Kim, L., Tong, S., Lu, X., Lindstrom, S., Pallansch, M.A., Weldon, W.C., Biggs, H. M., Uyeki, T.M., Pillai, S.K., 2020. First case of 2019 novel coronavirus in the United States. N. Engl. J. Med. 382, 929–936. https://doi.org/10.1056/NEJMoa2001191.

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, J., Gu, X., Cheng, Z., Yu, T., Xia, J., Wei, Y., Wu, W., Xie, X., Yin, W., Li, H., Liu, M., Xiao, Y., Gao, H., Guo, L., Xie, J., Wang, G., Jiang, R., Gao, Z., Jin, Q., Wang, J., Cao, B., 2020. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 395, 497–506. https://doi.org/10.1016/S0140-6736(20)30183-5.

Lai, C.-C., Liu, Y.H., Wang, C.-Y., Wang, Y.-H., Hsueh, S.-C., Yen, M.-Y., Ko, W.-C., Hsueh, P.-R., 2020a. Asymptomatic carrier state, acute respiratory disease, and pneumonia due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2): Facts and myths. J. Microbiol. Immunol. Infect. https://doi.org/10.1016/j.jmii.2020.02.012.

Lai, C.C., Shih, T.P., Ko, W.C., Tang, H.J., Hsueh, P.R., 2020b. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): The epidemic and the challenges. Int. J. Antimicrob. Agents 55, 105924. https://doi.org/10.1016/j.ijantimicag.2020.105924.

Li, J.Y., You, Z., Wang, Q., Zhou, Z.J., Qiu, Y., Luo, R., Ge, X.Y., 2020. The epidemic of 2019-novel-coronavirus (2019-nCoV) pneumonia and insights for emerging infectious diseases in the future. Microbes Infect. 22, 80–85. https://doi.org/10.1016/j.micinf.2020.02.002.

Liu, L., Wei, Q., Alvarez, X., Wang, H., Du, Y., Zhu, H., Jiang, H., Zhou, J., Lam, P., Zhang, L., Lackner, A., Qin, C., Chen, Z., 2011. Epithelial cells lining salivary gland ducts are early target cells of severe acute respiratory syndrome coronavirus infection in the upper respiratory tracts of rhesus macaques. J. Virol. 85, 4025–4030. https://doi.org/10.1128/jvi.02292-10.

Mills, C.E., Robins, J.M., Lipsitch, M., 2004. Transmissibility of 1918 pandemic influenza. Nature 432, 904–906. https://doi.org/10.1038/nature03063.

Scottish Government, 2020. Updated guidance for dental practices [WWW Document]. https://www.gdc-uk.org/docs/default-source/-covid-19/ocdo-scotland-letter.pdf (accessed 3.29.20).

To, K.K.-W., Tsang, O.T.-Y., Chik-Yan Yip, C., Chan, K.-H., Wu, T.-C., Chan, J.M.C., Leung, W.-S., Chik, T.S.-H., Choi, C.Y.-C., Kandamby, D.H., Lung, D.C., Tam, A.R., Poon, R.W.-S., Fung, A.Y.-F., Hung, I.F.-N., Cheng, V.C.-C., Chan, J.F.-W., Yuen, K.-Y., 2020. Consistent detection of 2019 novel coronavirus in saliva. Infect. Dis. Clin. https://doi.org/10.1093/cid/ciaa149.

van Doremalen, N., Bushmaker, T., Morris, D.H., Holbrook, M.G., Gamble, A., Williamson, B.N., Tamin, A., Harcourt, J.L., Thornburg, N.J., Gerber, S.I., Lloyd-Smith, J.O., de Wit, E., Munster, V. J., 2020. Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. N. Engl. J. Med. https://doi.org/10.1056/NEJMc2004973.

WHO, 2020a. WHO Virtual press conference on COVID-19 [WWW Document]. World Heal. Organ. https://www.who.int/docs/default-source/coronaviruse/transcripts/who-audio-emergencies-coronavirus-press-conference-full-and-final-11mar2020.pdf (accessed 3.29.20).

WHO, 2020b. WHO Novel Coronavirus (COVID-19) Situation [WWW Document]. World Heal. Organ. https://experience.arcgis.com/experience/685d0ace521648f8a5beeeee1b9125cd (accessed 3.29.20).

WHO, 2020c. Coronavirus disease 2019 Situation Report – 41 [WWW Document]. World Heal. Organ. https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200301-sitrep-41-covid-19.pdf (accessed 3.29.20).

Zuanazzi, D., Arts, E.J., Jorge, P.K., Mulyar, Y., Gibson, R., Xiao, Y., Bringel Dos Santos, M., Machado, M.A.A.M., Siqueira, W.L., 2017. Postnatal identification of Zika virus peptides from Saliva. J. Dent. Res. 96, 1078–1084. https://doi.org/10.1177/0022034517723325.

Journal of Dental Research
1–7
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Coronavirus Disease 2019 (COVID-19): Emerging and Future Challenges for Dental and Oral Medicine

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Abstract

The epidemic of coronavirus disease 2019 (COVID-19), originating in Wuhan, China, has become a major public health challenge for not only China but also countries around the world. The World Health Organization announced that the outbreaks of the novel coronavirus have constituted a public health emergency of international concern. As of February 26, 2020, COVID-19 has been recognized in 34 countries, with a total of 80,239 laboratory-confirmed cases and 2,700 deaths. Infection control measures are necessary to prevent the virus from further spreading and to help control the epidemic situation. Due to the characteristics of dental settings, the risk of cross infection can be high between patients and dental practitioners. For dental practices and hospitals in areas that are (potentially) affected with COVID-19, strict and effective infection control protocols are urgently needed. This article, based on our experience and relevant guidelines and research, introduces essential knowledge about COVID-19 and nosocomial infection in dental settings and provides recommended management protocols for dental practitioners and students in (potentially) affected areas.

Keywords: virology, infection control, dental public health, dental education, transmission, dental practice management

Introduction

On January 8, 2020, a novel coronavirus was officially announced as the causative pathogen of COVID-19 by the Chinese Center for Disease Control and Prevention (Li et al. 2020). The epidemics of coronavirus disease 2019 (COVID-19) started from Wuhan, China, last December and have become a major challenging public health problem for not only China but also countries around the world (Phelan et al. 2020). On January 30, 2020, the World Health Organization (WHO) announced that this outbreak had constituted a public health emergency of international concern (Mahase 2020). The novel coronavirus was initially named 2019-nCoV and officially as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). As of February 26, COVID-19 has been recognized in 34 countries, with a total of 80,239 laboratory-confirmed cases and 2,700 deaths (WHO 2020b).

Due to the characteristics of dental settings, the risk of cross infection may be high between dental practitioners and patients. For dental practices and hospitals in countries/regions that are (potentially) affected with COVID-19, strict and effective infection control protocols are urgently needed. This article, based on our experience and relevant guidelines and research, introduces the essential knowledge about COVID-19 and nosocomial infection in dental settings and provides recommended management protocols for dental practitioners and students in (potentially) affected areas.

What Is COVID-19?

Viral Etiology

According to recent research, similar to SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV), SARS-CoV-2 is zoonotic, with Chinese horseshoe bats (*Rhinolophus sinicus*) being the most probable origin (Chan et al. 2020; Lu et al. 2020) and pangolins as the most likely intermediate host (The Chinese Preventive Medicine Association 2020).

Epidemiologic Characteristics

Mode of Transmission. Based on findings of genetic and epidemiologic research, it appears that the COVID-19 outbreak

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started with a single animal-to-human transmission, followed by sustained human-to-human spread (Chan et al. 2020; Del Rio and Malani 2020). It is now believed that its interpersonal transmission occurs mainly via respiratory droplets and contact transmission (The Chinese Preventive Medicine Association 2020). In addition, there may be risk of fecal-oral transmission, as researchers have identified SARS-CoV-2 in the stool of patients from China and the United States (Holshue et al. 2020). However, whether SARS-CoV-2 can be spread through aerosols or vertical transmission (from mothers to their newborns) is yet to be confirmed (Chen, Guo, et al. 2020; WHO 2020c; Zhu et al. 2020).

Source of Transmission. Although patients with symptomatic COVID-19 have been the main source of transmission, recent observations suggest that asymptomatic patients and patients in their incubation period are also carriers of SARS-CoV-2 (Chan et al. 2020; Rothe et al. 2020). This epidemiologic feature of COVID-19 has made its control extremely challenging, as it is difficult to identify and quarantine these patients in time, which can result in an accumulation of SARS-CoV-2 in communities (The Chinese Preventive Medicine Association 2020). In addition, it remains to be proved whether patients in the recovering phase are a potential source of transmission (Rothe et al. 2020).

Incubation Period. The incubation period of COVID-19 has been estimated at 5 to 6 d on average, but there is evidence that it could be as long as 14 d, which is now the commonly adopted duration for medical observation and quarantine of (potentially) exposed persons (Backer et al. 2020; Li et al. 2020).

Fatality Rate. According to current data, the fatality rate (cumulative deaths divided by cumulative cases) of COVID-19 is 0.39% to 4.05%, depending on different regions of China, which is lower than that of SARS (severe acute respiratory syndrome; ≈10%) and MERS (Middle East respiratory syndrome; ≈34% (Malik et al. 2020) and higher than that of seasonal influenza (0.01% to 0.17%) according to data for 2010 to 2017 from the US Centers for Disease Control and Prevention (2020).

People at High Risk of Infection. Current observations suggest that people of all ages are generally susceptible to this new infectious disease. However, those who are in close contact with patients with symptomatic and asymptomatic COVID-19, including health care workers and other patients in the hospital, are at higher risk of SARS-CoV-2 infection. In the early stage of the epidemic, in an analysis of 138 hospitalized patients with COVID-19 in Wuhan, 57 (41%) were presumed to have been infected in hospital, including 40 (29%) health care workers and 17 (12%) patients hospitalized for other reasons (Wang et al. 2020). As of February 14, 2020, a total of 1,716 health care workers in China were infected with SARS-CoV-2, consisting of 3.8% affected patients nationally, 6 of that group who have died.

Clinical Manifestations

The majority of patients with COVID-19 represent relatively mild cases. According to recent studies (Guan et al. 2020; Yang et al. 2020) and data from the National Health Commission of China (2020b), the proportion of severe cases among all patients with COVID-19 in China was around 15% to 25%.

The majority of patients experienced fever and dry cough, while some also had shortness of breath, fatigue, and other atypical symptoms, such as muscle pain, confusion, headache, sore throat, diarrhea, and vomiting (Chen, Zhou, et al. 2020; Guan et al. 2020). Among patients who underwent chest computed tomography (CT), most showed bilateral pneumonia, with ground-glass opacity and bilateral patchy shadows being the most common patterns (Guan et al. 2020; Wang et al. 2020).

Among hospitalized patients in Wuhan, around one-fourth to one-third developed serious complications, such as acute respiratory distress syndrome, arrhythmia, and shock, and were therefore transferred to the intensive care unit (Chen, Zhou, et al. 2020; Huang et al. 2020; Wang et al. 2020). In general, older age and the existence of underlying comorbidities (e.g., diabetes, hypertension, and cardiovascular disease) were associated with poorer prognosis (Kui et al. 2020; Wang et al. 2020; Yang et al. 2020).

Diagnosis and Treatment

The diagnosis of COVID-19 can be based on a combination of epidemiologic information (e.g., a history of travel to or residence in affected region 14 d prior to symptom onset), clinical symptoms, CT imaging findings, and laboratory tests (e.g., reverse transcriptase polymerase chain reaction [RT-PCR] tests on respiratory tract specimens) according to standards of either the WHO (2020a) or the National Health Commission of China (2020a). It should be mentioned that a single negative RT-PCR test result from suspected patients does not exclude infection. Clinically, we should be alert of patients with an epidemiologic history, COVID-19–related symptoms, and/or positive CT imaging results.

So far, there has been no evidence from randomized controlled trials to recommend any specific anti-nCoV treatment, so the management of COVID-19 has been largely supportive (WHO 2020a). Currently, the approach to COVID-19 is to control the source of infection; use infection prevention and control measures to lower the risk of transmission; and provide early diagnosis, isolation, and supportive care for affected patients (Wang et al. 2020). A series of clinical trials are being carried out to investigate interventions that are potentially more effective (e.g., lopinavir, remdesivir; Del Rio and Malani 2020).

Infection Control in Dental Settings

Risk of Nosocomial Infection in Dental Settings

Dental patients who cough, sneeze, or receive dental treatment including the use of a high-speed handpiece or ultrasonic instruments make their secretions, saliva, or blood aerosolize

to the surroundings. Dental apparatus could be contaminated with various pathogenic microorganisms after use or become exposed to a contaminated clinic environment. Thereafter, infections can occur through the puncture of sharp instruments or direct contact between mucous membranes and contaminated hands (Kohn et al. 2003).

Due to the unique characteristics of dental procedures where a large number of droplets and aerosols could be generated, the standard protective measures in daily clinical work are not effective enough to prevent the spread of COVID-19, especially when patients are in the incubation period, are unaware they are infected, or choose to conceal their infection.

Effective Infection Control Protocols

Hand hygiene has been considered the most critical measure for reducing the risk of transmitting microorganism to patients (Larson et al. 2000). SARS-CoV-2 can persist on surfaces for a few hours or up to several days, depending on the type of surface, the temperature, or the humidity of the environment (WHO 2020c). This reinforces the need for good hand hygiene and the importance of thorough disinfection of all surfaces within the dental clinic. The use of personal protective equipment, including masks, gloves, gowns, and goggles or face shields, is recommended to protect skin and mucosa from (potentially) infected blood or secretion. As respiratory droplets are the main route of SARS-CoV-2 transmission, particulate respirators (e.g., N-95 masks authenticated by the National Institute for Occupational Safety and Health or FFP2-standard masks set by the European Union) are recommended for routine dental practice.

Recommended Measures during the COVID-19 Outbreak

Recommendations for Management

In January 2020, the National Health Commission of China added COVID-19 to the category of group B infectious diseases, which includes SARS and highly pathogenic avian influenza. However, it also suggested that all health care workers use protection measures similar to those indicated for group A infections—a category reserved for extremely infectious pathogens, such as cholera and plague.

Since then, in most cities of the mainland of China, only dental emergency cases have been treated when strict implementation of infection prevention and control measures are recommended. Routine dental practices have been suspended until further notification according to the situation of epidemics.

Additionally, dentistry-related quality control centers and professional societies in many provinces and cities have put forward their recommendations for dental services during the COVID-19 outbreak, which, as supplementary measures, should be helpful in ensuring the quality of infection control (Li and Meng 2020).

Current Status of Our School and Hospital

The School and Hospital of Stomatology, Wuhan University provided dental care (including oral and maxillofacial surgery) to around 890,000 patients last year and is home to 1,098 staff and 828 students. Our hospital does not have a fever clinic or belong to a designated one for patients with COVID-19. Any staff member who has fever, cough, sneezing, or COVID-19–related symptoms or has a close family member who is confirmed with the infection is advised to undergo a medical examination in a designated hospital and cease working. Since this epidemic, 9 of our colleagues have been confirmed to have COVID-19, including 3 doctors, 3 nurses, 2 administrative staff, and 1 postgraduate student (Fig. 1, Table). So far, there have been no further cases among colleagues or patients who had close contact with them. According to analyses of epidemiologic investigation and medical history, all these cases are without obvious aggregation, except 2 nurses from the same department (patients 2 and 3), and are unlikely to result from cross infection. The infection was possibly limited because medical masks and gloves worn during routine clinic work of dental practitioners prevented further transmission.

Despite the increasing number of confirmed cases during this period in Wuhan, we (169 staff involved in duty of dental emergency) have treated >700 patients with emergent dental treatment need since January 24 (Fig. 2), under the premise of adequate protection measures. All the dental procedures were recorded daily, and patients and their accompanying persons were requested to provide their phone number and home address in the case that either our staff or patients are suspected or confirmed with COVID-19 in the future. We have also provided consultations to >1,600 patients on our online platform since February 3. No further COVID-19 infection has been reported among our staff, which confirmed the effectiveness of our infection control measures in COVID-19 prevention within dental settings (Fig. 3).

According to the instructions from the Ministry of Education of China, all students, including those in our school, have been required to not return to school until further notification. Students are recommended to learn online after the Chinese Spring Festival on the mainland of China.

Recommendations for Dental Practice

Interim guidance on infection prevention and control during health care is recommended when COVID-19 infection is suspected (WHO 2020a). Up to now, there has been no consensus on the provision of dental services during the epidemic of COVID-19. On the basis of our experience and relevant guidelines and research, dentists should take strict personal protection measures and avoid or minimize operations that can produce droplets or aerosols. The 4-handed technique is beneficial for controlling infection. The use of saliva ejectors with low or high volume can reduce the production of droplets and aerosols (Kohn et al. 2003; Li et al. 2004; Samaranayake and Peiris 2004).

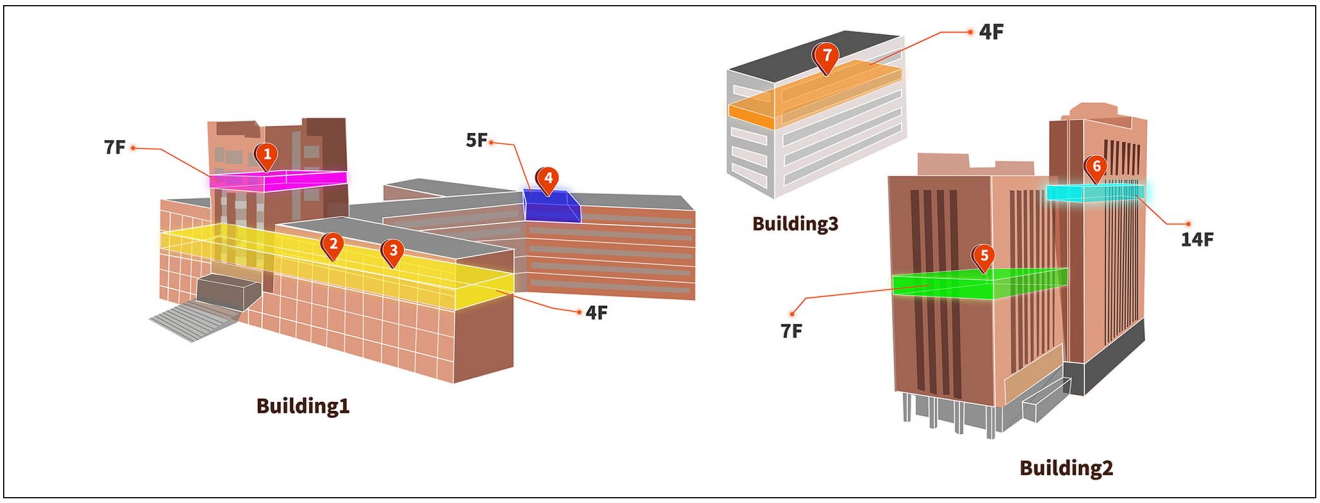


Figure 1. Location of staff and student confirmed with coronavirus disease 2019 (COVID-19) in the main buildings in the School and Hospital of Stomatology, Wuhan University. There are 3 main buildings in our hospital. Building 1 mainly contains outpatients, classrooms, and a library. Its air renewal system depends on air conditioners. Building 2 contains outpatients, wards, and administrative regions. This building was equipped with central air conditioners and a fresh air system. Building 3 is a research laboratory, and it also depends on air conditioners. See Table for details.

Table. A Brief Introduction to the Staff and Student Confirmed with COVID-19 in School and Hospital of Stomatology, Wuhan University.

Patient No.[a]	Occupation	Department	Workplace	No. of Persons on Same Floor or Dept	Are There Any Close Contacts Infected?	Date of Initial Symptom
1	Doctor	Preventive dentistry	Bldg 1	10	No	Jan 23
2	Nurse	Prosthodontics	Bldg 1	51	No	Jan 28
3	Nurse	Prosthodontics	Bldg 1	51	Family members[b]	Feb 4
4	Administrator	Library	Bldg 1	2	1 family member	Jan 27
5	Nurse	Oncology surgery	Bldg 2	37	Family members	Jan 31
6	Administrator	Teaching office	Bldg 2	18	Family members[b]	Jan 29
7	MD student	Research group[c]	Bldg 3	19	1 family member	Jan 28
8	Doctor	Zhongshang clinic[d]	Satellite clinic	18	1 family member	Jan 29
9	Doctor	Yichang clinic[d]	Satellite clinic	15	1 family member	Jan 29

COVID-19, coronavirus disease 2019.
[a]The staff and the student confirmed with COVID-19 did not contact one another closely, and most of them had been away since January 22 or 23, 2020, because of the Chinese Spring Festival.
[b]Family member with COVID-19–related symptom at least 1 d earlier.
[c]Patient 7 is a research group member supervised by a professor. No classmates or roommates of his were reported to be infected.
[d]Zhongshang clinic and Yichang clinic are 2 of 16 satellite clinics of our hospital. The former, located within Wuhan city, is 4 km away from the hospital, and the latter is 300 km away and located in Yichang city. They both solely depend on air conditioners. Patients 8 and 9 are a couple. They were reunited, took a high-speed train back home on January 21, and had respiratory symptoms after 8 d.

Evaluation of Patients. During the outbreak of COVID-19, dental clinics are recommended to establish precheck triages to measure and record the temperature of every staff and patient as a routine procedure. Precheck staff should ask patients questions about the health status and history of contact or travel (WHO 2020a). Patients and their accompanying persons are provided with medical masks and temperature measurement once they enter our hospital. Patients with fever should be registered and referred to designated hospitals. If a patient has been to epidemic regions within the past 14 d, quarantine for at least 14 d is suggested. In areas where COVID-19 spreads, nonemergency dental practices should be postponed (Kohn et al. 2003; Li et al. 2004; Samaranayake and Peiris 2004).

It was reported that dental practice should be postponed at least 1 mo for convalescing patients with SARS (Samaranayake and Peiris 2004). It is unknown yet whether the same suggestion should be recommended for patients with COVID-19.

Oral Examination. Preoperative antimicrobial mouth rinse could reduce the number of microbes in the oral cavity (Kohn et al. 2003; Marui et al. 2019). Procedures that are likely to induce coughing should be avoided (if possible) or performed cautiously (WHO 2020a). Aerosol-generating procedures, such as the use of a 3-way syringe, should be minimized as much as possible. Intraoral x-ray examination is the most common radiographic technique in dental imaging; however, it can stimulate saliva secretion and coughing (Vandenberghe et al. 2010). Therefore, extraoral dental radiographies, such as panoramic radiography and cone beam CT, are appropriate alternatives during the outbreak of COVID-19.

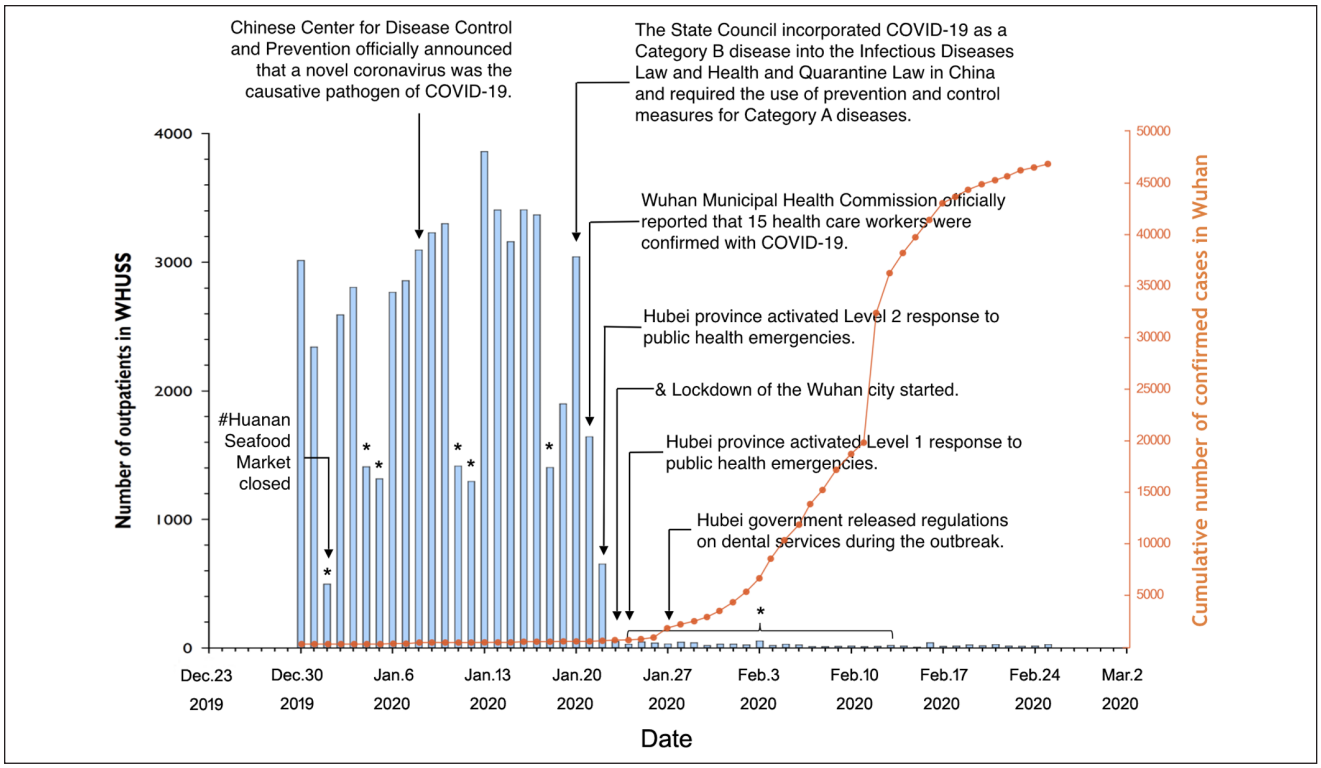


Figure 2. Number of outpatients treated at the School and Hospital of Stomatology, Wuhan University (WHUSS), and cumulative number of confirmed cases with coronavirus disease 2019 (COVID-19) in Wuhan city from December 30, 2019, to February 25, 2020. *Weekends and national holidays (including the extended spring festival holiday). #Huanan Seafood Market was closed in Wuhan city after the majority of the earliest COVID-19 cases were linked to the Huanan Seafood Wholesale Market. &Lockdown of Wuhan city started. According to the epidemic situation, the notification of city lockdown was made to stop any transportation, including airplane, train, and bus, from Wuhan city to prevent COVID-19 transmission, especially because of population movement during Chinese Spring Festival. Before January 21, WHUSS staff had only medical masks and gloves. On January 22, we started to use disposable surgical masks, N95 masks, and gowns. Goggles and protective suits were not available until January 28.

Treatment of Emergency Cases. Dental emergencies can occur and exacerbate in a short period and therefore need immediate treatment. Rubber dams and high-volume saliva ejectors can help minimize aerosol or spatter in dental procedures. Furthermore, face shields and goggles are essential with use of high- or low-speed drilling with water spray (Samaranayake et al. 1989). According to our clinic experience during the outbreak, if a carious tooth is diagnosed with symptomatic irreversible pulpitis, pulp exposure could be made with chemomechanical caries removal under rubber dam isolation and a high-volume saliva ejector after local anesthesia; then, pulp devitalization can be performed to reduce the pain. The filling material can be replaced gently without a devitalizing agent later according to the manufacturer's recommendation. We also met a patient who had a spontaneous toothache because of a cracked tooth without dental decay, and a high-speed handpiece had to be used to access cavity preparation. Given that the patient wanted to retain the tooth, she was scheduled as the last patient in the day to decrease the risk of nosocomial infection. After treatment, environmental cleaning and disinfection procedures were followed. Alternatively, patients could be treated in an isolated and well-ventilated room (Fig. 3) or negatively pressured rooms if available for suspected cases with COVID-19.

The treatment planning of tooth fracture, luxation, or avulsion is dependent on the age, the traumatic severity of dental tissue, the development of the apex, and the duration of tooth avulsion (Andersson et al. 2012; DiAngelis et al. 2012; Malmgren et al. 2012). If the tooth needs to be extracted, absorbable suture is preferred. For patients with facial soft tissue contusion, debridement and suturing should be performed. It is recommended to rinse the wound slowly and use the saliva ejector to avoid spraying. Life-threatening cases with oral and maxillofacial compound injuries should be admitted to the hospital immediately, and chest CT should be prescribed if available to exclude suspected infection because the RT-PCR test, besides time-consuming, needs a laboratory with pan-coronavirus or specific SARS-CoV-2 detection capacity.

Recommendations for Dental Education

Education-related challenges for medical and dental schools, as well as their affiliated hospitals, are significant. It was reported that open communication among students, clinical teachers, and administrative staff would enhance mutual trust and facilitate adequate cooperation (Park et al. 2016).

On the basis of our experience with SARS and relevant highly pathogenic infectious disease, we provide a few basic

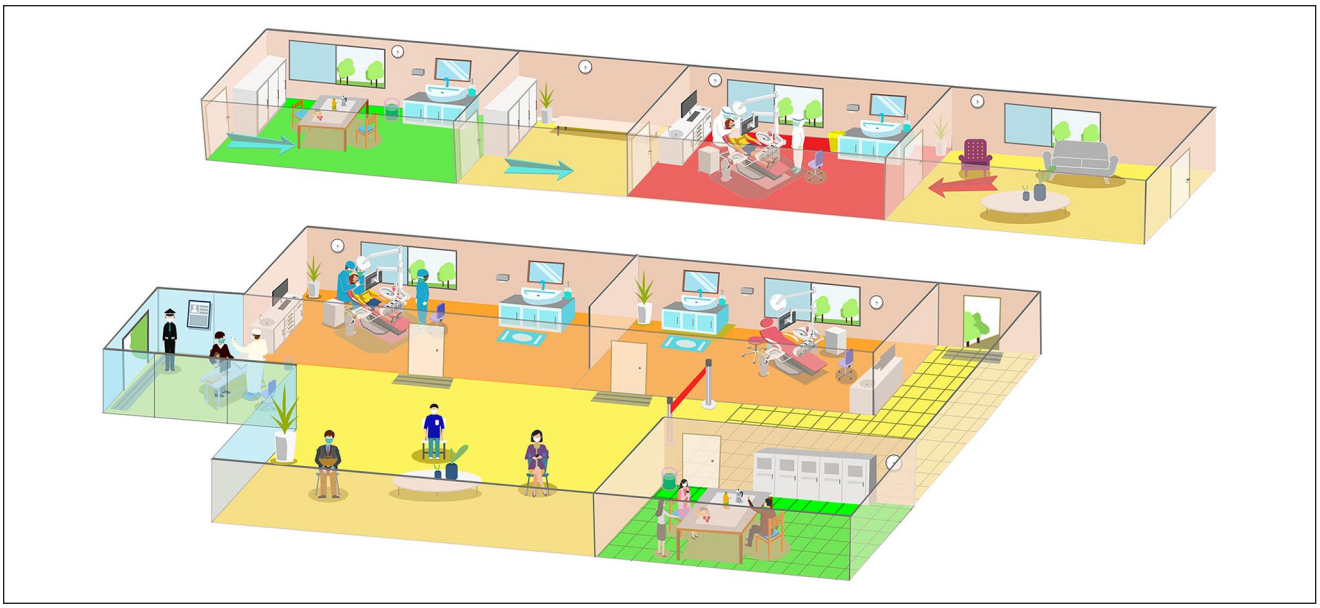


Figure 3. The personal protective equipment (PPE) diagram for divisions in the emergency care area at the School and Hospital of Stomatology, Wuhan University, during the coronavirus disease 2019 (COVID-19) outbreak. Yellow: triage and waiting area. Orange: dental clinic. Red: isolation clinic. Green: resting area for staff only. As shown in the diagram, our triage staff in the yellow area wear disposable surgical mask, cap, and work clothes. In the orange area, dental staff is provided with PPE, including disposable N95 masks, gloves, gowns, cap, shoe cover, and goggles or face shield. The area is disinfected once every half day. Before February 21, all the patients were treated in this area. The isolation clinic in the red area was set up on February 21. It is designed for patients who are suspected with COVID-19, who are recovering from COVID-19 (but <1 mo after they are discharged from hospital), or who need dental procedures producing droplets and/or aerosols. Separate entrances for patients (red arrow) and staff (blue arrow) are provided in the area. Dental staff should wear protective clothing besides the aforementioned PPE. In addition, the entire isolation area is disinfected immediately after the treatment is over and the patient has left. The grid area behind the red line is for staff only. Staff can have a rest in the room (green area). They are recommended to enter the room by turn and to keep wearing medical masks unless they are eating or drinking.

recommendations for dental education during an outbreak: First, during the outbreak period, online lectures, case studies, and problem-based learning tutorials should be adopted to avoid unnecessary aggregation of people and associated risk of infection (Patil et al. 2003). Existing smart devices and applications have already made it possible for students to listen to and review lectures whenever and wherever possible. In fact, our students started online learning from February 17. Second, it is worth advocating to encourage students to engage in self-learning, make full use of online resources, and learn about the latest academic developments. Third, during this period, it is easy for students to be affected by disease-associated fear and pressure, and dental schools should be prepared to provide psychological services to those who need them (Wong et al. 2004).

With the increased knowledge of viral features, epidemiologic characteristics, clinical spectrum, and treatment, efficient strategies have been taken to prevent, control, and stop the spread of COVID-19. The infection prevention and control strategies that we have adopted are determined by the fact that we are in the center of COVID-19. Other regions should follow the recommendations from the disease control centers for infection prevention and control according to the local epidemic situation.

What should we do to improve the current infection prevention and control strategies after the epidemic? How should we respond to similar contagious diseases in the future? These are open questions in need of further discussion and research.

We must be constantly aware of infectious threats that may challenge the current infection control regimen, especially in dental practices and schools of dental medicine.

Author Contributions

L. Meng, contributed to conception, design, data acquisition, and analysis, drafted and critically revised the manuscript; F. Hua, contributed to design and data acquisition, drafted and critically revised the manuscript; Z. Bian, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

Acknowledgments

The article was supported by grants from the National Natural Science Foundation of China (Nos. 81870761, 81970923, 81901044), The National Key Research and Development Program of China (2016YFC1000505), and the Key Research Program of the Provincial Department of Science and Technology (No. 2017ACA181). The authors acknowledge technical assistance from Chujian Jiang and clinical advice from Prof. Yong Cheng and Tianjuan Xia and all the other staff and students from the School and Hospital of Stomatology, Wuhan University, China, who contributed directly or indirectly to the article. Fortunately, 5 staff confirmed to have COVID-19 have been convalescing and discharged from hospital. The authors declare no

potential conflicts of interest with respect to the authorship and/or publication of this article.

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References

Andersson L, Andreasen JO, Day P, Heithersay G, Trope M, DiAngelis AJ, Kenny DJ, Sigurdsson A, Bourguignon C, Flores MT. 2012. International Association of Dental Traumatology guidelines for the management of traumatic dental injuries: 2. Avulsion of permanent teeth. Dent Traumatol. 28(2):88–96.

Backer JA, Klinkenberg D, Wallinga J. 2020. Incubation period of 2019 novel coronavirus (2019-nCoV) infections among travellers from Wuhan, China, 20–28 January 2020. Euro Surveill. 25(5). doi:10.2807/1560-7917.ES.2020.2825.2805.2000062.

Centers for Disease Control and Prevention. 2020. Disease burden of influenza; [accessed 2020 Feb 25]. https://www.cdc.gov/flu/about/burden/index.html.

Chan JF, Yuan S, Kok KH, To KK, Chu H, Yang J, Xing F, Liu J, Yip CC, Poon RW, et al. 2020. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet. 395(10223):514–523.

Chen H, Guo J, Wang C, Luo F, Yu X, Zhang W, Li J, Zhao D, Xu D, Gong Q, et al. 2020. Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records. Lancet [epub ahead of print 12 Feb 2020] in press. doi:10.1016/S0140-6736(20)30360-3.

Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y, et al. 2020. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 395(10223):507–513.

Del Rio C, Malani PN. 2020. 2019 novel coronavirus-important information for clinicians. JAMA [epub ahead of print 5 Feb 2020] in press. doi:10.1001/jama.2020.1490.

DiAngelis AJ, Andreasen JO, Ebeleseder KA, Kenny DJ, Trope M, Sigurdsson A, Andersson L, Bourguignon C, Flores MT, Hicks ML. 2012. International Association of Dental Traumatology guidelines for the management of traumatic dental injuries: 1. Fractures and luxations of permanent teeth. Dent Traumatol. 28(1):2–12.

Guan W-J, Ni Z-Y, Hu Y, Liang W-H, Ou C-Q, He J-X, Liu L, Shan H, Lei C-L, Hui DS, et al. 2020. Clinical characteristics of 2019 novel coronavirus infection in China. medRxiv. doi:10.1101/2020.1102.1106.20020974.

Holshue ML, DeBolt C, Lindquist S, Lofy KH, Wiesman J, Bruce H, Spitters C, Ericson K, Wilkerson S, Tural A, et al. 2020. First case of 2019 novel coronavirus in the United States. N Engl J Med [epub ahead of print 31 Jan 2020] in press. doi:10.1056/NEJMoa2001191.

Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, et al. 2020. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 395(10223):497–506.

Kohn WG, Collins AS, Cleveland JL, Harte JA, Eklund KJ, Malvitz DM; Centers for Disease Control and Prevention. 2003. Guidelines for infection control in dental health-care settings—2003. https://www.cdc.gov/mmwr/preview/mmwrhtml/rr5217a1.htm.

Kui L, Fang YY, Deng Y, Liu W, Wang MF, Ma JP, Xiao W, Wang YN, Zhong MH, Li CH, et al. 2020. Clinical characteristics of novel coronavirus cases in tertiary hospitals in Hubei province. Chin Med J (Engl) [epub ahead of print 7 Feb 2020] in press. doi:10.1097/CM1099.0000000000000744.

Larson EL, Early E, Cloonan P, Sugrue S, Parides M. 2000. An organizational climate intervention associated with increased handwashing and decreased nosocomial infections. Behav Med. 26(1):14–22.

Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, Ren R, Leung KSM, Lau EHY, Wong JY, et al. 2020. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. N Engl J Med [epub ahead of print 29 Jan 2020] in press. doi:10.1056/NEJMoa2001316.

Li R, Leung K, Sun F, Samaranayake L. 2004. Severe acute respiratory syndrome (SARS) and the GDP. Part II: implications for GDPs. Br Dent J. 197(3):130–134.

Li ZY, Meng LY. 2020. Prevention and control of new coronavirus infection in department of stomatology. Chin J Stomatol [epub ahead of print 14 Feb 2020] in press. doi:10.3760/cma.j.issn.1002-0098.2020.0001.

Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, Wang W, Song H, Huang B, Zhu N, et al. 2020. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet. 395(10224):565–574.

Mahase E. 2020. China coronavirus: WHO declares international emergency as death toll exceeds 200. BMJ. 368:m408.

Malik YS, Sircar S, Bhat S, Sharun K, Dhama K, Dadar M, Tiwari R, Chaicumpa W. 2020. Emerging novel coronavirus (2019-nCoV)— current scenario, evolutionary perspective based on genome analysis and recent developments. Vet Q [epub ahead of print 8 Feb 2020] in press. doi:10.1080/01652176.2020.1727993.

Malmgren B, Andreasen JO, Flores MT, Robertson A, DiAngelis AJ, Andersson L, Cavalleri G, Cohenca N, Day P, Hicks ML, et al. 2012. International Association of Dental Traumatology guidelines for the management of traumatic dental injuries: 3. Injuries in the primary dentition. Dent Traumatol. 28(3):174–182.

Marui VC, Souto MLS, Rovai ES, Romito GA, Chambrone L, Pannuti CM. 2019. Efficacy of preprocedural mouthrinses in the reduction of microorganisms in aerosol: a systematic review. J Am Dent Assoc. 150(12):1015–1026.

National Health Commission of China. 2020a. The diagnosis and treatment protocol for novel coronavirus pneumonia (interim sixth edition) [accessed 2020 Feb 19]. http://www.gov.cn/zhengce/zhengceku/2020-02/19/content_5480948.htm.

National Health Commission of China. 2020b. An update of novel coronavirus pneumonia outbreak as of 24:00 on February 25 [accessed 2020 Feb 26]. http://www.nhc.gov.cn/xcs/yqtb/list_gzbd.shtml.

Park SW, Jang HW, Choe YH, Lee KS, Ahn YC, Chung MJ, Lee K-S, Lee K, Han T. 2016. Avoiding student infection during a Middle East respiratory syndrome (MERS) outbreak: a single medical school experience. Korean J Med Educ. 28(2):209–217.

Patil N, Chan Y, Yan H. 2003. SARS and its effect on medical education in Hong Kong. Med Educ. 37(12):1127–1128.

Phelan AL, Katz R, Gostin LO. 2020. The novel coronavirus originating in Wuhan, China: challenges for global health governance [epub ahead of print 30 Jan 2020] in press. JAMA. doi:10.1001/jama.2020.1097.

Rothe C, Schunk M, Sothmann P, Bretzel G, Froeschl G, Wallrauch C, Zimmer T, Thiel V, Janke C, Guggemos W, et al. 2020. Transmission of 2019-nCoV infection from an asymptomatic contact in Germany [epub ahead of print 30 Jan 2020] in press. N Engl J Med. doi:10.1056/NEJMc2001468.

Samaranayake L, Reid J, Evans D. 1989. The efficacy of rubber dam isolation in reducing atmospheric bacterial contamination. ASDC J Dent Child. 56(6):442–444.

Samaranayake LP, Peiris M. 2004. Severe acute respiratory syndrome and dentistry: a retrospective view. J Am Dent Assoc. 135(9):1292–1302.

The Chinese Preventive Medicine Association. 2020. An update on the epidemiological characteristics of novel coronavirus pneumonia (COVID-19). Chin J Epidemiol. 41(2):139–144.

Vandenberghe B, Jacobs R, Bosmans H. 2010. Modern dental imaging: a review of the current technology and clinical applications in dental practice. Eur Radiol. 20(11):2637–2655.

Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, Wang B, Xiang H, Cheng Z, Xiong Y, et al. 2020. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China. JAMA [epub ahead of print 7 Feb 2020] in press. doi:10.1001/jama.2020.1585.

Wong JG, Cheung EP, Cheung V, Cheung C, Chan MT, Chua SE, McAlonan GM, Tsang KW, Ip MS. 2004. Psychological responses to the SARS outbreak in healthcare students in Hong Kong. Med Teach. 26(7):657–659.

World Health Organization. 2020a. Clinical management of severe acute respiratory infection when novel coronavirus (2019-nCoV) infection is suspected: interim guidance [accessed 2020 Feb 17]. https://www.who.int/publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected.

World Health Organization. 2020b. Coronavirus disease 2019 (COVID-19): situation report-36 [accessed 2020 Feb 26]. https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200225-sitrep-36-covid-19.pdf?sfvrsn=2791b4e0_2.

World Health Organization. 2020c. Questions and answers on coronaviruses [accessed 2020 Feb 26]. https://www.who.int/news-room/q-a-detail/q-a-coronaviruses.

Yang Y, Lu Q, Liu M, Wang Y, Zhang A, Jalali N, Dean N, Longini I, Halloran ME, Xu B, et al. 2020. Epidemiological and clinical features of the 2019 novel coronavirus outbreak in China. medRxiv. doi:10.1101/2020.1102.1110.20021675.

Zhu H, Wang L, Fang C, Peng S, Zhang L, Chang G, Xia S. 2020. Clinical analysis of 10 neonates born to mothers with 2019-nCoV pneumonia. Transl Pediatr. 9(1):51–60. doi:10.21037/tp.2020.02.06.

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Letter to the Editor

Journal of Dental Research
1
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Coronavirus Disease 2019 (COVID-19): Emerging and Future Challenges for Dental and Oral Medicine

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We read with great interest the article by Meng et al. (2020) on the dental challenges raised by the current pandemic of coronavirus disease 2019 (COVID-19). They recommended the use of preoperative antimicrobial mouthrinse since the oral cavity is a high-risk route for COVID-19 infection. Following this, 2 recent reports by Kirk-Bayley et al. (2020) and Carrouel et al. (2020) also supported the use of antimicrobial mouthrinse to reduce the risk of COVID-19 infection. However, we believe that a word of caution is currently needed before recommending the use of generic antimicrobial products because they can induce a detrimental shift on the oral ecosystem (Willis and Gabaldón 2020). For instance, it is now recognized that the use of antimicrobial mouthrinse (chlorhexidine) inhibits bacterial species that are essential to promote vasodilation and to reduce blood pressure through an oral nitrate/nitrite/nitric oxide pathway (Bryan et al. 2017; Bescos et al. 2020). This is relevant in the current pandemic since people with hypertension, which is commonly associated with impaired nitric oxide availability, have the greatest risk of developing severe COVID-19 infection (Zhou et al. 2020). Furthermore, oral bacterial nitrite synthesis may be essential to improve the first line of immunologic response against viral infections (Rimmelzwaan et al. 1999). Research examining oral health and the oral microbiome is urgently needed as it may help to enhance our knowledge on the course of COVID-19 infection. We encourage researchers and dentists treating patients with COVID-19 to contact us to develop collaborative research in this area.

Acknowledgments

The authors received no financial support and declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

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References

Bescos R, Ashworth A, Cutler C, Brookes ZL, Belfield L, Rodiles A, Casas-Agustench P, Farnham G, Liddle L, Burleigh M, et al. 2020. Effects of chlorhexidine mouthwash on the oral microbiome. Sci Rep. 10(1):5254.

Bryan NS, Tribble G, Angelov N. 2017. Oral microbiome and nitric oxide: the missing link in the management of blood pressure. Curr Hypertens Rep. 19(4):33.

Carrouel F, Conte MP, Fisher J, Gonçalves LS, Dussart C, Llodra JC, Bourgeois D. 2020. COVID-19: a recommendation to examine the effect of mouthrinses with β-cyclodextrin combined with citrox in preventing infection and progression. J Clin Med. 9(4):E1126.

Kirk-Bayley J, Challacombe S, Sunkaraneni S, Combes J. 2020. The use of povidone iodine nasal spray and mouthwash during the current COVID-19 pandemic may protect healthcare workers and reduce cross infection. SSRN [accessed 2020 Apr 23]. https://papers.ssrn.com/sol3/papers.cfm?abstract_id=3563092

Meng L, Hua F, Bian Z. 2020. Coronavirus disease 2019 (COVID-19): emerging and future challenges for dental and oral medicine. J Dent Res. 99(5):481–487.

Rimmelzwaan GF, Baars MMJW, de Lijster P, Fouchier RAM, Osterhaus ADME. 1999. Inhibition of influenza virus replication by nitric oxide. J Virol. 73(10):8880–8883.

Willis JR, Gabaldón T. 2020. The human oral microbiome in health and disease: from sequences to ecosystems. Microorganisms. 8(2):308.

Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, Xiang J, Wang Y, Song B, Gu X, et al. 2020. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 395(10229):1054–1062.

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Effects of Chlorhexidine mouthwash on the oral microbiome

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Following a single blind, cross-over and non-randomized design we investigated the effect of 7-day use of chlorhexidine (CHX) mouthwash on the salivary microbiome as well as several saliva and plasma biomarkers in 36 healthy individuals. They rinsed their mouth (for 1 min) twice a day for seven days with a placebo mouthwash and then repeated this protocol with CHX mouthwash for a further seven days. Saliva and blood samples were taken at the end of each treatment to analyse the abundance and diversity of oral bacteria, and pH, lactate, glucose, nitrate and nitrite concentrations. CHX significantly increased the abundance of Firmicutes and Proteobacteria, and reduced the content of Bacteroidetes, TM7, SR1 and Fusobacteria. This shift was associated with a significant decrease in saliva pH and buffering capacity, accompanied by an increase in saliva lactate and glucose levels. Lower saliva and plasma nitrite concentrations were found after using CHX, followed by a trend of increased systolic blood pressure. Overall, this study demonstrates that mouthwash containing CHX is associated with a major shift in the salivary microbiome, leading to more acidic conditions and lower nitrite availability in healthy individuals.

Chlorhexidine (CHX) has been commonly used in dental practice as antiseptic agent since 1970, due to its long-lasting antibacterial activity with a broad-spectrum of action[1]. Since then, many clinical trials have shown effective results of CHX for the clinical management of dental plaque and gingival inflammation and bleeding[2–4]. This is supported by other studies using *in vitro* methods and reporting positive results of CHX in reducing the proliferation of bacterial species associated with periodontal disease, such as *Enterobacteria*, *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, as well as different species of *Actinomyces* and *Streptococcus*, including *Streptococcus mutans*, which is considered the main etiological agent of dental caries[4,5]. Other studies have also reported that the use of CHX was effective in the treatment of halitosis, especially in reducing the levels of halitosis-related bacteria colonising the dorsal surface of the tongue[6].

The anti-microbial activity of CHX however, has been extensively studied using *in vitro* culture methods, which limit the identification and cultivation of all microorganisms in the environment[4]. To the best of our knowledge, only one recent study has investigated the effect of CHX mouthwash on mixed bacterial communities (microbiome) of the tongue using new genome sequencing techniques such as 16 S rRNA[7]. The study found differences in over 10 different species colonizing the tongue, and a lower microbial diversity after using CHX for a week, but did not analyse other parameters related to oral health such as pH, lactate production or buffering capacity[7]. Additionally, we and others have recently shown that the use of CHX in healthy subjects can attenuate the nitrate-reducing activity of oral bacteria by at least 80%[8–11]. This in turn leads to lower nitrite availability and an increase of blood pressure, suggesting that the oral microbiome can regulate cardiovascular health in healthy individuals and hypertensive patients[8,11].

CHX is widely available over the counter and is used in healthy patients, but it is unknown whether it promotes a healthy oral microbiome, or it may cause a shift to a microbiome associated with disease. Thus, the main aim of this study was to investigate the effects of 7-day use of CHX mouthwash on the oral microbiome of healthy participants, and its impact on several saliva markers such as pH, buffering capacity, lactate and glucose levels. We also investigated saliva and plasma concentrations of nitrate and nitrite with respect to blood pressure changes.

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Age (years)	26 ± 1
Gender (F:M)	25:11
Weight (kg)	65.4 ± 2.0
Height (cm)	170.6 ± 1.9
Systolic blood pressure	103.6 ± 1.2
Diastolic blood pressure	62.8 ± 1.1
Mean arterial blood pressure	76.4 ± 1.0

Table 1. Main characteristic of participants (mean ± SEM).

Results

Thirty six healthy participants successfully completed this study (Table 1).

Oral microbiome analysis. Changes in the abundance of phyla are shown in Fig. 1A,B. The ratio between the main two phyla (Firmicutes/Bacteroidetes) is shown in Fig. 1C. CHX increased the abundance of Firmicutes (FDR < 0.001) and Proteobacteria (FDR < 0.001) and lowered the abundance of Bacteroidetes (FDR < 0.001), TM7 (FDR < 0.001), SR1 (FDR < 0.001) and Fusobacteria (FDR = 0.043).

Changes at genus level are presented in Fig. 1D,E. Within the phylum Firmicutes, the genus *Bulleidia* (FDR = 0.023) and *Streptococcus* (FDR = 0.020) were increased after using CHX, while 4 other genera decreased: *Clostridium* (FDR = 0.035), *Megasphera* (FDR = 0.001), *Catonella* (FDR < 0.001) and *Lachnoanaerobaculum* (FDR < 0.001). Regarding Proteobacteria, CHX led to an increase in *Neisseria* (FDR = 0.004), *Hylemonella* (FDR = 0.004) and *Eikenella* (FDR < 0.001) as well as a reduction in *Campylobacter* (FDR = 0.035). Changes in Bactoroidetes were led by an increase of *Capnocytophaga* (FDR < 0.001) and a decrease in *Prevotella* (FDR < 0.001). A significant reduction in non-assigned genera was found after CHX treatment (FDR = 0.004).

Figures 1F,G show the main changes at family level. Three families within the phylum Firmicutes increased after using CHX: *Erysipelotrichaceae* (FDR = 0.019), *Streptococcaceae* (FDR = 0.012) and *Carnobacteriaceae* (FDR = 0.012), whilst three other families decreased: *Clostridiaceae* (FDR = 0.027), *Mogibacteriaceae* (FDR < 0.001) and *Lachnospiraceae* (FDR = 0.005). The increase of the phylum Proteobacteria after CHX treatment was attributable to an increase in the abundance of 3 families: *Neisseriaceae* (FDR = 0.003), *Comamonadaceae* (FDR = 0.004) and *Enterobacteriaceae* (FDR = 0.005). This was also accompanied by a decrease of the family *Campylobacteraceae* (FDR = 0.027). On the other hand, lower abundance of the phylum Bacteroidetes was attributable to lower abundance of 2 families: *Prevotellaceae* (FDR < 0.001) and *Paraprevotellaceae* (FDR < 0.001) and an increase of the family *Flavobacteriaceae* (FDR < 0.001). Within the phylum Fusobacteria, the family *Fusobacteriaceae* (FDR = 0.003) showed the greatest reduction following CHX treatment, whilst in the phylum TM7, *F16* (FDR = 0.004) levels showed the greatest reduction. Finally, although the abundance of Actinobacteria did not significantly change after using CHX compared to placebo at phylum level, some families such as *Actinomycetaceae* (FDR = 0.007) and *Corynebacteriaceae* (FDR = 0.001) belonging to this phyla were significantly reduced by CHX. Finally, regarding alpha diversity, a significant decrease in the Shannon index was found after using CHX compared to placebo (FDR = 0.001) (Fig. 2A). Beta diversity was also significantly affected by CHX as shown by greater dissimilarity of the Bray-Curtis plot compared to placebo (Fig. 2B).

Saliva and plasma markers. Salivary pH and buffering capacity were significantly reduced after using CHX compared to placebo (Fig. 3A,B). This was accompanied by a significant increase of salivary lactate and glucose (Fig. 3C,D). CHX also led to lower oral nitrate-reducing capacity (Fig. 3E), which in turn, led to lower saliva and plasma nitrite availability (Fig. 3F,H) and increased salivary nitrate concentration (Fig. 3G).

Correlations. We found several moderate correlations between the abundance of oral bacteria and salivary biomarkers after using the placebo and CHX mouthwash (Fig. 4). In the placebo condition, greater abundance of Proteobacteria was negatively correlated with greater ability to form nitrite in the mouth (oral nitrate-reducing capacity) (Fig. 4A). Furthermore, greater abundance of Proteobacteria was associated with lower diastolic blood pressure (Fig. 4D). Plasma nitrite was negatively correlated with Bacteroidetes (Fig. 4B) and positively correlated with Actinobacteria (Fig. 4E). Greater abundance of SR1 was also correlated with higher pH salivary values (Fig. 4C).

All these correlations changed after using CHX. We only found a positive correlation between Fusobacteria and saliva glucose (Fig. 4F), and a negative correlation between Actinobacteria and Proteobacteria and lactate and nitrite in saliva (Fig. 4G,H), respectively.

Blood pressure. When CHX was administered systolic blood pressure increased although it was not statistically significant (Fig. 5A).

Discussion

This study showed that CHX mouthwash significantly changed the oral microbiome towards greater abundance of Firmicutes and Proteobacteria species, with lower abundance of Bacteroidetes, TM7, SR1 and Fusobacteria. These changes were associated with an increase in oral acidic conditions, represented by lower salivary pH. Saliva lactate and glucose concentrations were also elevated after using CHX. Additionally, CHX disrupted the ability of oral bacteria to reduce nitrate into nitrite, which may support our finding of lower circulatory nitrite bioavailability.

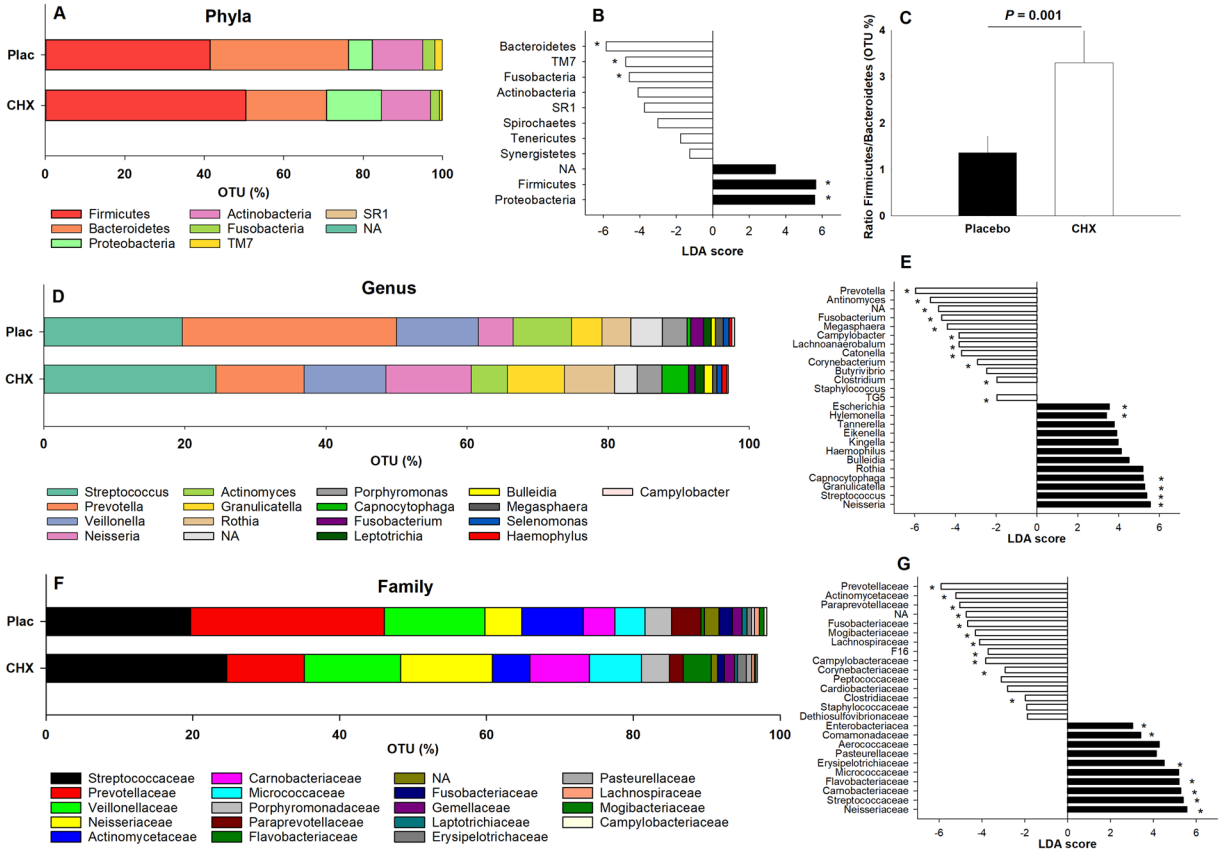


Figure 1. Absolute abundance and Linear Discriminant Analysis (LDA) scores in the main bacterial phyla (**A**,**B**), genus (**D**,**E**) and family (**F**,**G**) after a 7-day treatment with placebo and chlorhexidine (CHX). Figure C shows the ratio between the two main phyla (Firmicutes:Bacteroidetes) following each treatment. Operational Taxonomic Units (OTUs) with an asterisk were statistically significant (False Discovery Rate > 0.05).

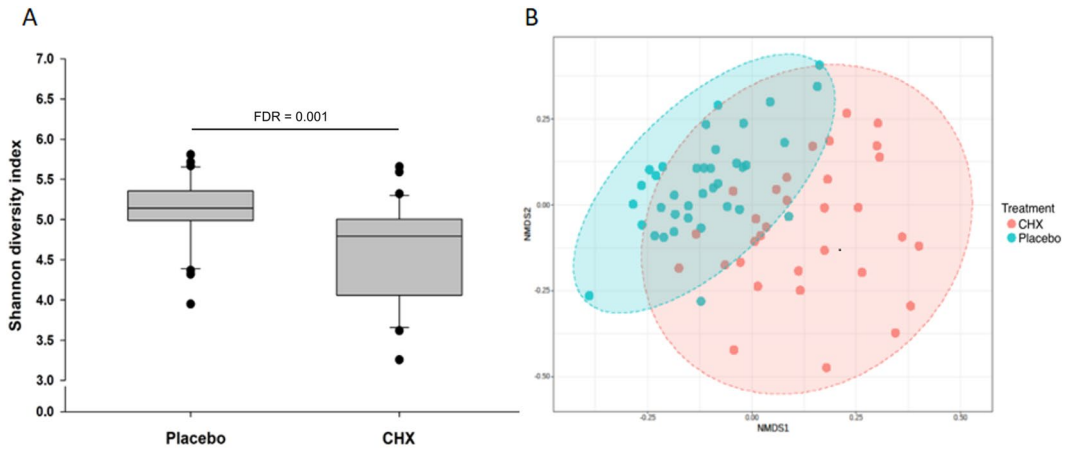


Figure 2. Shannon's index representing alpha-diversity (**A**) and Bray-Curtis index representing beta-diversity (**B**) (each dot represents an individual sample and ellipsis represents the 95% confidence regions for group) after a 7-day treatment with placebo and chlorhexidine (CHX).

To the best of our knowledge, this is the first study showing the impact of 7-day use of CHX on the oral microbiome. A large body of literature suggests that mouthwashes with CHX are effective in reducing dental plaque accumulation, gingival inflammation and bleeding[2–4]. However, the view about oral bacteria and oral health has changed substantially over the last few years[12]. Current approaches, using genome sequencing to identify and quantify the microorganisms in dental biofilms, have revealed a much more complex ecosystem than previously appreciated[13]. Results from this study showed that CHX led to an increase in the abundance of some genera such as *Neisseria*, *Streptococcus and Granulicatella*, and lowered the abundance of *Actinomyces*, but did not affect the

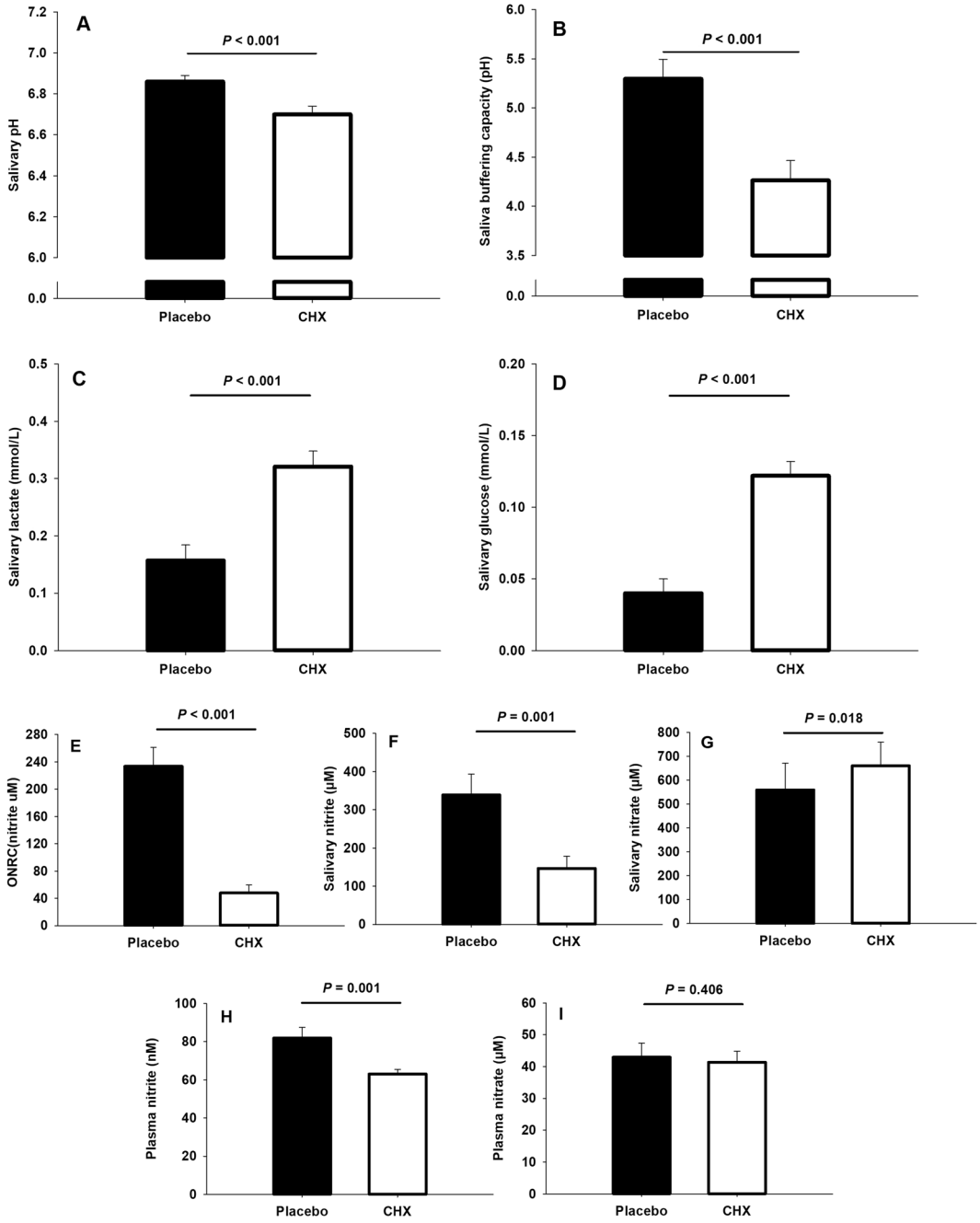


Figure 3. Saliva pH (**A**), saliva buffering capacity (**B**) and concentration of salivary lactate (**C**), glucose (**D**), nitrite (**F**), nitrate (**G**), as well as the oral nitrate-reducing capacity of bacteria (ONRC) (**E**) and concentration of plasma nitrite (**H**) and nitrate (**I**) after a 7-day treatment with placebo and chlorhexidine (CHX).

abundance of *Veillonella*. However, it remains difficult to determine whether these microbial changes suggest a shift towards a healthy oral environment, or whether they may increase the risk of oral disease, as both increases and decreases in the bacteria associated with caries and periodontal disease have been reported[14]. Consequently, additional studies are required to investigate bacterial communities during different disease states, with and without CHX. Nevertheless, in the current study, we were able to associate genome sequencing measurements with other general markers of oral health, which allowed us to analyse more in detail the impact of mouthwash containing CHX on oral and systemic health.

Lower microbial diversity as represented by the Shannon's index was found after using CHX. This result is in agreement with another recent study showing lower diversity of bacteria colonizing the tongue[7]. These findings are relevant from a dental viewpoint since lower diversity of oral bacteria has been related to greater risk of oral diseases[13]. This may reflect the ecological pressure of lowered environmental pH. Healthy biofilms are associated with an active balance between slow rates of acid production and compensatory alkali generation, resulting in

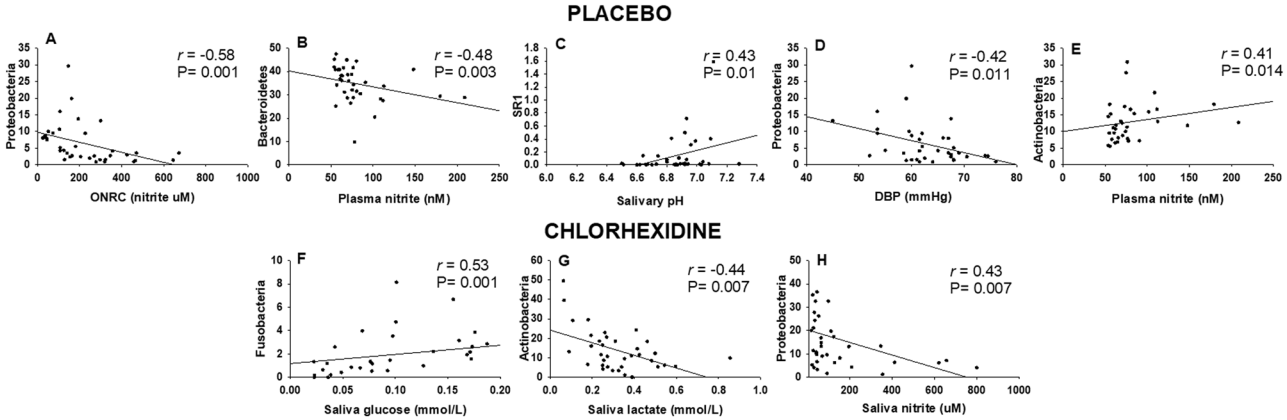


Figure 4. Moderate degree and significant Pearson correlations ($r > 0.40$; $P < 0.05$) found between abundance of oral bacteria (Operational Taxonomic Units [OTUs] %) at phylum level and salivary markers after the placebo and chlorhexidine treatment. In the placebo condition, abundance of Proteobacteria was negatively correlated to the oral nitrate-reducing capacity of bacteria (ONRC) (**A**), and with lower levels of diastolic blood pressure (**D**). Abundance of Bacteroidetes was negatively associated with plasma nitrite (**B**), while abundance of Actinobacteria was positively correlated (**E**). Abundance of the phylum SR1 was positively associated with greater salivary pH. Following 7-day use of chlorhexidine, the abundance of Fusobacteria was correlated with greater concentration of glucose in saliva (**F**). Abundance of Actinobacteria was negatively correlated with saliva lactate (**G**), and abundance of Proteobacteria was also negatively correlated with saliva nitrite concentration (**H**).

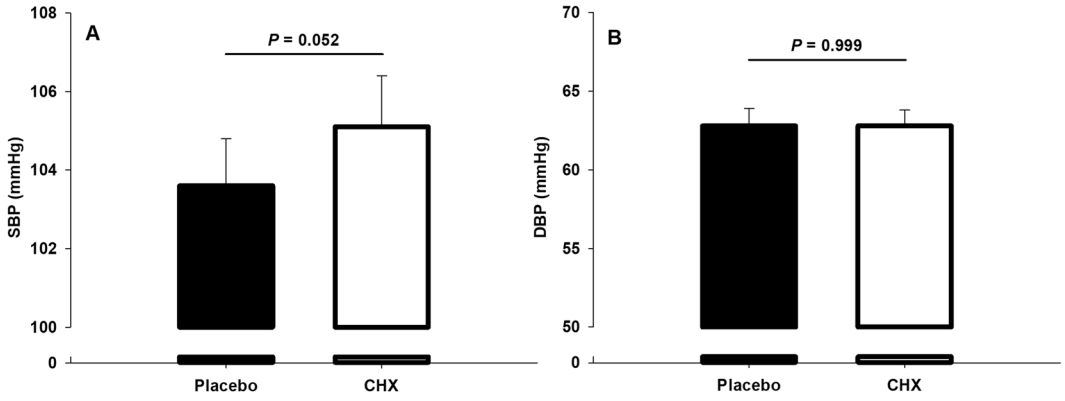


Figure 5. Changes in systolic (SBP) and diastolic (DBP) blood pressure after 7-day use of placebo and chlorhexidine (CHX).

an environment with a broadly neutral pH[12]. Surprisingly, the effect of CHX in salivary pH has only been investigated acutely in both *in vivo*[15] and *in vitro* conditions[16], but, no previous study analysed the impact of this antibacterial compound over a period of days in healthy individuals. Our results showing lower saliva pH after using CHX are relevant, since decreased pH in saliva is associated with demineralization of tooth enamel and risk of caries, tooth loss and other dental problems[17]. Oral pH may differ between different oral conditions: whilst saliva pH is more alkaline in chronic gingivitis, it tends to be more acidic in chronic periodontitis[18]. Thus, in terms of salivary pH, CHX could therefore be more useful for managing gingivitis than periodontitis.

Saliva's composition is another factor to pay attention when analysing the antimicrobial effectiveness of CHX. Several *in vitro* studies have indicated that saliva has a neutralizing effect on CHX[19–21]. Since CHX is a strongly cationic molecule it can react with anionic chemicals, resulting in inactivation of antimicrobial activity. We did not analyse the antimicrobial interaction between saliva and CHX in this study, but, we investigated the effect of CHX in several saliva markers. We found that CHX increased saliva lactate concentration and reduced its buffering capacity. These changes are commonly associated with greater risk of oral disease[22]. Regarding bacteria, we found a negative correlation between the phylum Actinobacteria and saliva lactate concentration. This phylum comprises a large variety of Gram-positive bacteria and is known for its high production of bioactive compounds, including those with antimicrobial activity such as lantibiotics[23]. For instance, *Nisin* is one of the best known antibiotics for its highly effective bactericidal activity against most lactic acid bacteria[24]. Another important bacterial change associated with CHX administration was an increase of the major phyla Firmicutes, mainly comprised of an increase of the genus *Streptococcus*. This genus contains several families of lactic acid bacteria that are able to produce large quantities of this compound in the mouth[25]. On the other hand, we also found a significant

decrease in the abundance of Bacteroidetes after using CHX. This was the second most abundant phyla in the oral cavity and some genera from this phyla such as Veillonella has been shown to be important in maintaining the acid/base conditions in the mouth[26]. Overall, these findings indicate that CHX promotes acidification of saliva by changing the ratio abundance of different families of bacteria that are essential to maintain the acid/base conditions in the mouth of healthy people.

Oral nitrite synthesis is another factor to take into account, with regards to the acid/base conditions of the oral cavity[27]. Nitrite is a nitrogen compound that forms naturally in the mouth by the action of oral bacteria that can use exogenous (diet) or endogenous (nitric oxide synthesis) nitrate sources[28]. Species within the genus *Veillonella* and *Actinomyces* have been suggested to lead this reaction in the oral cavity[29]. Importantly, CHX had a detrimental effect lowering the abundance of bacteria from these groups and reducing nitrite availability. Thus, the detrimental effect of CHX on oral nitrite synthesis is another key point requiring further attention by dental professionals, since nitrite has been shown to have an inhibitory effect in the growth of periodontal bacteria which can also help to reduce the acid production from these strains[27,30].

On the other hand, nitrite synthesis in the mouth has been shown to play a key role in cardiovascular control by enhancing circulatory nitrite availability. The vasodilatory effects of nitrite are well described by previous studies using intra-arterial infusions or dietary supplements with this anion[31,32]. Some recent studies, but not all[9,33], have also found that the use of CHX mouthwash from 3 to 7 days led to higher blood pressure in healthy[8] and hypertensive individuals[34]. Participants from these studies had higher values of blood pressure compared to participants in our current study. In agreement with our results, Sundqvist *et al.*[33] did not show a raise in blood pressure in a young and healthy group of females after using CHX for three days. Additional studies are required to improve our understanding about the hypertensive effect of CHX in males and females with different resting blood pressure levels and physiological status, especially, after new evidence has shown that CHX raised the mortality rate in hospitalized patients[35]. Overall, current studies seem to indicate that the use of CHX mouthwash leads to an increase of blood pressure, and this may be more accentuated in people with high blood pressure levels[8,9,11,33].

This study has some limitations. For instance, it would be interesting to analyse the effect of CHX in patients with different oral health conditions such as gingivitis or periodontitis. We assessed the oral health status of participants using a medical questionnaire, but it would be useful to undertake a full oral and dental examination, to analyse more in detail the concurrent effect of CHX on markers of periodontal health. Treatments were not randomized in this study due to the lack of available data indicating the time needed for the full recovery of the oral microbiome after one-week use of CHX. Consequently, there was not a wash out period between treatments. Furthermore, we analysed the microbiome in saliva as it provides an average of the oral microbiome, but bacterial communities can significantly differ among sites in distinct microbial niches in the oral cavity, therefore where the effects of CHX may also differ.

In conclusion this study indicates that a 7-day use of CHX mouthwash has a significant impact on the oral microbiome, as well as shift to an acidic environment, favourable for increased dental caries, and a reduction of the amount of oral nitrate-reducing bacteria, which contribute to cardiovascular health. Thus, these findings add to the growing body of evidence that the applications of CHX mouthwash should be more carefully considered, and that CHX could have detrimental effects on the healthy microbiome, and in turn cardiovascular health, requiring further investigation.

Methods

This study was approved by the Ethics Committee of the Faculty of Health & Human Sciences (University of Plymouth) and was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving human subjects. All the participants provided written consent to participate in this study. This study was also registered on http://www.clinicaltrials.gov (NCT03871777; date of registration: 12/03/2019). Parts of the data presented herein were extracted from a study that examined the dietary consumption of nitrate in healthy vegetarian and omnivore subjects and the impact of inhibiting the nitrate-reducing activity of oral bacteria using CHX mouthwash in blood pressure[9].

Main protocol. Following a single blinded, non-randomized, cross over design participants visited the laboratory twice. Before the first trial, each participant received 14 tubes containing 10 mL placebo mouthwash (ultrapure unflavoured water), with which they rinsed their mouth for 1 min, twice a day for 7 days taking the final tube the night before the trial. Individuals were excluded from this study if they were smokers, using mouthwash or tongue scrapes, suffering from gingivitis or periodontitis, or exhibited a medical condition (e.g hypertension, diabetes). For standardisation, they were also given the same toothpaste to use throughout the duration of the study. Participants visited the laboratory on the eighth day between 8 and 10 am, having fasted overnight. Additionally, at least 24 h prior to their visit, they were sent written instructions via email to avoid drinks containing caffeine, such as tea or coffee, before the test and to refrain from strenuous exercise. Basic anthropometrical (weight and height) and physiological (blood pressure) parameters were measured before the collection of a plasma sample and a non-stimulated salivary sample (3 mL) as previously indicated[9]. Then, the oral nitrate-reducing capacity was also measured. At the end of the visit, the participant was given a further one-week supply of antibacterial mouthwash containing 0.2% CHX (Corsodyl Mint, GlaxoSmithKline, UK), instructed to use it as per the previous mouthwash (1 min, twice a day) and requested to return to the laboratory in 7 days to repeat all measurements in the same order.

Bacterial analysis. Saliva pellets were extracted and frozen at −80 °C in a single sterile tube prior to metagenomic sequencing of the oral microbiome. DNA extraction of saliva and sequencing was performed as previously described at the Systems Biology Centre in Plymouth University (UK)[9].

Saliva lactate, glucose and pH. Saliva concentrations of lactate and glucose were measured using a biochemistry analyser (YSI 2300 Stat Plus, YSI Life Sciences, USA). Salivary pH was measured using a single electrode digital pH meter (Lutron Electronic Enterprise Co Ltd., Model PH-208, Taiwan) that was calibrated following the manufacturer's instructions.

Saliva buffering capacity. 250 µL of saliva was mixed with 750 µL of HCl (0.0033 m/L) and shaken for 20 min. Then, salivary pH was measured using a single electrode digital pH meter (Lutron Electronic Enterprise Co Ltd., Model PH-208, Taiwan).

Saliva and plasma concentration of nitrate and nitrite. Whole blood was collected into lithium-heparin tubes (BD Vacutainer®, Becton Dickinson, Plymouth, UK) and rapidly centrifuged (4,000 rpm, 4 °C, 10 min). The plasma was then separated, and frozen at −80 °C until further analyses of nitrate and nitrite. Both anions were measured in saliva and plasma using ozone-based chemiluminescence as previously described[36].

Oral-nitrate reducing capacity. Participants were instructed to hold 10 mL of water containing sodium nitrate (80 µmol) in their mouth for 5 min. The mouth rinse was collected into a sterile Falcon tube and centrifuged (4,000 rpm, 4 °C) for 10 min. The supernatant was collected and stored at −80 °C before measurement of absolute nitrite concentration as indicated above.

Blood pressure measurement. Participants rested in a supine position for 30 min, before three successive readings were taken (four if variation in systolic or diastolic blood pressure of >4 mmHg was found), using an oscillometric device (Connex ProBP 3400 Digital Blood Pressure Device, Welch Allyn UK Ltd.) with 1 min rest between readings. The second and third readings were averaged to determine mean clinical blood pressure.

Statistical analyses. General data are presented as mean (95% confidence interval). Normal distribution of the sample was assessed using Shapiro-Wilk test. Differences between treatments (placebo vs CHX) were analysed using paired *t*-tests (data normally distributed) or Wilcoxon test (data non-normally distributed). Operational Taxonomic Units (OTUs) assigned to the major salivary bacterial phyla, and genera were analysed using the linear discriminant analysis (LDA) effect size (LEfSe) method[37]. The False Discovery Rate (FDR) was used at an alpha of 0.05 as previously indicated[38]. The Pearson correlation test was used to investigate relationship between relative oral bacterial abundance (OTUs %) and salivary markers. Bioinformatics analysis was performed using the OTUs_biom table generated in with MicrobiomeAnalysit[39].

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request. All data generated or analysed during this study are included in this published article.

Received: 2 October 2019; Accepted: 26 February 2020;
Published online: 24 March 2020

References

1. Löe, H. & Rindom Schiøtt, C. The effect of mouthrinses and topical application of chlorhexidine on the development of dental plaque and gingivitis in man. *J Period Res.* **5**, 79–83 (1970).
2. Afennich, F., Slot, D., Hossainian, N. & Van der Weijden, G. The effect of hexetidine mouthwash on the prevention of plaque and gingival inflammation: a systematic review. *Int J Dent Hyg*. 9 (2011).
3. Gunsolley, J. C. Clinical efficacy of antimicrobial mouthrinses. *J Dent.* **38**, S6–S10 (2010).
4. James, P. *et al*. Chlorhexidine mouthrinse as an adjunctive treatment for gingival health. *Cochrane Database Syst Rev*. (2017).
5. Eick, S., Goltz, S., Nietzsche, S., Jentsch, H. & Pfister, W. Efficacy of chlorhexidine digluconate–containing formulations and other mouthrinses against periodontopathogenic microorganisms. *Quintessence Int*. 42 (2011).
6. Fedorowicz, Z., Aljufairi, H., Nasser, M., Outhouse, T. L. & Pedrazzi, V. Mouthrinses for the treatment of halitosis. *Cochrane Database Syst Rev*. (2008).
7. Tribble, G. D. *et al*. Frequency of Tongue Cleaning Impacts the Human Tongue Microbiome Composition and Enterosalivary Circulation of Nitrate. *Front Cell Infect Microbiol* **9**, 39–39 (2019).
8. Kapil, V. *et al*. Physiological role for nitrate-reducing oral bacteria in blood pressure control. *Free Rad. Biol Med.* **55**, 93–100 (2013).
9. Ashworth, A. *et al*. Dietary intake of inorganic nitrate in vegetarians and omnivores and its impact on blood pressure, resting metabolic rate and the oral microbiome. *Free Rad Biol Med.* **138**, 63–72 (2019).
10. Govoni, M., Jansson, E. A., Weitzberg, E. & Lundberg, J. O. The increase in plasma nitrite after a dietary nitrate load is markedly attenuated by an antibacterial mouthwash. *Nitric Oxide.* **19**, 333–337 (2008).
11. Bondonno, C. P. *et al*. Antibacterial Mouthwash Blunts Oral Nitrate Reduction and Increases Blood Pressure in Treated Hypertensive Men and Women. *Am J Hypert.* **28**, 572–575 (2015).
12. Rosier, B. T., Marsh, P. D. & Mira, A. Resilience of the Oral Microbiota in Health: Mechanisms That Prevent Dysbiosis. *J Dent Res.* **97**, 371–380 (2018).
13. Sanz, M. *et al*. Role of microbial biofilms in the maintenance of oral health and in the development of dental caries and periodontal diseases. Consensus report of group 1 of the Joint EFP/ORCA workshop on the boundaries between caries and periodontal disease. *J Clin Period*. 44 (2017).
14. Chen, H. & Jiang, W. Application of high-throughput sequencing in understanding human oral microbiome related with health and disease. *Front Microbiol.* **5**, 508 (2014).
15. Belardinelli, P. A., Morelatto, R. A., Benavidez, T. E., Baruzzi, A. M. & Lopez de Blanc, S. A. Effect of two mouthwashes on salivary pH. *Acta Odontol Latinoam.* **27**, 66–71 (2014).

16. Andreadis, G., Topitsoglou, V. & Kalfas, S. Acidogenicity and acidurance of dental plaque and saliva sediment from adults in relation to caries activity and chlorhexidine exposure. *J Oral Microbiol.* **7**, 26197 (2015).
17. Lenander-Lumikari, M. & Loimaranta, V. Saliva and dental caries. *Adv Dent Res.* **14**, 40–47 (2000).
18. Baliga, S., Muglikar, S. & Kale, R. Salivary pH: A diagnostic biomarker. *J Ind Soc Period.* **17**, 461–465 (2013).
19. Portenier, I., Waltimo, T., Orstavik, D. & Haapasalo, M. Killing of Enterococcus faecalis by MTAD and chlorhexidine digluconate with or without cetrimide in the presence or absence of dentine powder or BSA. *J Endod.* **32**, 138–141 (2006).
20. Spijkervet, F. K. *et al*. Chlorhexidine inactivation by saliva. *Oral Surg Oral Med Oral Pathol* **69**, 444–449 (1990).
21. Abouassi, T. *et al*. Does human saliva decrease the antimicrobial activity of chlorhexidine against oral bacteria? *BMC Res Notes* **7**, 711 (2014).
22. Seethalakshmi, C., Reddy, R. C. J., Asifa, N. & Prabhu, S. Correlation of Salivary pH, Incidence of Dental Caries and Periodontal Status in Diabetes Mellitus Patients: A Cross-sectional Study. *J Clin Diagn Res* **10**, ZC12–ZC14 (2016).
23. Gomes, K. M., Duarte, R. S. & de Freire Bastos, M. D. C. Lantibiotics produced by Actinobacteria and their potential applications (a review). *Microbiology.* **163**, 109–121 (2017).
24. Ouwehand, A. C. & Vesterlund, S. In Lactic Acid Bacteria: Microbiological and Functional Aspects Vol. 139 (eds Salminen, S., von Wright, A., & Ouwehand, A.) 375–396 (Marcel Dekker, Inc, 2004).
25. De Soet, J., Nyvad, B. & Kilian, M. Strain–Related Acid Production by Oral Streptococci. *Caries Res.* **34**, 486–490 (2000).
26. Brooks, G. A. The science and translation of lactate shuttle theory. *Cell Metab.* **27**, 757–785 (2018).
27. Kilian, M. *et al*. The oral microbiome–an update for oral healthcare professionals. *Br Dent J.* **221**, 657–666 (2016).
28. Lundberg, J. O., Weitzberg, E. & Gladwin, M. T. The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. *Nat Rev Drug Discov.* **7**, 156–167 (2008).
29. Hyde, E. R. *et al*. Metagenomic analysis of nitrate-reducing bacteria in the oral cavity: implications for nitric oxide homeostasis. *PLoS One.* **9**, e88645 (2014).
30. Allaker, R. P., Silva Mendez, L. S., Hardie, J. M. & Benjamin, N. Antimicrobial effect of acidified nitrite on periodontal bacteria. *Oral Microbiol Immunol* **16**, 253–256 (2001).
31. Dejam, A. *et al*. Nitrite Infusion in Humans and Nonhuman Primates. *Circulation.* **116**, 1821–1831 (2007).
32. Montenegro, M. F. *et al*. Blood Pressure–Lowering Effect of Orally Ingested Nitrite Is Abolished by a Proton Pump Inhibitor. *Hypertension.* **69**, 23–31 (2017).
33. Sundqvist, M. L., Lundberg, J. O. & Weitzberg, E. Effects of antiseptic mouthwash on resting metabolic rate: A randomized, double-blind, crossover study. *Nitric Oxide.* **61**, 38–44 (2016).
34. Kapil, V. *et al*. Sex differences in the nitrate-nitrite-NO• pathway: Role of oral nitrate-reducing bacteria. *Free Rad Biol Med.* **126**, 113–121 (2018).
35. Deschepper, M., Waegeman, W., Eeckloo, K., Vogelaers, D. & Blot, S. Effects of chlorhexidine gluconate oral care on hospital mortality: a hospital-wide, observational cohort study. *Int Care Med*. 44 (2018).
36. Liddle, L. *et al*. Changes in body posture alter plasma nitrite but not nitrate concentration in humans. *Nitric Oxide.* **72**, 59–65 (2018).
37. Segata, N. *et al*. Metagenomic biomarker discovery and explanation. *Genome Biol.* **12**, R60 (2011).
38. Benjamini, Y. & Hochberg, Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *J Royal Statistical Soc. Series B (Methodological)* **57**, 289–300 (1995).
39. Dhariwal, A. *et al*. MicrobiomeAnalyst: a web-based tool for comprehensive statistical, visual and meta-analysis of microbiome data. *Nucleic Acids Res.* **45**, W180–W188 (2017).

Acknowledgements

This study was funded by research grants of the Northcott Devon Medical Foundation (UK), the Seale-Hayne Trust (Devon, UK) and the Institute of Health & Community (University of Plymouth, UK). The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

Author contributions

R.B. contributed to conception, design, data acquisition, analysis, interpretation and drafted the manuscript; A.A. contributed to conception, data acquisition, analysis and drafted the manuscript; C.C. contributed to data analysis and drafted the manuscript; Z.L.B. contributed to interpretation and drafted the manuscript; L.B. contributed to interpretation and drafted the manuscript; A.R. contributed to analysis, interpretation and critically revised the manuscript; P.C.A. contributed to analysis, interpretation and critically revised the manuscript; G.F., L.L., M.B., D.W., M.H., C.E. contributed to analysis and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

Competing interests

The authors declare no competing interests.

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Can J Anesth/J Can Anesth (2020) 67:732–745
https://doi.org/10.1007/s12630-020-01620-9

REVIEW ARTICLE/BRIEF REVIEW

Preparing for a COVID-19 pandemic: a review of operating room outbreak response measures in a large tertiary hospital in Singapore

Se préparer pour la pandémie de COVID-19: revue des moyens déployés dans un bloc opératoire d'un grand hôpital tertiaire au Singapour

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Received: 2 March 2020 / Revised: 4 March 2020 / Accepted: 4 March 2020 / Published online: 11 March 2020
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Abstract *The coronavirus disease 2019 (COVID-19) outbreak has been designated a public health emergency of international concern. To prepare for a pandemic, hospitals need a strategy to manage their space, staff, and supplies so that optimum care is provided to patients. In addition, infection prevention measures need to be implemented to reduce in-hospital transmission. In the operating room, these preparations involve multiple stakeholders and can present a significant challenge. Here, we describe the outbreak response measures of the anesthetic department staffing the largest (1,700-bed) academic tertiary level acute care hospital in Singapore (Singapore General Hospital) and a smaller regional hospital (Sengkang General Hospital). These include engineering controls such as identification and preparation of an isolation operating room, administrative measures such as modification of workflow and processes, introduction of personal protective equipment for staff, and formulation of clinical guidelines for anesthetic management. Simulation was valuable in evaluating the feasibility of new operating room set-ups or workflow. We also discuss how the hierarchy of controls can be used as a framework to plan the necessary measures during each phase of a pandemic, and review the evidence for the measures taken. These containment measures are necessary to optimize the quality of care provided to COVID-19 patients and to reduce the risk of viral transmission to other patients or healthcare workers.*

Résumé *L'épidémie liée au coronavirus 2019 (COVID-19) a été qualifiée d'urgence de santé publique de portée internationale. La préparation face à une pandémie nécessite de la part d'un hôpital l'élaboration de stratégies pour gérer ses infrastructures, ses processus, son personnel et ses patients; il doit notamment instaurer des mesures de prévention des infections pour réduire la transmission intrahospitalière. Pour un bloc opératoire, ces préparations impliquent la participation de nombreux acteurs et peuvent constituer un véritable défi. Nous décrivons les mesures prises en réponse à l'épidémie par le département d'anesthésie qui sert le plus grand hôpital universitaire de soins aigus (1700 lits) de Singapour (Singapore General Hospital) et un plus petit hôpital régional (Sengkang General Hospital). Cela a été obtenu grâce à des expertises d'ingénierie, telles que l'identification et la préparation d'une salle d'opération en isolation, des mesures administratives telles que la modification du déroulement des activités et des processus, l'introduction d'équipements de protection individuels pour le personnel et — enfin — la formulation de lignes directrices cliniques pour la gestion anesthésique. La*

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simulation a été importante pour évaluer la faisabilité de toutes nouvelles modifications des salles d'opération ou d'un nouveau flux de travail. Dans le contexte des différentes phases d'une pandémie, nous discutons de l'application d'une hiérarchie de contrôles comme cadre des modifications à chaque niveau de contrôle et nous passons aussi en revue les données probantes soutenant les mesures prises. Ces mesures de confinement sont nécessaires pour optimiser la qualité des soins procurés aux patients atteints de COVID-19 et pour réduire le risque de transmission du virus à d'autres patients ou employés du domaine de la santé.

On 30 January 2020, the World Health Organization (WHO) declared the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) outbreak a public health emergency of international concern.[1] Human-to-human transmission of SARS-CoV-2 was established and at the time of writing this article, it has caused coronavirus disease (COVID-19) in over 90,000 people in 72 countries, with a case fatality rate of 2.3% in China and 1.6% outside China.[2–8]

Singapore General Hospital (SGH) confirmed Singapore's first case of COVID-19 on 23 January 2020.[9,10] Local transmission was subsequently reported[11] and by 7 February 2020, Singapore raised the Disease Outbreak Response System Condition (DORSCON) alert level to orange, indicating that the disease is severe and spreads easily from person to person (but is not spread widely and is being contained).[12,13]

Current understanding of SARS-CoV2 transmission is based on extrapolation from past experience with the SARS-CoV outbreak in 2003.[14–17] Possible modes of transmission include respiratory droplets, contact, fomites, and the fecal-oral route.[18,19] At present, there is no conclusive evidence of airborne transmission of SARS-CoV-2, but past experience suggests that SARS-CoV can be transmitted via aerosol-generating procedures, most commonly tracheal intubation.[20,21] As with most respiratory viruses, it is considered most contagious when the patient is symptomatic, but SARS-CoV-2 has been transmitted by asymptomatic individuals.[22] Early data suggests that transmissibility of SARS-CoV-2 is moderate, with a basic reproduction number of approximately 2.[23,24]

Transmission within healthcare facilities to healthcare workers has been documented: 3.8% of COVID-19 cases have occurred in healthcare workers, causing five deaths in China.[7] During the SARS outbreak in 2003, anesthesiologists also contracted SARS in the line of duty.[25] With the institution of infection control measures, several hospitals successfully operated on patients with SARS without any transmission of SARS-CoV within the operating room complex.[26,27]

The Division of Anesthesiology at SGH developed disease outbreak response measures in coordination with surgical, nursing, and other allied health staff. The two goals of these measures were to facilitate the care of patients with known or suspected COVID-19 who needed surgery, and to reduce the risk of perioperative viral transmission to healthcare workers and other patients. At the time of writing, no patients with COVID-19 have required surgery, but we expect this to change in the near future as the number of cases increases globally.

This article aims to describe the measures taken to address these goals, including identification and set-up of an isolation operating room (OR), modification of workflow and processes, management of staff, and formulation of clinical guidelines for anesthetic management.

Hospital measures related to surgery and anesthesia

Increasing capacity

To cope with the anticipated increased influx of COVID-19 patients, we decided to reduce elective surgery.[28] Day surgery, cancer surgery, and other urgent operations continued as normal. We increased the turnaround time between elective surgeries to accommodate additional infection prevention measures.

Management of patients, visitors, and staff

All patients presenting to the hospital were screened using a standard questionnaire. Patients who fulfilled the criteria for suspected SARS-CoV-2 infection were isolated, referred to an infectious diseases specialist, and tested for the virus. Elective surgery was postponed if the patient had travelled to affected areas. Visitor numbers were also restricted.[28]

Staff were asked to refrain from unnecessary travel to certain destinations as advised by the Singapore Ministry of Health.[29] Staff returning from these destinations were required to take a 14-day leave of absence. We also separated staff caring for COVID-19 patients and those caring for other patients to reduce the risk of in-hospital

transmission. Staff who had cared for COVID-19 patients continued with usual clinical work with a surgical mask on and monitored themselves for fever and respiratory symptoms. If inadequate personal protective equipment (PPE) was worn at the time of close contact, the staff member was taken off duty while an infectious diseases team assessed the risk of transmission and planned subsequent management. Temperatures of all staff were taken twice daily using hospital-issued oral digital thermometers, and entered into electronic records monitored by administrative personnel. Web-based forms were created to facilitate ease of entry using personal smartphones. Staff members who developed a fever or respiratory symptoms were screened at the staff clinic.

Communications and staff support

In the early phase of the outbreak, the situation was rapidly evolving so case definitions and hospital guidelines were modified frequently. Communication channels including a COVID-19 website were set up and daily email updates were sent to all staff. Staff used social media, such as *Workplace* from Facebook and encrypted instant messaging, to approach senior management with ideas or concerns.[13]

Resources, including reading material and a helpline, were set up in the hospital to help staff cope with COVID-19-related anxiety and burnout.[13]

Operating room management

An OR with a negative pressure environment is ideal to reduce dissemination of the virus beyond the OR.[5,30] A standard OR is usually designed to be at positive pressure relative to surrounding air. Nevertheless, a high frequency of air changes (25 per hr) rapidly reduces viral load within the OR.

A small OR complex comprising three separate ORs was designated for surgery in patients suspected or confirmed to have COVID-19. This was separate from the main OR complex and reduced the risk of contaminating other ORs and other patients. Elective surgeries on non-COVID-19 patients were relocated to the main OR complex. Each OR had its own ventilation system with an integrated high-efficiency particulate air (HEPA) filter. Traffic and flow of contaminated air were minimized by locking all doors to the OR during surgery, with only one possible route for entry/exit via the scrub room.

New workflows were created for activation and use of the designated isolation OR. These workflows considered coordination of staff, movement of surgical and anesthetic equipment, infection prevention practices, and decontamination following the procedure. An additional anesthesiologist was called in to care for the COVID-19 patient, while the usual on-call specialist continued with emergency surgery in the main OR.

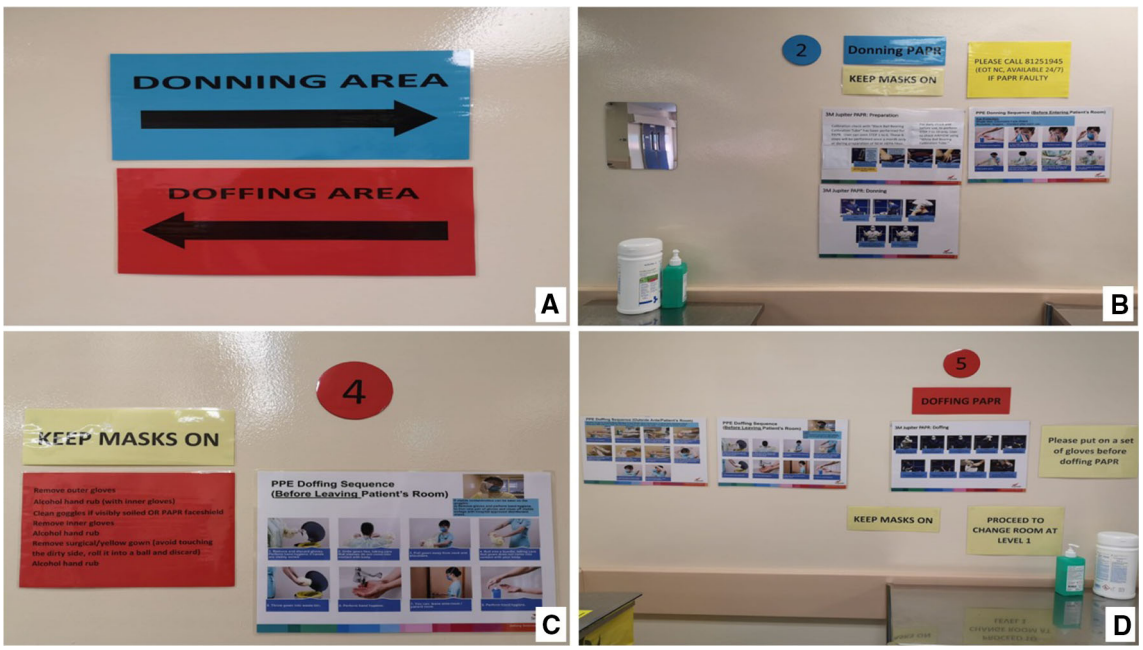


Fig. 1 Personal protective equipment donning and doffing areas. Steps are colour-coded (A: blue for clean and red for contaminated) and marked in numerical sequence at designated areas just outside the OR, with posters to guide staff on the process. Step 1: Put on N95. Step 2: Donning PAPR (B). Step 3: Enter OR through scrub room. Step 4: Clean external surface of the PAPR face shield (C), remove surgical gown and outer gloves. Step 5: Doffing PAPR (D). OR = operating room; PAPR = powered air-purifying respirator

Because more time is needed to prepare for surgery in a suspected/confirmed case of COVID-19, daily routines were set up regardless of whether surgery would happen. These included routine checks and cleaning of anesthetic machines and powered air-purifying respirator (PAPR) sets. Designated areas were prepared for donning and doffing of PPE. Steps were numbered in sequence and posters were put up to guide staff (Fig. 1).

Management of staff in the division of anesthesiology

Personal protective equipment

A comprehensive program for the use of PPE was enforced. Healthcare personnel were medically cleared and trained in the use of PPE. They were also taught how to clean, disinfect, store, and inspect their PPE for any damage. All staff were fitted with the National Institute of Occupational Safety and Health (NIOSH)-certified N95 respirators. If staff did not fit into available N95 masks, they would use the CleanSpace^{R} HALO^{TM} PAPR (CleanSpace Technology Pty Ltd, Artarmon, NSW, Australia). Personal goggles (model 9302-245; Uvex, Germany) were also issued to every member of staff.

Powered air-purifying respirator training

Refresher training for the use and maintenance of the PAPR was conducted. Educators from the Department of Occupational and Environmental Medicine trained designated trainers within the department who then went on to train the 180 anesthesia staff members (consultants and trainees) on the Jupiter and Proflow PAPR models (3M, St Paul, MN, United States of America). In addition, mask fitting was done for all users of the CleanSpace^{R} HALO^{TM}.

Segregation of staff

The anesthesia division staffs a tertiary hospital (SGH) and a smaller regional hospital (Sengkang General Hospital). Staff were segregated between the two to reduce cross infection between hospitals. Locum employment and large group meetings were suspended. Web-based conferencing was used to enable communication. Attendance was taken at all face-to-face meetings to facilitate contact tracing should the need arise.

Minimizing exposure and spread

Routine postoperative visits were suspended and replaced by phone calls where applicable to reduce movement of staff around the hospital. Non-urgent preoperative assessment clinic visits were also postponed. Pregnant and immunocompromised staff did not care for patients with COVID-19.

Measures for infection prevention

Routine infection prevention measures

Once community spread of the virus had been confirmed, the hospital instituted guidelines for airborne and contact precautions during all aerosol-generating procedures. These included wearing N95 masks, eye protection, gown, and gloves. Routine care of low-risk patients (no fever or respiratory symptoms, no history of recent travel or close contact with a COVID-19 patient) required only the use of a simple surgical mask to protect against droplet transmission.

Infection prevention when caring for the COVID-19 patient

The minimum standard of PPE for any staff caring for a patient with confirmed/suspected COVID-19 infection is a fitted, NIOSH-certified N95 respirator, eye protection (either goggles or full-face shield), cap, gown, and gloves.[5] Double gloves may be considered and the outer pair should be changed when contaminated.[31] As transmission remains possible despite N95 protection,[32] staff participating in aerosol-generating procedures can wear a PAPR (Fig. 2).

The patient, wearing a surgical face mask, should be transported from the isolation unit along a designated route with minimal contact with others.[31]

Only selected equipment and drugs should be brought into the OR to reduce the number of items that need cleaning or discarding following the procedure.[33] Single-use equipment should be selected where possible.[26] Consent and charting are done electronically with touch-screen devices to facilitate decontamination. Anesthetic monitors, laptop computers, and ultrasound machine surfaces are covered with plastic wrap to decrease the risk of contamination and to facilitate cleaning (Fig. 3).

The patient should be reviewed, induced, and recovered within the OR itself to restrict contamination to just one room.[27] The number of staff involved in the surgery should be limited.[5] Movement of staff in and out of the OR should also be restricted.[31]

After surgery, the anesthetic breathing circuit and the canister of soda lime are discarded to eliminate the negligible risk of circuit contamination.[25,26,34] After disposing of single-use equipment in well-marked biohazard bags, all instruments are sent for decontamination and sterile reprocessing. Surfaces of all medical devices are cleaned with quaternary ammonium chloride disinfectant wipes. The OR is then



Fig. 2 Staff in powered air-purifying respirator (PAPR) during an aerosol-generating procedure



Fig. 3 High-touch equipment within the operating room are wrapped with plastic sheets to facilitate decontamination. A) Anesthesia workstation. B) Back of anesthesia workstation. C) Exposed surfaces wrapped with plastic. D) Laptop for nursing documentation

cleaned with sodium hypochlorite 1000 ppm and treated with hydrogen peroxide vaporization or ultraviolet-C irradiation. All staff then shower and change into a clean set of scrubs. Names of all participating staff members are recorded to facilitate contact tracing. More time is needed for decontamination, so the turn-around time following surgery is increased.

Anesthesia of patients with suspected/confirmed COVID-19

A study of 1,099 patients with COVID-19 showed that 18.7% present with shortness of breath, 41.3% require oxygen supplementation, 5% become critically ill, and 2.3% require invasive mechanical ventilation.[35] The median duration of symptoms and hospital length of stay are both between 12 and 13 days.[10,35] To reduce risks to both patient and staff, only emergency and not elective surgery should be performed on COVID-19-infected patients.[33] If a few hours' delay is permissible, it is preferable to await the results of the viral swab before proceeding. In patients confirmed to have COVID-19 who require urgent surgery (e.g., cancer or cardiac surgery), the risks and benefits of proceeding or postponing need to be weighed.

A team huddle should take place before surgery to ensure everyone understands the plan for anesthesia and surgery. This enables seamless teamwork and ensures that all necessary drugs and equipment have been prepared. It also minimizes the need to leave and re-enter the OR to bring in missing equipment. All staff should be aware that communication is more difficult after PPE (and especially PAPR) have been donned, and pay special attention to facilitate communication during the procedure.[26] All staff should be protected in appropriate PPE, and the staff number should be restricted to the minimum necessary for patient safety.[5] Given the relative lack of familiarity with the new changes in workflow, an OR coordinator is assigned to each team caring for the COVID-19 patient. This coordinator directs all team members and guides them on their roles and infection prevention measures. A core group of anesthesiologists, who are familiar with the isolation OR processes, serve as coordinators.

Anesthesia should be planned with two goals in mind: patient safety and infection prevention. Infection prevention entails reducing aerosol-generating procedures (i.e., airway manipulation, face mask ventilation, open airway suctioning, patient coughing) as far as possible.[33] Regional anesthesia is preferred over general anesthesia. Awake intubation techniques should be avoided because both patient coughing and atomized local anesthetic may lead to aerosolization of the virus.[25,33] If a regional technique is chosen, the patient should wear a surgical face mask at all times.[5] If sedation is administered, supplemental oxygen may be administered via nasal prongs underneath the surgical mask.[25] Non-invasive positive pressure ventilation and high flow nasal cannulae such as the transnasal humidified rapid-insufflation ventilatory exchange should be avoided to reduce the risk of viral aerosolization.

Before anesthesia induction, a HEPA filter should be connected to the patient end of the breathing circuit, and another between the expiratory limb and the anesthetic machine.[33] Equipment should be prepared to reduce the need for circuit disconnections—e.g., any circuit extensions should be attached before starting the case. A definitive airway with an endotracheal tube is preferred over a supraglottic airway device because it has a better seal. A video-laryngoscope is recommended because a PAPR hood or goggles may hamper vision during direct laryngoscopy. A video-laryngoscope also keeps the intubator further from the patient's airway during intubation.[36]

Pre-oxygenation should be carried out via a well-fitting face mask.[25] Rapid sequence induction should be carried out to reduce the need for bag-mask ventilation.[25] If bag-mask ventilation cannot be avoided, small tidal volumes (at low pressure) should be administered.[25] Deep anesthesia and neuromuscular blockade should be achieved before attempting intubation, and the latter can be assessed by train-of-four monitoring. Ensure full expiration into the face mask before lifting it off the patient's face. The most experienced operator should intubate.[25] Following intubation, the cuff should be inflated and the circuit connected before initiating positive pressure ventilation. Closed, in-line tracheal suction should be used instead of open suction.[33] Minimizing circuit disconnections is ideal, but if this is unavoidable, ensure positive pressure ventilation is ceased, turn the adjustable pressure limiting valve to zero, and consider clamping the endotracheal tube prior to disconnection. This technique may also be used before switching a patient from intensive care from the transport ventilator to the anesthetic machine. The patient should be preoxygenated and the duration of disconnection should be kept to a minimum to avoid exacerbating hypoxia in critically ill COVID-19 patients with respiratory failure.

Anti-emetics should be administered to reduce postoperative retching. A rigid suction catheter may be used to reduce the chance of contaminating the surroundings with the soft flexible suction catheter. All equipment in contact with patient secretions should be carefully manipulated. This includes the suction device, endotracheal tube, nasogastric tube (if used) and even the tape used to cover the eyes. Following extubation, the

patient should wear a surgical face mask. Supplemental oxygen may be administered via nasal prongs underneath the face mask.[25] Venturi masks should be avoided as they may aerosolize the virus.[25] The patient should recover within the OR itself.

Considerations specific to individual disciplines

Interventional radiology (IR)

As far as possible, portable cases that can be done under local anesthesia should be done so in the isolation ward within the airborne infection isolation room (AIIR).[28] If the procedure requires anesthetic support, it should take place in the isolation OR. If the procedure is more complex and requires specialized equipment such as a computed tomography scan, it should take place in a dedicated room within the IR facility with its own control room.

As the IR suite is regularly used for procedures, it would have to be cleared and prepared for a patient with COVID-19. This would mean postponing or cancelling other elective procedures and discharging any patients recovering within the post-anesthetic care unit. Unnecessary equipment should be removed from the procedure room. Remaining equipment (such as image intensifier, lead shield) should be covered with plastic sheets. Signs are placed at doors to alert other staff not to enter the room without PPE.

A similar workflow can be replicated as in the isolation OR with similar principles of reducing exposure of staff, ensuring appropriate PPE, reducing contamination, and subsequent decontamination. All processes from pre-induction checks to recovery should be done within the procedure room.[27]

Obstetrics

Current limited evidence suggests that there is no vertical transmission of the virus to the neonate if the mother is infected in late pregnancy.[37] Considerations specific to obstetrics include protecting the neonate and preventing transmission of the virus. In theory, a lower segment Cesarean delivery may reduce fecal-oral transmission, but this is may not be favoured because it has more risks.[38]

Cesarean deliveries should be performed in the isolation OR if the mother is a known or suspected COVID-19 patient. At birth, the neonate should be isolated and be cared for by a dedicated neonatal team in a separate room from the mother.[38,39] We use the induction room for this purpose as it has the necessary oxygen supply and suction apparatus. If this environment is unfamiliar to obstetric and neonatal teams, simulation is conducted to familiarize all the staff with the planned workflow.

Cardiothoracic surgery

The National Heart Centre Singapore (NHCS) performs all elective cardiac surgery within its own OR complex, which is connected to the main SGH building via a link bridge. Emergency surgeries done after office hours are usually performed in the major OR complex in the main SGH building. To isolate known/suspected COVID-19 patients requiring emergency cardiac surgery, the largest OR within the outbreak OR complex was chosen. This accommodated additional equipment such as the cardiopulmonary bypass machine, intra-aortic balloon pump, transesophageal echocardiography machine, and fluid management system. Because the main NHCS operating complex was further away, equipment such as a dedicated bypass machine and other essential cardiac equipment such as cell saver, cerebral oximetry, fluid warmer, and disposable bronchoscope were stored in the isolation OR complex. The use of a heated humidifier was discouraged to avoid viral aerosolization.

Equipment kits for peripheral arterial and central venous cannulation were assembled and contained all necessary items such as syringes and gauze (Fig. 4). This was done to reduce contamination of common equipment stores. To familiarize cardiothoracic anesthetic staff with the new set-up in the isolation OR, they were given photographs showing the location of essential drugs and equipment.

In situ simulation

In situ simulation was important for testing the preparedness of our isolation OR and procedures. *In situ* simulation is simulation training that takes place in the actual clinical environment. It has been used to improve reliability and safety in high-risk or high-stress environments,[40–42] and has been shown to improve clinical skills, teamwork, patient safety, and behaviour.[43] We conducted a series of simulations and "walk-throughs" of various scenarios with different surgical disciplines. The final scenario involved a crisis situation that required the surgical and anesthesia participants to perform resuscitation while wearing PPEs and PAPRs. The objectives of these simulations were to proactively seek latent threats and potential breaches in infection control, especially during vulnerable and stressful moments of crisis. We also wanted to evaluate the responsiveness of our activation workflow and adaptability of the isolation OR to cater to a variety of surgeries. These simulation exercises allowed us to identify and address unexpected

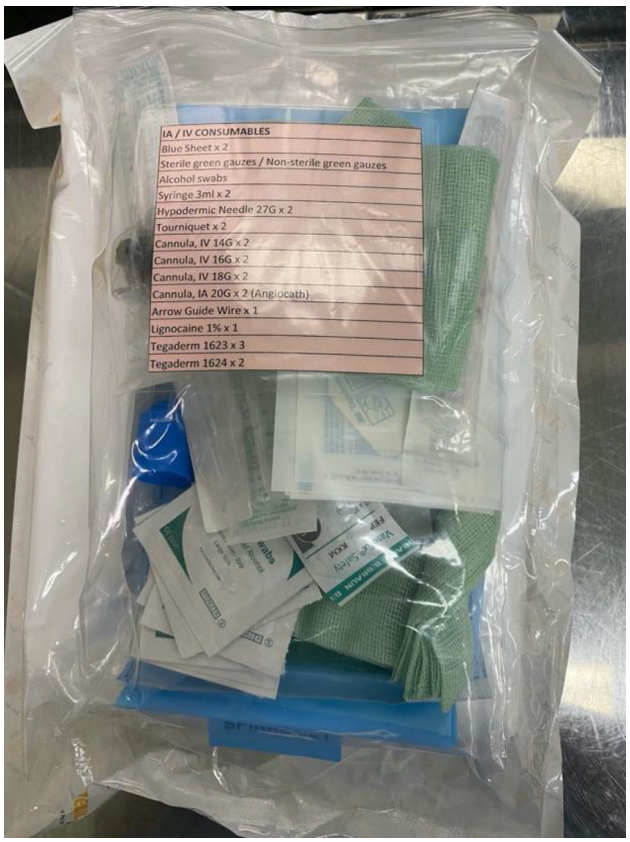


Fig. 4 Pre-packed sets of equipment

problems that were not apparent in the initial planning, such as lack of oversight and coordination, environment limitations, unsatisfactory equipment set-up, communication difficulties, lack of familiarity with protective equipment, infection control breaches, and inadequate support during crisis. These led to many downstream interventions, including appointing an OR coordinator to oversee this multi-step and multidisciplinary activation process to ensure that essential steps that may potentially compromise patient and staff safety were not missed. By rectifying deficiencies, we were able to effectively improve OR responsiveness and readiness by streamlining the ward transport process and refining the OR activation workflow. This was reflected in faster activation times with each *in situ* simulation.

Discussion

As part of disease containment measures, our anesthetic division implemented the above procedures in phases to decrease the risk of SARS-CoV2 transmission. In the initial phase, the emphasis was on identifying and isolating imported cases. Once local community transmission had been reported, surveillance was enhanced to identify cases, including cases with no previous travel to affected areas or previous contact with infected persons. At that stage, routine PPE was also enhanced in case of undiagnosed COVID-19. However, if there is widespread global transmission, containment will no longer be feasible and the goals will shift towards risk mitigation instead, to reduce the impact on society.[44,45]

Containment measures may be categorized according to the hierarchy of controls approach,[46] which has been used to effectively control exposure to occupational hazards (Fig. 5).

To control exposure to infectious diseases in the healthcare environment, options for hazard control, such as elimination and substitution controls, are often limited or not feasible. Nevertheless, transmission of respiratory pathogens (such as SARS-CoV2) can often be reduced through exposure control by means of engineering, administrative controls, and PPE.[5,28] Figure 6 shows the summary of the measures adopted.

Engineering controls refer to the placement of barriers between the hazard and healthcare personnel. Ideally, AIIR

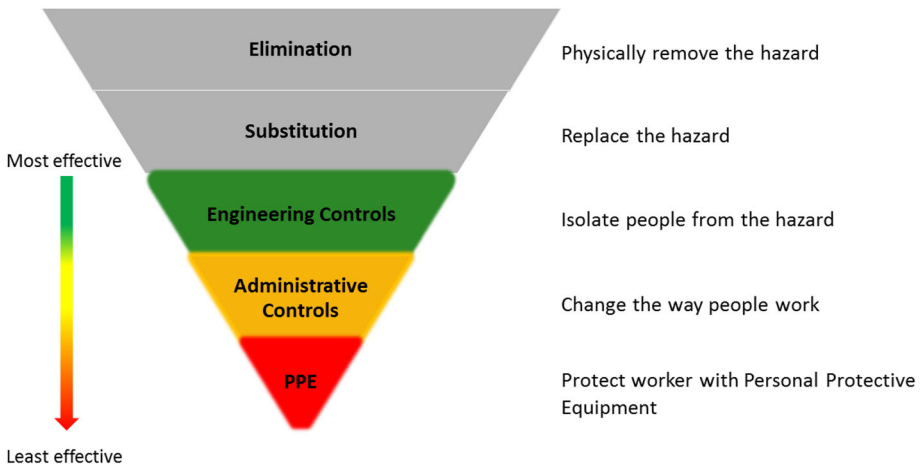


Fig. 5 Hierarchy of controls to control exposure to occupational hazards. The five layers can be implemented concurrently or sequentially. To control exposure to infectious diseases in the healthcare environment, elimination and substitution controls are often limited or not feasible. Effectiveness of control measures generally decreases down the layers



Fig. 6 Summary of the measures implemented in the operating room and anesthetic department to enhance infection prevention in a COVID-19 pandemic

should be used as ORs for surgical patients who had airborne infections.[28] Nevertheless, in our institution, all the ORs are positive pressure rooms and we did not have AIIR which could be used as ORs. Therefore, a separate OR complex was set aside for surgery involving COVID-19 patients. Disposable anesthetic and surgical equipment were used whenever possible to decrease the risk of virus exposure to OR sterile processing technicians. HEPA filters were used at the patient's end of the breathing circuit and also between the expiratory limb of the circuit and the anesthetic machine to decrease the risk of environmental contamination.

Administrative controls refer to policies mandated by healthcare institutions to reduce or prevent exposure to hazards. One of the first policies implemented was to discourage unnecessary travel to high-risk areas affected by COVID-19, and to impose a leave of absence upon return from these areas. It is likely that travel restrictions in China over the Chinese New Year holiday limited the spread of infections.[7,47] In the first few weeks of the outbreak, cases outside China emerged in Bangkok, Hong Kong, and Singapore—all cities with high volumes of air traffic from China.[48] COVID-19 infections diagnosed in six countries were traced back to one single conference.[49] At least one medical conference in Singapore was cancelled because of transmission risk.

Other administrative controls in the hospital environment include the screening and isolation of high risk patients. Location-based segregation of staff was implemented and large group meetings were suspended to reduce potential cross-infection among healthcare personnel. In a pandemic, should the numbers of COVID-19 patients increase dramatically, OR staff should be segregated into those caring for infected patients and those caring for other patients, to reduce the possibility of in-hospital transmission.[28] Anesthetic pre-assessment, induction, and postoperative recovery of each patient should be conducted in a single OR with restricted staff numbers.[28] *In situ* simulation was invaluable in helping us identify latent threats and gaps in workflow. It allowed members of the operating team to understand the challenges of operating in an unfamiliar environment with additional PPE, and allowed us to identify unexpected deficiencies that were not apparent in the initial planning stage. Together with other authors who have used simulation-based training to prepare for disease outbreaks,[50,51] we highly recommend it to refine processes, enhance communication, and build staff confidence.

Personal protective equipment is the final line of protection for healthcare workers especially in the context of community transmission, as patients without known contact history may also be carriers of the virus. In line with Occupational Safety and Health Administration Respiratory Protection Standards, all healthcare personnel were trained on the indications and proper use of PPE.

Twenty percent of anesthesiologists in our division failed fit-testing for N95 respirators (1860, 1860S, 1870+ by 3M^{TM} [3M, St Paul, MN, USA]), increasing the risk of them becoming infected should they be exposed.[52] Alternative respiratory protection should be made available to these staff, or they should be allocated duties away from high-risk areas. This has implications on anesthetic departments in particular, as our routine work involves aerosol-generating procedures. Staff who failed to be fitted for the N95 respirator may use the PAPR instead. In our department, they were fitted for a PAPR with a facepiece (CleanSpace^{R} HALO^{TM}). This offers better protection than a hooded PAPR with a loose fitting facepiece.[53] Sufficient PAPR devices should therefore be available to cater for different operational and staffing needs and advance consideration must be given to purchasing additional sets. Furthermore, eye protection is important because inoculation of the conjunctival surface may result in COVID-19 infection[5,54,55] Goggles were therefore issued to each member of staff.

There is risk of a shortage of N95 respirators during any pandemic, especially if it becomes prolonged. Measures taken to mitigate this included earlier mentioned engineering and administrative controls to decrease the number of healthcare personnel requiring respiratory protection. Alternatives such as PAPRs may be used and practices may be introduced to extend the use of each N95 respirator. The latter include extended use and limited re-use.[56] Extended use refers to the practice of wearing the same N95 respirator for repeated close contact encounters with several patients, without removing the respirator between patient encounters. Studies have shown that most healthcare personnel could tolerate wearing N95 respirators for up to eight to 12 hr.[57,58] Nevertheless, because most healthcare workers need to take breaks during shifts to eat, drink, and use the toilet, extended use of N95 beyond four hours is unlikely in most settings.[59] Re-use refers to the practice of using the same N95 respirator for multiple encounters with patients but removing it after each encounter. The Association for Professionals in Infection Control and Epidemiology

Table 1 Recommended practices for extending the use and/or re-using an N95 respirator

Avoid removing, adjusting, or touching the respirator (both outside and inside surfaces)
Discard the respirator if it becomes grossly contaminated or damaged or if breathing through it becomes difficult
Perform hand hygiene before and after handling/touching the respirator
Store the respirator in a clean, dry location to avoid contamination and maintain its integrity. It can be stored in a single-use breathable container, or hung in a designated area
Inspect the respirator and perform a seal check before each use

position paper, developed from various guidelines (including the United States Centers for Disease Control and Prevention, Food and Drug Administration, Occupational Safety and Health Administration, WHO and the Institute of Medicine), recommend extended use over re-use.[60]

For cases with highly suspected or confirmed COVID-19 infection, we adopted a single-use policy for these respirators because extended use and limited re-use of these respirators carries risks, such as droplet spray contamination, degradation of filtration efficacy and mask fit with time, cross-contamination during storage, and contamination during doffing and donning of the respirators.

Nevertheless, we adopted a re-use policy for N95 respirators for cases with a low suspicion of COVID-19 infection. The recommended practices for extending the use and/or reusing a respirator can be found in Table 1.[60] This includes performing hand hygiene before and after handling of the respirator; inspecting and performing a seal check before each use; avoiding the removal, adjustment, and contact with the inner and outer surfaces of the respirator; storing it in a clean dry location to avoid contamination and to maintain its integrity; and discarding the respirator if it is grossly contaminated or damaged or if breathing through it becomes difficult.

Training staff to use PPE competently is essential. In an observational study, 90% of staff did not use the correct doffing sequence or technique, or use the appropriate PPE.[61] According to a survey conducted by Amrita John *et al*., 11 of 74 (14.9%) physicians did not have prior training in the use of PPE.[62] Emphasis must be placed on proper doffing technique and staff must be reminded not to touch their eyes, face, or mucous membranes as these may lead to self-contamination. In our institution, trainers from the Infection Control and Occupational Medicine departments were initially overwhelmed by requests to conduct PAPR training. To mitigate this, we arranged for these trainers to teach and test a small group of anesthetic consultants on the correct use of PAPR. This group then went on to teach all 180 staff in the division.

Powered air-purifying respirators have a higher protective factor than N95 respirators,[63] but there is no conclusive evidence to show that PAPRs decrease the likelihood of viral transmission, especially airborne transmission.[36] The concurrent use of N95 respirators with PAPR to further decrease the risk of viral transmission may sound tempting, but there is no strong evidence of an added protective effect.[64] Disadvantages of such concurrent use include increased communication difficulties,[65] claustrophobic reactions,[66] and increased risk of infection from doffing the additional layers of PPE (Table 2).[67] It is more important to emphasize proper doffing and cleaning of PAPR for re-use to prevent contamination and cross-infection.

A recent review of 22 studies revealed that human coronaviruses such as SARS-CoV, Middle East Respiratory Syndrome (MERS) coronavirus, or endemic human coronaviruses can persist (i.e., maintain viral morphology or ability to infect cells) on inanimate surfaces such as metal, glass, or plastic for up to nine days. Nevertheless, these can be efficiently inactivated by surface disinfection procedures with 62–71% ethanol, 0.5% hydrogen peroxide or 0.1% sodium hypochlorite within one minute.[68] Other biocidal agents such as 0.05–0.2% benzalkonium chloride or 0.02% chlorhexidine digluconate

Table 2 Pros and cons of powered air-purifying respirator (PAPR)

Pros	Cons
Higher protective factor than N95 respirators	No definitive evidence that PAPR reduces likelihood of viral transmission for potential airborne infections
Provides eye protection (hooded models only)	Inability to auscultate for heart and lung sounds (for hooded PAPR)
More comfortable to wear than N95 respirator	Challenges in communication
Can be used if user has facial hair (not possible with N95 respirator)	Patient apprehension (especially among pediatric patients)
Hooded models do not require fit-testing (unlike N95 respirator)	Training on use, doffing, and care of PAPR is needed to prevent contamination
Eliminates unexpected poor N95 respirator fit	Requires decontamination after use
Less likely to be dislodged when managing an agitated patient	More expensive than N95 respirator
PAPRs with hood may provide additional protection against contamination compared with typical gear worn with N95 mask	Inability to re-use disposable filters between patients (need large supply of filters)
	Need to train staff repeatedly to maintain competency if not frequently used
	Risk of battery failure and inadvertent exposure

are less effective. The disinfection process at our institution involves cleaning of all medical devices with quaternary ammonium chloride disinfectant wipes and cleaning of the OR with sodium hypochlorite followed by hydrogen peroxide vaporization or ultraviolet-C irradiation.

The mechanical action of wiping removes organic debris. The disinfectant solution released by the wipe on the target surface is responsible for the bactericidal activity. Quaternary ammonium compounds have good cleaning and deodorization properties with a broad spectrum of biocidal and sporostatic activity, including against lipid-enveloped viruses such as coronaviruses.[69] Hypochlorite is affordable, has a rapid onset, and has a large bactericidal spectrum with no toxic residues.[69]

Hydrogen peroxide vaporization is effective against a range of bacteria and viruses within hospital settings,[70] including transmissible gastroenteritis coronavirus of pigs, a surrogate for the SARS-CoV.[71] Ultraviolet-C irradiation effectively inactivates aerosolized viruses, bacteria, and fungi.[72]

Conclusions

Preparation for a pandemic involves considering the different levels in the hierarchy of controls as well as the different phases of the pandemic. In the OR setting, these measures include the modification of infrastructure and processes, management of staff and patients, infection prevention strategies, and clinical recommendations. The OR is a complex environment with multiple stakeholders including anesthesiologists, surgeons, nurses, OR attendants, and technicians; it can be a challenge to align the interests and concerns of all parties. Nevertheless, we believe that these containment measures are necessary to optimize the quality of care provided to COVID-19 patients and to reduce the risk of viral transmission to other patients or healthcare workers.

Acknowledgements The authors would like to acknowledge the Division of Anaesthesiology, Singapore General Hospital.

Conflicts of interest None.

Funding statement None.

Editorial responsibility This submission was handled by Dr. Hilary P. Grocott, Editor-in-Chief, *Canadian Journal of Anesthesia.*

References

1. *World Health Organization.* Statement on the second meeting of the International Health Regulations (2005) Emergency Committee regarding the outbreak of novel coronavirus (2019-nCoV). Available from URL: https://www.who.int/news-room/detail/30-01-2020-statement-on-the-second-meeting-of-the-international-health-regulations-(2005)-emergency-committee-regarding-the-outbreak-of-novel-coronavirus-(2019-ncov) (accessed March 2020).
2. *Huang C*, *Wang Y*, *Li X*, *et al*. Clinical features of patients infected with 2019 novel coronavirus in Wuhan. China. Lancet 2020; 395: 497-506.
3. *Wang D*, *Hu B*, *Hu C*, *et al*. Clinical characteristics of 138 hospitalized patients with, novel coronavirus–infected pneumonia in Wuhan, China. JAMA 2019; DOI: https://doi.org/10.1001/jama.2020.1585.
4. *Chen N*, *Zhou M*, *Dong X*, *et al*. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 2020; 395: 507-13.
5. *Centers for Disease Control and Prevention*. Coronavirus Disease 2019 (COVID-19) Situation Summary. Available from URL: https://www.cdc.gov/coronavirus/2019-ncov/summary.html (accessed March 2020).
6. Operations Dashboard for ArcGIS. Available from URL: https://doc.arcgis.com/fr/operations-dashboard (accessed March 2020).
7. *Wu Z*, *McGoogan JM*. Characteristics of and important lessons from the coronavirus disease, (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. JAMA 2019; DOI: https://doi.org/10.1001/jama.2020.2648.
8. *World Health Organization*. Coronavirus disease 2019 (COVID-19) Situation Report - 43. 03 March 2020. Available from URL: https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200303-sitrep-43-covid-19.pdf?sfvrsn=2c21c09c_2 (accessed March 2020).
9. *Abdullah Z, Solamat H*. Singapore confirms first case of Wuhan virus. CNA. Available from URL: https://www.channelnewsasia.com/news/singapore/wuhan-virus-pneumonia-singapore-confirms-first-case-12312860 (accessed March 2020).
10. *Young BE*, *Ong SW*, *Kalimuddin S*, *et al*. Epidemiologic features and clinical course of patients infected with SARS-CoV-2 in Singapore. JAMA 2020; DOI: https://doi.org/10.1001/jama.2020.3204.
11. *Ministry of Health Singapore*. Confirmed cases of local transmission of novel coronavirus infection in Singapore. Available from URL: https://www.moh.gov.sg/news-highlights/details/confirmed-cases-of-local-transmission-of-novel-coronavirus-infection-in-singapore (accessed March 2020).
12. *Ministry of Health Singapore.* Risk assessment raised to DORSCON Orange. 2020. Available from URL: https://www.moh.gov.sg/news-highlights/details/risk-assessment-raised-to-dorscon-orange (accessed March 2020).
13. *Wong JE*, *Leo YS*, *Tan CC*. COVID-19 in Singapore-current experience: critical global issues that require attention and action. JAMA 2020; DOI: https://doi.org/10.1001/jama.2020.2467.
14. *World Health Organization*. Q&A on coronavirus (COVID-19). Available from URL: https://www.who.int/news-room/q-a-detail/q-a-coronaviruses (accessed March 2020).
15. *European Centre for Disease Prevention and Control.* COVID-19. Available from URL: https://www.ecdc.europa.eu/en/novel-coronavirus-china (accessed March 2020).
16. *Centers for Disease Control and Prevention.* Isolation Precautions. Available from URL: https://www.cdc.gov/infectioncontrol/guidelines/isolation/index.html (accessed March 2020).
17. *Poutanen SM*, *McGeer AJ*. Transmission and control of SARS. Curr Infect Dis Rep 2004; 6: 220-7.
18. *Yeo C*, *Kaushal S*, *Yeo D*. Enteric involvement of coronaviruses: is faecal-oral transmission of SARS-CoV-2 possible? Lancet

Gastroenterol Hepatol 2020; DOI: https://doi.org/10.1016/S2468-1253(20)30048-0.
19. *Centers for Disease Control and Prevention.* How COVID-19 spreads. Available from URL: https://www.cdc.gov/coronavirus/2019-ncov/about/transmission.html (accessed March 2020).
20. *Scales DC*, *Green K*, *Chan AK*, *et al*. Illness in intensive care staff after brief exposure to severe acute respiratory syndrome. Emerg Infect Dis 2003; 9: 1205-10.
21. *Loeb M*, *McGeer A*, *Henry B*, *et al*. SARS among critical care nurses. Toronto. Emerg Infect Dis 2004; 10: 251-5.
22. *Rothe C*, *Schunk M*, *Sothmann P*, *et al*. Transmission of 2019-nCoV infection from an asymptomatic contact in Germany. N Engl J Med 2020; DOI: https://doi.org/10.1056/NEJMc2001468.
23. *Riou J*, *Althaus CL*. Pattern of early human-to-human transmission of Wuhan, novel coronavirus (2019-nCoV), December 2019 to January 2020. Euro Surveill 2019; DOI: https://doi.org/10.2807/1560-7917.ES.2020.25.4.2000058.
24. *Li Q, Guan X, Wu P, W, et al.* Early Transmission dynamics in Wuhan, China, of novel coronavirus–infected pneumonia. N Engl J Med 2020; DOI: https://doi.org/10.1056/nejmoa2001316.
25. *Kamming D*, *Gardam M*, *Chung F*. Anaesthesia and SARS. Br J Anaesth 2003; 90: 715-8.
26. *Tan TK*. How severe acute respiratory syndrome (SARS) affected the department of anaesthesia at Singapore General Hospital. Anaesth Intensive Care 2004; 32: 394-400.
27. *Ter Chee VW*, *Khoo ML*, *Lee SF*, *Lai YC*, *Chin NM*. Infection control measures for operative procedures in severe acute respiratory syndrome–related patients. Anesthesiology 2004; 100: 1394-8.
28. *World Health Organization.* Infection prevention and control of epidemic- and pandemic-prone acute respiratory diseases in health care. WHO guidelines 2007.
29. *Ministry of Health Singapore.* Additional precautionary measures in response to escalating global situation. Available from URL: https://www.moh.gov.sg/news-highlights/details/additional-precautionary-measures-in-response-to-escalating-global-situation (accessed March 2020).
30. *Ti, LK, Ang LS, Foong TW, Ng BS*. What we do when a COVID-19 patient needs an operation: operating room preparation and guidance. Can J Anesth 2020; 67. DOI: https://doi.org/10.1007/s12630-020-01617-4.
31. *Peng PW*, *Wong DT*, *Bevan D*, *Gardam M*. Infection control and anesthesia: lessons learned from the Toronto SARS outbreak. Can J Anesth 2003; 50: 989-97.
32. *Christian MD*, *Loutfy M*, *McDonald LC*, *et al*. Possible SARS coronavirus transmission during cardiopulmonary resuscitation. Emerg Infect Dis 2004; 10: 287-93.
33. *Tompkins BM*, *Kerchberger JP*. Special article: personal protective equipment for care of pandemic influenza patients: a training workshop for the powered air purifying respirator. Anesth Analg 2010; 111: 933-45.
34. *Wilkes AR*, *Benbough JE*, *Speight SE*, *Harmer M*. The bacterial and viral filtration performance of breathing system filters. Anaesthesia 2000; 55: 458-65.
35. *Guan WJ*, *Ni ZY*, *Hu Y*, *et al*. Clinical characteristics of coronavirus disease, in China. N Engl J Med 2019; DOI: https://doi.org/10.1056/NEJMoa2002032.
36. *Wax RS*, *Christian MD*. Practical recommendations for critical care and anesthesiology teams caring for novel coronavirus (2019-nCoV) patients. Can J Anesth 2020; DOI: https://doi.org/10.1007/s12630-020-01591-x.
37. *Chen H*, *Guo J*, *Wang C*, *et al*. Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records. Lancet 2020; DOI: https://doi.org/10.1016/S0140-6736(20)30360-3.
38. *Favre G*, *Pomar L*, *Qi X*, *Nielsen-Saines K*, *Musso D*, *Baud D*. Guidelines for pregnant women with suspected SARS-CoV-2 infection. Lancet Infect Dis 2020; DOI: https://doi.org/10.1016/S1473-3099(20)30157-2.
39. *Centers for Disease Control and Prevention.* Coronavirus Disease 2019 (COVID-19). Interim considerations for infection prevention and control of coronavirus disease 2019 (COVID-19) in inpatient obstetric healthcare settings. Available from URL: https://www.cdc.gov/coronavirus/2019-ncov/hcp/inpatient-obstetric-healthcare-guidance.html (accessed March 2020).
40. *Kurup V*, *Matei V*, *Ray J*. Role of in-situ simulation for training in healthcare: opportunities and challenges. Curr Opin Anaesthesiol 2017; 30: 755-60.
41. *Patterson MD*, *Geis GL*, *Falcone RA*, *LeMaster T*, *Wears RL*. In situ simulation: detection of safety threats and teamwork training in a high risk emergency department. BMJ Qual Saf 2013; 22: 468-77.
42. *Mastoras G*, *Poulin C*, *Norman L*, *et al*. Stress testing the resuscitation room: latent threats to patient safety identified during interprofessional in situ simulation in a Canadian Academic emergency department. AEM Educ Train 2019; DOI: https://doi.org/10.1002/aet2.10422.
43. *Fent G*, *Blythe J*, *Farooq O*, *Purva M*. In situ simulation as a tool for patient safety: a systematic review identifying how it is used and its effectiveness. BMJ Simul Technol Enhanced Learn 2015; DOI: https://doi.org/10.1136/bmjstel-2015-000065.
44. *World Health Organization.* Pandemic influenza preparedness and response. A WHO guidance document - 2009. Available from URL: https://apps.who.int/iris/bitstream/handle/10665/44123/9789241547680_eng.pdf;jsessionid=1F82125A878C00D08B9AC204EB320742?sequence=1 (accessed March 2020).
45. *Ministry of Health Singapore*. MOH pandemic readiness and response plan for influenza and other acute respiratory diseases - April 2014. Available from URL: https://www.moh.gov.sg/docs/librariesprovider5/diseases-updates/interim-pandemic-plan-public-ver-_april-2014.pdf (accessed March 2020).
46. *Centers for Disease Control and Prevention*. Hierarchy of Controls - NIOSH Workplace Safety and Health Topics. Available from URL: https://www.cdc.gov/niosh/topics/hierarchy/default.html (accessed March 2020).
47. *Chen S*, *Yang J*, *Yang W*, *Wang C*, *Bärnighausen T*. COVID-19 control in China during mass population movements at New Year. Lancet 2020; DOI: https://doi.org/10.1016/S0140-6736(20)30421-9.
48. *Bogoch II*, *Watts A*, *Thomas-Bachli A*, *Huber C*, *Kraemer MU*, *Khan K*. Pneumonia of unknown aetiology in Wuhan, China: potential for international spread via commercial air travel. J Travel Med 2020; DOI: https://doi.org/10.1093/jtm/taaa008.
49. *Vaswani K*. Coronavirus: why Singapore is so vulnerable to coronavirus spread. BBC News, 13 Feb 2020. Available from URL: https://www.bbc.com/news/world-asia-51480613 (accessed March 2020).
50. *Abualenain JT*, *Al-Alawi MM*. Simulation-based training in Ebola personal protective equipment for healthcare workers: experience from King Abdulaziz University Hospital in Saudi Arabia. J Infect Public Health 2018; 11: 796-800.
51. *National Academies of Sciences, Engineering, and Medicine*. The Ebola Epidemic in West Africa: Proceedings of a Workshop. DC: The National Academies Press; 2016.
52. *Lee K*, *Slavcev A*, *Nicas M*. Respiratory protection against Mycobacterium tuberculosis: quantitative fit test outcomes for five type N95 filtering-facepiece respirators. J Occup Environ Hyg 2004; 1: 22-8.
53. *Occupational Safety and Health Administration; US Department of Labor.* Assigned protection factors for the revised respiratory protection standard - 2009. Available from URL: https://www.osha.gov/Publications/3352-APF-respirators.pdf (accessed March 2020).

54. *Chang D*, *Xu H*, *Rebaza A*, *Sharma L*, *Dela Cruz CS*. Protecting health-care workers from subclinical coronavirus infection. Lancet Respir Med 2020; DOI: https://doi.org/10.1016/S2213-2600(20)30066-7.
55. *Li JP*, *Lam DS*, *Chen Y*, *Ting DS*. Novel coronavirus disease 2019 (COVID-19): the importance of recognising possible early ocular manifestation and using protective eyewear. Br J Ophthalmol 2020; 104: 297-8.
56. *Centers for Disease Control and Prevention.* Recommended guidance for extended use and limited reuse of n95 filtering facepiece respirators in healthcare settings - NIOSH Workplace Safety and Health Topic. Available from URL: https://www.cdc.gov/niosh/topics/hcwcontrols/recommendedguidanceextuse.html (accessed March 2020).
57. *Radonovich LJ Jr*, *Cheng J*, *Shenal BV*, *Hodgson M*, *Bender BS*. Respirator tolerance in health care workers. JAMA 2009; 301: 36-8.
58. *Rebmann T*, *Carrico R*, *Wang J*. Physiologic and other effects and compliance with long-term respirator use among medical intensive care unit nurses. Am J Infect Control 2013; 41: 1218-23.
59. *Fisher EM*, *Shaffer RE*. Considerations for recommending extended use and limited reuse of filtering facepiece respirators in health care settings. J Occup Environ Hyg 2014; 11: D115-28.
60. *Rebmann T*, *Alexander S*, *Bartley J*, *et al*. APIC position paper: extending the use and/or reusing respiratory protection in healthcare settings during disasters. Washington, DC: APIC; 2009 .
61. *Phan LT*, *Maita D*, *Mortiz DC*, *et al*. Personal protective equipment doffing practices of healthcare workers. J Occup Environ Hyg 2019; 16: 575-81.
62. *John A*, *Tomas ME*, *Cadnum JL*, *et al*. Are health care personnel trained in correct use of personal protective equipment? Am J Infect Control 2016; 44: 840-2.
63. *Board on Health Sciences Policy*. Institute of Medicine. Defining PAPRs and Current Standards. DC: National Academies Press (US); 2015 .
64. *Roberge RJ*. Evaluation of the rationale for concurrent use of N95 filtering facepiece respirators with loose-fitting powered air-purifying respirators during aerosol-generating medical procedures. Am J Infect Control 2008; 36: 135-41.
65. *Coates MJ*, *Jundi AS*, *James MR*. Chemical protective clothing; a study into the ability of staff to perform lifesaving procedures. J Accid Emerg Med 2000; 17: 115-8.
66. *Laird IS*, *Goldsmith R*, *Pack RJ*, *Vitalis A*. The effect on heart rate and facial skin temperature of wearing respiratory protection at work. Ann Occup Hyg 2002; 46: 143-8.
67. *Nicolle L*. SARS safety and science/La science et les mesures de sécurité contre le SRAS. Can J Anesth 2003; DOI: https://doi.org/10.1007/BF03018360.
68. *Kampf G*, *Todt D*, *Pfaender S*, *Steinmann E*. Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents. J Hosp Infect 2020; DOI: https://doi.org/10.1016/j.jhin.2020.01.022.
69. *Song X*, *Vossebein L*, *Zille A*. Efficacy of disinfectant-impregnated wipes used for surface disinfection in hospitals: a review. Antimicrob Resist Infect Control 2019; DOI: https://doi.org/10.1186/s13756-019-0595-2.
70. *Pottage T*, *Richardson C*, *Parks S*, *Walker JT*, *Bennett AM*. Evaluation of hydrogen peroxide gaseous disinfection systems to decontaminate viruses. J Hosp Infect 2010; 74: 55-61.
71. *Goyal SM*, *Chander Y*, *Yezli S*, *Otter JA*. Evaluating the virucidal efficacy of hydrogen peroxide vapour. J Hosp Infect 2014; 86: 255-9.
72. *Kim DK*, *Kang DH*. UVC LED irradiation effectively inactivates aerosolized viruses, bacteria, and fungi in a chamber-type air disinfection system. Appl Environ Microbiol 2018; DOI: https://doi.org/10.1128/AEM.00944-18.

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FUNCTION american physiological society

FUNCTION, 2020, 1(1): zqaa002

doi: 10.1093/function/zqaa002
Advance Access Publication Date: 14 May 2020
Evidence Review

EVIDENCE REVIEW

Potential Role of Oral Rinses Targeting the Viral Lipid Envelope in SARS-CoV-2 Infection

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Abstract

Emerging studies increasingly demonstrate the importance of the throat and salivary glands as sites of virus replication and transmission in early COVID-19 disease. SARS-CoV-2 is an enveloped virus, characterized by an outer lipid membrane derived from the host cell from which it buds. While it is highly sensitive to agents that disrupt lipid biomembranes, there has been no discussion about the potential role of oral rinsing in preventing transmission. Here, we review known mechanisms of viral lipid membrane disruption by widely available dental mouthwash components that include ethanol, chlorhexidine, cetylpyridinium chloride, hydrogen peroxide, and povidone-iodine. We also assess existing formulations for their potential ability to disrupt the SARS-CoV-2 lipid envelope, based on their concentrations of these agents, and conclude that several deserve clinical evaluation. We highlight that already published research on other enveloped viruses, including coronaviruses, directly supports the idea that oral rinsing should be considered as a potential way to reduce transmission of SARS-CoV-2. Research to test this could include evaluating existing or specifically tailored new formulations in well-designed viral inactivation assays, then in clinical trials. Population-based interventions could be undertaken with available mouthwashes, with active monitoring of outcome to determine efficacy. This is an under-researched area of major clinical need.

Key words: coronavirus; lipid; envelope; oropharynx; virus; respiratory

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The Viral Lipid Envelope

In common with many viruses, such as influenza and herpes simplex, coronaviruses are surrounded by a fatty layer, called a "lipid envelope," into which the spike glycoproteins required for infection are inserted (Figure 1). Viral envelopes are acquired at host cell membranes—some at the plasma membrane, others at internal cell membranes such as the nuclear membrane,

Submitted: 1 May 2020; **Revised:** 4 May 2020; **Accepted:** 8 May 2020

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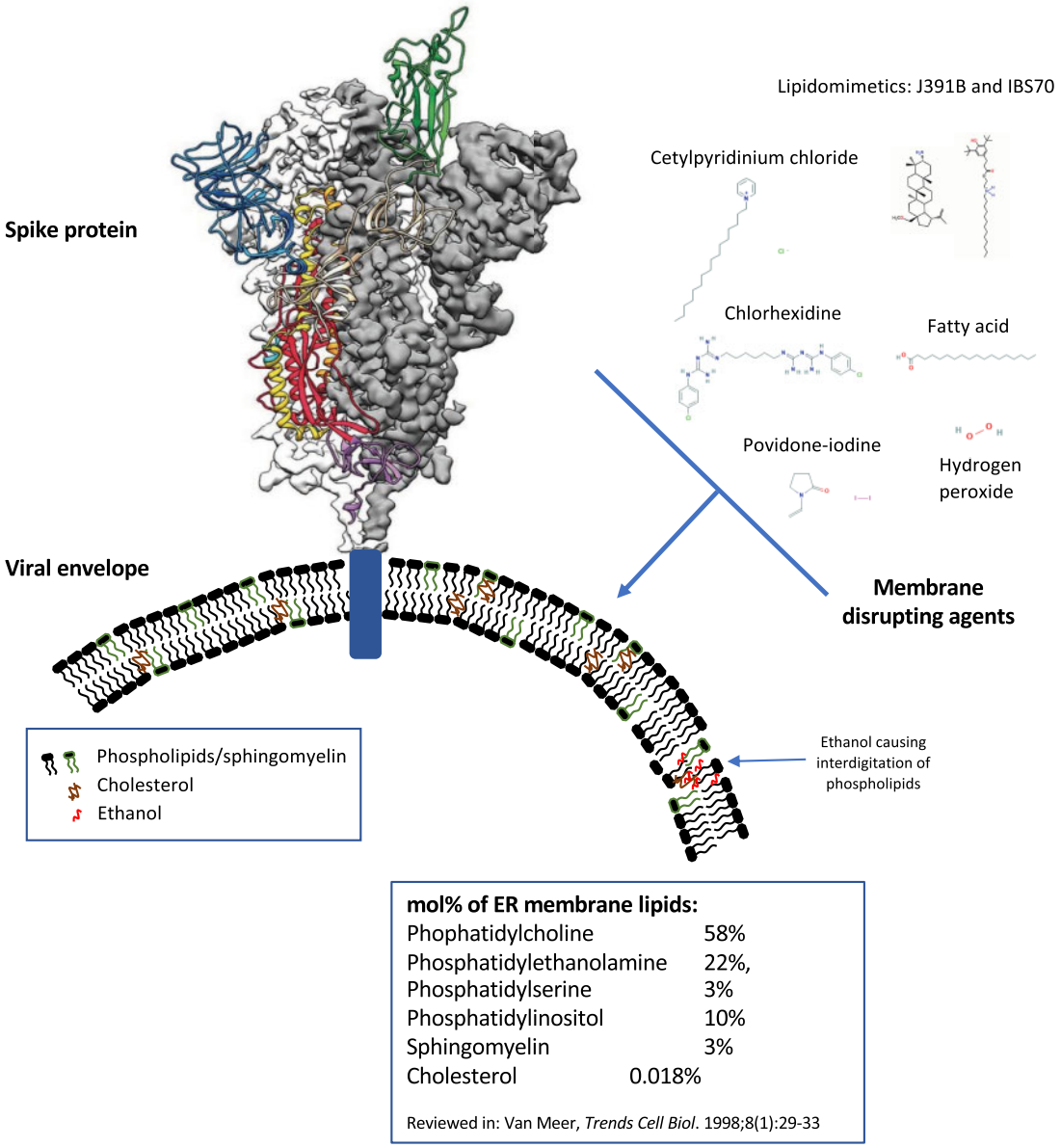


Figure 1. Cartoon Representation of the SARS-CoV-2 Glycoprotein, Embedded in the Viral Envelope, along with Membrane Disrupting Agents. Ribbon diagram was obtained from Wrapp et al.,[1] chemical structures were from PubChem (https://pubchem.ncbi.nlm.nih.gov/) and Nieto-Garai et al.[2]

endoplasmic reticulum, and Golgi complex.[3,4] During this, viral proteins are incorporated at the expense of host cell proteins, creating the shed viral particle.[5] Thus, for most viruses, the envelope lipids are considered to be the same as the host membranes (phospholipids, sphingolipids, and some cholesterol). Lipid composition is not the same across subcellular membranes with mammalian plasma membranes having higher cholesterol and sphingolipid content.[6–12] While the lipid makeup of the envelope of SARS-CoV-2 has not been characterized yet, coronaviruses are known to bud from the endoplasmic reticulum Golgi intermediate compartment (ERGIC), before being transported by exocytosis in cargo vesicles.[13,14] This indicates their composition will be related to endoplasmic reticulum membrane, which contains more phosphatidylcholine, but less cholesterol and sphingolipids than the plasma membrane.[6–12] A recent report demonstrated that coronavirus (HCoV-229E) regulates host lipid metabolism in response to infection, in common with many other viruses.[15–17] However, no information on the virus lipid envelope composition was provided, and its specific composition has not been determined experimentally.

The Soap/Alcohol Virucidal Public Health Advice Relating to Surface Neutralization

It is widely known that interfering with the lipid envelope represents a virucidal strategy to target many coronaviruses, with a large body of work evidencing the impact of many agents.[18,19] For a summary, refer to Kampf et al., a systematic review providing tables showing data from different original publications for inactivation of coronaviruses by biocidal agents in suspension tests.[19] During the 2003 SARS-CoV outbreak, viral material

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was detected on hospital surfaces, leading to the idea that surface decontamination would be an important approach. At that time, various compounds were considered, including ethanol at high concentrations of 60%–70% (v/v), since these doses had been found to be highly effective against several viral pathogens, including coronaviruses.[19–21] Recent studies on SARS-CoV-2 also support this, with high concentrations being highly effective as shown in a recent preprint.[22] The historical reason for only testing high concentrations in microbicidal research has been that these give broad-spectrum activity toward bacteria, viruses, and fungi, and thus for use on inanimate surfaces/fomites where the target microbes are unknown would be always preferred. The consensus view is that enveloped viruses, such as herpesviruses, orthomyxoviruses, paramyxoviruses, and coronaviruses are highly sensitive to 60%–70% (v/v) ethanol with almost immediate inactivation, while nonenveloped viruses are less or not susceptible.[19–22]

We are now widely encouraged to use soap or 60%–70% alcohol-based gels to inactivate SARS-CoV-2, based on the view that these agents damage the lipid envelope, including in recent World Health Organisation (WHO) and Environmental Protection Agency recommendations (https://apps.who.int/iris/bitstream/handle/10665/331138/WHO-WPE-GIH-2020.1-eng.pdf; https://www.epa.gov/pesticide-registration/list-n-disinfectants-use-against-sars-cov-2; https://www.who.int/gpsc/tools/GPSC-HandRub-Wash.pdf) . At the same time, there has been no discussion of oral antiviral strategies, apart from a recent response to an article in the *British Medical Journal* calling for protection for healthcare workers against infection (https://www.bmj.com/content/369/bmj.m1324/rr-5). Properly designed clinical trials that address this issue are currently lacking in the literature. Current WHO interim guidance on clinical management of SARS-CoV-2 in the home is focused on the use of personal protection, including face masks, along with hand, clothing, and surface sanitation, to reduce risk of airborne and direct spread of the virus, but does not mention oral hygiene (https://apps.who.int/iris/handle/10665/331133). Thus, its utility in the setting of SARS-CoV-2 has not been considered systematically, and there is a lack of either positive or negative robust clinical evidence.

Mouthwashes vary widely in composition; however, some commercially available formulations contain ethanol at 14%–27% (w/v) in the United Kingdom, Europe, and the United States. Prompted by this, we reviewed the available scientific literature to establish whether oral treatment using ethanol-based or other types of mouthwashes could present a strategy to either dampen or reduce viral load, to potentially restrict virus transmission in the current pandemic situation, particularly for vulnerable individuals or healthcare workers. We found that there is a paucity of data systematically testing the impact of lower (less toxic) ethanol concentrations on enveloped virus inactivation, with most simply reiterating the use of the higher concentrations described above.[18,20,21,23–26] We also found a paucity of robust clinical studies in this area that address in a randomized double-blind manner the impact of oral rinsing on objective measures, specifically neutralization of enveloped viruses, including coronaviruses.

Viral Load, Saliva/Throat Virus, and Disease Severity in SARS-CoV-2

It is becoming increasingly recognized that the throat is a major site of replication and shedding of virus in COVID-19 illness, and that viral load is important.[27] Throat and sputum are abundant in particles, which peak 5–6 days after symptom onset, and decline thereafter.[28,29] Viral load correlates with older age,[29] and a study of 76 patients in Nanchang, China, showed that those with severe SARS-CoV-2 tend to have higher viral load and longer virus-shedding period than those with mild disease.[30] Similarly, viral load was linked with lung disease severity in a study of 12 patients with pneumonia.[31] Many asymptomatic individuals have modest levels of detectable viral RNA in their oropharynx for at least 5 days, which is similar to individuals with clinical symptoms.[32] Data from GTEX gene expression data indicate that angiotensin converting enzyme (ACE2) (a key receptor for COVID-19) expression is higher in salivary glands than lungs, suggesting that these could be a major source of new viral particles.[33] A recent study using mobility data and Bayesian inference inferred that a high rate of undocumented infections is responsible for rapid spread of SARS-CoV-2.[34] Taken together, these data suggest that the potential for transmission is high early in the disease. While further studies are needed to better understand the relationship between viral load and symptom severity, it is expected that higher levels of viral shedding in the throat or lungs might be associated with an increased ability to infect others. To date, the relationship between lung and throat viral load in terms of disease severity, is not clear, and how dampening throat virus load may impact on resulting lung disease or viral transmission is not known.

The route of SARS-CoV-2 infection is currently considered to be via respiratory droplets, similar to SARS-CoV,[35] and the virus particle is viable in aerosols for up to 3 h.[36] Although we do not yet know the minimal infectious dose, the high rate of transmission indicates this is likely to be relatively low. If correct, then strategies to reduce the number of infective virus particles in mucous membranes through promoting their removal or inactivation could contribute to reducing risk of transmission. Thus, assuming that the throat is a major site of replication in early stages (even before symptoms are apparent), the oral washing using agents that could damage or destroy the lipid envelope has the potential to reduce viral load in the oropharynx.

At this time, there is incomplete information on how SARS-CoV-2 moves from the throat and nose to the lungs, and this could include (1) viral shedding, (2) the aspiration of necrotic cell debris, or (3) direct infection of neighboring cells. Assuming viral shedding is involved, the oral rinses that target the viral lipid envelope represent a potential method to remove/rinse or inactivate infective particles generated in the throat. The specific intracellular replication cycle for SARS-CoV-2 in humans is not yet known. Based on nonsynchronized replication cycles that take <24 h, virus is likely to be secreted almost constantly.[37,38] Oral agents will impact only on virus that is extracellular or actively budding. Therefore, the persistence of treatment will be important. How long mouthwash components retain an ability to interact with biomembranes in the mouth is unclear, and more research is required.

The Impact of Lower Ethanol Concentration on Biomembranes

When considering lower (nontoxic, more economical) ethanol concentrations, the literature on mammalian cells (from where the lipid envelope originates) provides a close comparator. We also reviewed studies on model membrane vesicles comprising phospholipids such as phosphatidylcholine; however, as these are protein-free, the impact of nonlipid components on ethanol

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toxicity is not accounted for. Bacterial pathogens contain very different membranes in lipid and protein composition, including lipopolysaccharides and peptidoglycans, so they are not considered here. Below we summarize the literature on impact of ethanol on cell/model membranes (Table 1).

Low Concentrations of Ethanol Cause Swelling, Interdigitation, and Leakage in Model Membranes

Biophysical studies in the 1980s and 1990s compared various alcohols (ethanol, methanol, butanol, and propanol) for their ability to perturb model phospholipid membranes. Many were optimizing generation of lipid vesicles for drug delivery; however, the toxicity of most short-chain alcohols prohibits oral use. Here, we reviewed reports on the properties of ethanol on model membranes. Few studies directly investigated lysis, instead focusing on membrane fluidity, permeability, interdigitation, thickness, and other parameters. In one study, ethanol >3.4 M (20% v/v) resulted in membranes not being considered "stable".[39] Ethanol addition causes rapid swelling of phosphatidylcholine vesicles from around 30 nm diameter at 0 M, up to between 80 and 110 nm diameter, at 1.1–1.5 M (6.5%–8.8% v/v).[55] *Interdigitation* refers to the process whereby the presence of short-chain alcohols enables the methyl group of the fatty acyl chains to move beyond the midplane of the bilayer, penetrating the opposite monolayer, and appears to be an event that precedes and promotes vesicle fusion and leakage.[56,57] Several studies demonstrate that ethanol promotes interdigitation.[58] In one, ethanol at above 2 M (11.8% v/v) led to formation of *interdigitated* phospholipid sheets from small unilamellar vesicles (SUVs), which then annealed to form larger interdigitation-fusion vesicles (IFVs).[40] This means that ethanol at this concentration can deform small phospholipid vesicles leading to fusion and formation of larger structures. During this process, leakage of contents from vesicles is seen.[40,42,55,59] Three studies compared membranes consisting of either phosphatidylcholine alone, phosphatidylcholine/phosphatidylethanolamine mixtures, or phosphatidylcholine/cholesterol mixtures, and showed that all became permeable at ethanol concentrations around 0.6–2.1 M (3.5%–12.3%, v/v).[43,44,55] Elsewhere, ethanol at rather lower concentrations of 86 mM (0.5% v/v) caused lysis of phosphatidylcholine vesicles during repeated cycling through phase transition temperatures.[41] Partitioning of ethanol into the membrane can be altered through the presence of additional biologically relevant lipid species such as cholesterol or gangliosides.[43,60,61] This indicates that complex biological membranes may respond very differently, and not only the presence of other lipid types, but also the impact of proteins need to be taken into account. Nonetheless, it is clear that model membranes are sensitive to ethanol at concentrations far lower than the 60%–70% currently recommended for inactivation of virus on hard surfaces, and at amounts contained in widely available mouthwashes (Section 6).

Impact of Ethanol on Mammalian Cell Membranes

We also reviewed the impact of ethanol on mammalian cells in vitro. Of direct relevance to coronaviruses, a study on corneal epithelial cells showed that a 30-s incubation with 20% ethanol led to around 40% loss of viability, which increased to 70% loss at 40% ethanol. There was significant leakage of intracellular contents following 20% ethanol for 30 s.[48] This short incubation also altered inflammatory responses, differentiation, and epithelial marker expression.[48] Several studies on the impact of ethanol on red blood cells were also found. Sonmez et al. showed that around 1 M (5.9% v/v) causes ~10% cell lysis, but higher amounts were not tested.[49] A variety of effects on red cells have been shown including potassium leakage and hemolysis, at moderate concentrations around 3–4 M (18%–23.5% v/v). However, incubation times of 15 min or greater were generally used.[50–52] Last, a study on an intestinal cell line (Caco-2) showed that ethanol >5%–10% causes loss of viability, leakage of contents, and disruption of tight junctions, with a long incubation time of 60 min.[53] Since the membrane composition of coronaviruses is expected to match ERGIC (Section 1), these studies provide strong evidence that low ethanol will directly impact on the SARS-CoV-2 membrane also.

So far, we found only studies that tested the impact of reduced ethanol amounts on enveloped viruses. Both were conducted in vitro, and show positive outcomes in relation to virus denaturation.

- In 2007, Roberts and Lloyd found that 20% ethanol completely inactivated three enveloped viruses: Sindbis, herpes simplex-1, and vaccinia, in vitro, while having no effect on the nonenveloped poliovirus-1.[46] Inactivation was measured by inhibition of plaque-forming units in a viral infectivity assay, but direct impact on viral envelope was not determined. This study used a rather basic system, in the absence of a soil load, which is nowadays recommended under the American Society for Testing and Materials (ASTM) Committee E35 on Pesticides, Antimicrobials and Alternative Control Agents (https://www.astm.org/Standards/E2197.htm), or in the United Kingdom, the equivalent British Standard (BS) Norme Européenne (EN) standard (BS EN 14476:2013+A2:2019 Chemical disinfectants and antiseptics. Quantitative suspension test for the evaluation of virucidal activity in the medical area. Test method and requirements (Phase 2/Step 1), https://shop.bsigroup.com/ProductDetail?pid=000000000030401479). Also, it was conducted at 22°C, rather than the more relevant 36.8°C oral temperature, where the impact of denaturation agents would be greater.
- In 2017, Siddharta et al. tested WHO recommended formulations against enveloped viruses, including coronavirus. Focusing on WHO formulation I, which contains 85% (v/v) ethanol, 0.725% (v/v) glycerol, and 0.125% (v/v) hydrogen peroxide, they measured in vitro infectivity in the presence of a soil load (0.5% w/v bovine serum albumin). A 30-s exposure of a dilution containing 34% (v/v) ethanol (40% of neat) completely prevented subsequent viral replication.[47]

These studies indicate that relatively dilute ethanol will be highly effective against enveloped viruses. However, there is an urgent need to determine how coronaviruses are impacted by dilute alcohol under biologically relevant conditions (mucosa, mouth, etc.), and whether in combination with nontoxic, membrane disrupting agents, oral inactivation of SARS-CoV-2 could be achieved. A minimum amount of ethanol, for example, 10%–30% (v/v) would be effective, and contact time will also be an important parameter that may reduce ethanol exposure required. Ethanol impacts membrane properties of artificial lipid membranes, causing leakage of contents even in the absence of complete lysis. The ability of the virus to infect host cells could also be modified by inducing biophysical changes to the virus membrane which impact on protein function. The spike glycoprotein which is required for SARS-CoV-2 infectivity contains a transmembrane domain that is inserted into the viral envelope,[1] and

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Table 1. In vitro and in vivo data supporting the effects of ethanol on biomembranes or enveloped viruses

Reference	Study type	Ethanol	Results
Ly and Longo[39]	Model membrane vesicles (membrane fluidity, permeability, interdigitation, thickness, etc.)	Ethanol >3.4 M (20% v:v)	Membranes not considered "stable"; interdigitation; rapid swelling of PC vesicles
Ahl et al.[40]	Formation of *interdigitated* PL sheets from SUV	Ethanol above 2 M (11.8% v:v)	Formed larger IFVs; leakage of contents of vesicles
Hunt et al.[41]	Repeated cycling through transition phase of model membranes	Ethanol 86 mM (0.5% v:v)	Lysis of PC vesicles
Komatsu et al.[42–44]	Leakage of dye from vesicles made of PC, PE/PC, or PC/cholesterol.	0.6–2.1 M (3.5%–12.3%, v/v)	Calcein leaks out at low ethanol concentrations. Rapid swelling of vesicles.
Dennison et al.[45]	In vitro—Herpes, influenza, rotavirus, and adenovirus	26.9% ethanol (v:v) with essential oils	Enveloped viruses (herpes and influenza) were significantly impacted
IADR abstract 2010	H1N1 Influenza A pandemic strain, in vitro	21.6% ethanol, 30-s rinse	>99.99% reduction in infectivity
Roberts and Lloyd[46]	Three enveloped viruses: Sindbis, herpes simplex-1 and vaccinia, in vitro	20% (v:v) ethanol	Completely inactivated
Siddharta et al.[47]	Enveloped viruses; in vitro infectivity WHO formulation I in the presence of coronavirus.	30-s exposure of a dilution containing 34% (v:v) ethanol	Completely prevented subsequent viral replication
Oh et al.[48]	Mammalian cell membranes: Corneal epithelial cells	20% ethanol; 30-s incubation	40% loss of viability; high level of leakage of intracellular contents
Sonmez et al.[49]	Mammalian cell membranes: Red blood cells	1M (5.9% v:v) ethanol	Approximately 10% cell lysis
Chi and Wu[50] and Tyulina et al.[51,52]	Mammalian cell membranes: Red blood cells	Moderate concentrations around 3–4M (18%–23.5%).	Potassium leakage and hemolysis
Wang et al.[53]	Mammalian cell membranes: Intestinal cell line (Caco-2)	Ethanol >5%–10%: long incubation time of 60 min	Loss of viability, leakage of contents, and disruption of tight junctions
Meiller et al.[54]	In vivo human study	21.6% ethanol, 30-s rinse	Recoverable virions of herpes simplex types I and II to 0 post rinse; at 30 min all lower than prerinse, 11/20 remained 0
Meiller et al.[54]	In vivo human repeat study	21.6% ethanol, 30-s rinse	0 recoverable virions in 18/20 post rinse and 12/20 at 30 min; at 60 min all less than baseline
Sattar et al. (unpublished data)	Finger pads of adults; Dried inocula; human respiratory coronavirus 229E	Hand gels with 60% and 70% ethanol exposed for 20 s	Viability titer of the virus was reduced by >99.99% in both cases

Studies cited in our text are summarized above for type, ethanol amount, and outcome. They are listed in order of model membranes, followed by in vitro studies on viruses, studies on mammalian cell membranes, then in vivo studies. Ethanol concentrations were listed also, in some cases, whether v/v or w/v was used was not provided in the study. In all studies, refer to the primary literature for full information on the impact of ethanol on the membrane. PC, Phosphatidylcholine, PE, phosphatidylethanolamine.

it is well known that lipid membrane biophysical perturbations can impact on conformation and function of many transmembrane proteins in mammalian cells. In this regard, the lipid membrane of HIV-1 was recently demonstrated to stabilize viral membrane glycoproteins and regulates their sensitivity to neutralization by antibodies.[62] Thus, lower concentrations of ethanol could alter pathogenicity without complete neutralization of viral particles. Research is required to determine the impact of ethanol or other agents on the infective activity of the SARS-CoV-2 spike protein in vivo.

Membrane Perturbation Without Lysis can Dampen Enveloped Virus Infectivity

The concept that perturbing the membrane could inactivate viruses has recently been tested in relation to membrane-disrupting agents, and an in vitro screen of 1000 compounds identified a series of lipidomimetics that can alter the membrane and dampen infectivity of HIV-1.[2] Active agents included lipids related to cholesterol, sphingosine, or aliphatic lipids with long-chain fatty acids which blocked at the stage of entry into the host cell. The impact appeared to result from the lipids being incorporated into the membrane and inducing changes in lipid order and buoyant density of the particles. In support of this, a study in the 1970s showed that fatty acids and monoglycerides of 16–18 carbon chain length are highly effective in vitro, reducing survival of herpes simplex virus to around 50% at concentrations down to 0.2 μM.[63] These studies use compounds that are nontoxic to mammalian cells and show great promise, thus research is needed to determine whether they are also active against the envelope of SARS-CoV-2 both in vitro and in vivo.

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Mouthwash Preparations that Show Activity Against Enveloped Viruses in Published Studies

We investigated the potential for commercially available mouthwashes to disrupt viral lipid envelopes, either due to ethanol (Table 1), or other active agents, through reviewing available literature.

Three industry-sponsored studies from the Universities of Maryland, Texas–Houston Health Sciences Centre, and State University New York tested this using a widely available formulation that combines 21%–26% ethanol with essential oils (eucalyptol 0.092%, menthol 0.042%, methyl salicylate 0.060%, and thymol 0.064% w/v). Notably, there is published evidence that eucalyptus oil and thymol have significant antiviral properties toward herpes simplex virus at these concentrations, hypothesized to relate to disruption of the viral lipid envelope.[64]

- The virucidal actions of 21% (v/v) ethanol with essential oils toward an enveloped virus were reported in humans in vivo in 2005. A 30-s rinse reduced infectious virions of herpes simplex types I and II to effectively zero.[54] Specifically, 18/20 people demonstrated no virions postrinse, and after a 30-min rinse, virions remained at zero for 11/20 subjects, with all subjects remaining lower than prerinse levels. In contrast, rinsing with distilled water reduced mean virions considerably less post 30-s rinse, and levels had largely returned to baseline by 30 min. This indicates that the mouthwash had a specific and significant impact on virion recovery. In a repeat trial, 18/20 subjects had zero virions post 30-s rinse, with 12/20 remaining at zero at 30 min. At 60 min, all 20 were still shedding virions at 1–2 $\log_{10}$ lower than baseline, demonstrating a modest impact on viral titer. Longer contact times (eg, 60-s rinses) were not tested.[54] Herpesviruses differ from coronaviruses in that the former can erupt periodically from where they reside in the nerves; so, using mouthwash may temporarily reduce the level and it may then help promote resolution of the lesion. On the contrary, coronaviruses will be shed almost constantly when actively replicating.
- A study in 1995 tested 26.9% ethanol with essential oils against herpes, influenza, rotavirus, and adenovirus in vitro. Here, an impact on the viral lipid envelope was speculated since herpes and influenza were significantly impacted, while adenovirus and rotavirus (nonenveloped) were not.[45]
- A follow-up unpublished study in 2010 determined that a 30-s in vitro exposure to 21.6% ethanol with essential oils led to >99.99% reduction of infectivity of H1N1 Influenza A pandemic strain (https://iadr.abstractarchives.com/abstract/2010dc-131191/evaluation-of-h1n1-antiviral-properties-of-an-essential-oil-containing-mouthrinse).

These studies provide proof-of-concept that mouthwashes containing essential oils with 21%–27% ethanol can inactivate enveloped viruses, both in the lab and in humans, with the likely mechanism being damage to the lipid envelope. Here, ethanol in combination with essential oils may provide a more effective formulation. Thus, these types of mouthwash may be effective against SARS-CoV-2, although studies have not been conducted. While other commercially available ethanol mouthwashes generally contain lower levels without essential oils, an impact on membrane biology may remain theoretically possible, and studies are required.

Chlorhexidine

Chlorhexidine is widely used for oral health in the United Kingdom, being especially effective against Gram-positive bacteria, but to a lesser extent Gram-negative bacteria and fungi.[65] Due to its positive charge, it reacts with the negatively charged microbial surface, penetrating into the cell and causing leakage. A report on its in vitro viricidal effectiveness at 0.12% has indicated it can reduce the viral concentration of enveloped but not nonenveloped viruses.[66] However, this limited in vitro study only considered the immediate postexposure, and no further time points were included in the experimental design. Chlorhexidine is often formulated with ethanol at lower concentrations, which may in part explain its virucidal impact. A recent review of coronavirus literature identified that chlorhexidine exposure for 10 min only weakly inactivated coronavirus strains in suspension tests although the concentration used was low at 0.02%.[19,24] Chlorhexidine formulations have been shown to retain oral antimicrobial activity for up to 12 h.[67] It is a more effective antimicrobial in vivo because it binds to clean oral surfaces and is released over time (substantivity).[67] Despite lower activity toward coronaviruses, a combination of chlorhexidine with alcohol may offer a useful strategy for reducing viral load over longer times.

Chlorhexidine mouthwashes have been a critical clinical tool for over 40 years to reduce oral bacterial flora and prevent infection and mucositis in cancer patients receiving chemotherapy and radiotherapy.[68–70] However, there are no reported studies assessing the impact of mouthwashes in specifically preventing or treating viral infections in neutropenic patients. Last, a recent meta-analysis showed that chlorhexidine (rinse or gel) can reduce risk of ventilator-associated pneumonia in patients undergoing mechanical ventilation, although causative organisms were not described.[71]

Povidone-Iodine

Povidone-iodine (PVP-I) mouthwash has been widely studied in relation to broad-spectrum antimicrobial and virucidal actions. At 0.23%, which is routinely used in Japan, this rapidly inactivates SARS-CoV, MERS-CoV, influenza virus A (H1N1), and rotavirus in vitro.[72] A second study also showed that PVP-I (0.23%) is equivalent to 70% ethanol in inactivating SARS-CoV in vitro.[73] Indeed, based on in vitro and limited clinical studies, in Japan, the Ministry of Health, Labour and Welfare supported daily gargling as a protective measure to prevent upper respiratory tract infections.[74] A small number of human studies supporting this in the case of PVP-I have shown reduced incidence of both bacterial and viral (influenza) infection through repeated gargling.[72,75] In one rather limited study, the absence rate in middle schools in Yamagata City was compared over 3 months, where PVP-I gargling was encouraged in 1 school, versus 7 where it was not. A reduction of absence due to colds and influenza from a mean of 25.5% (no gargling) down to 19.8% ($P < 0.05$) was found.[76] In another study, a group of 23 patients gargled more than 4 times/day for up to 2 h. Here, acute exacerbation of chronic respiratory infection was reduced by around 50%.[75] This mouthwash is not available in the United Kingdom, although may still be purchased in Germany and other countries. As a 1% solution, PVP-I is available in Hong Kong, Korea, Singapore, Malaysia, Philippines, and Taiwan. The importance of higher concentrations of PVP-I as a broad-spectrum antimicrobial agent for topical uses is indicated by its inclusion on the World Health Organization's List of Essential Medicines (https://www.who.int/medicines/publications/essentialmedicines/en/). It should be noted that rare allergic reactions have been reported for PVP-I.[77]

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Chlorinated Water or Hypertonic Saline Rinsing

Studies from Japan surprisingly found that gargling with chlorinated tap water reduced respiratory infections, and in one, was even better than PVP-I. In one, three groups of around 130 age- and gender-matched human subjects were studied (three 15-s gargles with 20 mL, at least 3/day for 60 days).[78] Tap water reduced incidence of common cold by 36%, while PVP-I was not effective. It was speculated that chlorine in the water may have contributed, since levels in Japan were above concentrations that are known to have viricidal activity including toward enveloped species.[79] However, information on the virucidal impact of chlorine comes from in vitro studies, including 1 with a 30 min contact time, and its impact in vivo on enveloped viruses, through gargling tap water is not known.[79] In 2008, another trial calculated economics of the activity in two groups of around 120 subjects gargling water for 60 days and concluded that this was a cost-effective strategy for upper respiratory infection prevention.[80] Last, a recent study showed that gargling and nasal rinsing with hypertonic saline could reduce symptoms, duration of illness, and viral shedding. However, this was a pilot, nonblinded, self-reported study and so cannot be considered definitive.[81] A follow-up study in vitro with enveloped and non/enveloped viruses including human coronavirus 229E suggested that this may have been related to altered intracellular chloride levels and peroxidase activities.[82] None of the in vivo studies addressed the issue of which pathogens were contributing to illness, and so cannot be extrapolated to coronaviruses.

Separate to oral rinsing it is worth noting that nasal rinsing with saline is a popular method promoted to clear nasal passages for sufferers of colds and allergies. Given that virus is recovered in the nasopharynx, a similar consideration of how this might be used as preventative measure could be made. As for mouthwash, clinical studies have not systematically examined how effective nasal rinsing is for preventing respiratory infections. Notably, rare reports of serious illness when not properly cleaned, due to the presence of parasitic amoebae in unboiled tap water, has led to recommendations on careful disinfection of rinsing syringes being made by CDC (https://www.cdc.gov/parasites/naegleria/sinus-rinsing.html).

Hydrogen Peroxide

Hydrogen peroxide causes oxygen-free radical-induced disruption of lipid membranes and is widely used as an agent for tooth whitening. Studies, including a recent systematic review, report that coronavirus 229E and other enveloped viruses are inactivated at concentrations around 0.5%.[19,83] While higher concentrations of hydrogen peroxide (>5%) will induce damage to both soft and hard tissues, within the range of concentrations used in mouthwashes for whitening at 1%–3% little damage is reported.[84] Within the oral environment, hydrogen peroxide is rapidly inactivated due to the presence of host- and bacteria-derived catalase activity in saliva and other endogenous peroxidases.[85] The impact of peroxidases could theoretically be reduced by using a prerinse with water, although this is untested. A consideration with this agent is that it can have potential proviral activities, although so far this was only seen in vitro.[86–88]

Quaternary ammonium compounds

These are widely used as microbicidal agents that interfere with protein or lipid components on the cell surface, particularly Gram-positive or Gram-negative bacteria. Their virucidal activities are not widely reported although some reports against enveloped viruses have been made in the literature relating to surface disinfection.[79] Among this group of compounds, cetylpyridinium chloride (CPC) was recently shown to have activity against influenza both in vitro and in vivo, through direct attack on the viral envelope, with in vitro EC50 being 5–20 μg/mL.[89] CPC is used in medicated oral rinses at concentrations 0.025%–0.075% w/v (250–750 μg/mL) in the United Kingdom, while lozenges sold in some countries contain 1.4–3.0 mg of CPC.

Current Policies Relating to Oral Health and Use of Microbicides in Dental Practice and with Immunosuppressed Individuals

Dental practitioners are at elevated risk of exposure to SARS-CoV-2, and there are guidelines that advocate use of mouthwash clinically. Previously published CDC guidelines for infection control in the dental setting have cited the potential usefulness of preprocedural mouthwashes in reducing the spread of airborne pathogens of all types.[90] Indeed, studies have addressed how virucidal components including CPC and chlorhexidine can be effective in reducing bacterial contamination in this setting, although virus inactivation was not tested.[91,92] Meng et al. in an experience-based review of their practice in Wuhan, recommended preprocedural mouthwash to reduce the oral microbial load in patients undergoing dental treatment in patients with SARS-CoV-2.[93] Last, two recent papers aimed at providing guidelines for endodontists in relation to SARS-CoV-2 advocated preprocedural mouth rinse with 0.2% PVP-I.[94,95] Given that the dental community recognize, the potential for oral mouth washing in relation to reducing infection risk, extrapolating these guidelines to the wider community is worth a full discussion.

The Urgent Need for Research

Many questions need to be addressed in relation to whether oral hygiene could represent a viable approach to dampen transmission of SARS-CoV-2, and research is required to address this.

In relation to oral hygiene, we need to determine:

- Can we reduce viral load in the oropharynx through oral rinsing?
- If we can reduce load, then which oral rinse would be clinically effective: The current choice includes 20%–30% ethanol, lipid-based membrane disruptors, PVP-1, CPC, hydrogen peroxide, simple chlorinated tap water or WHO formulation I diluted to 30% of neat?
- Would a combination of agents in lower amounts be better tolerated, reducing adverse effects, and remain effective?
- What combinations or agents, contact time and frequency of use would induce antiviral activity and reduce infectivity of SARS-CoV-2?

Available research approaches include:

Statistical epidemiological studies could establish on a population level whether mouth rinsing is associated with reduced rates of throat and respiratory infections including SARS-CoV-2. Purchasing data of health-related products to model health linkages could be used. New applications to conduct widespread monitoring of SARS-CoV-2 symptoms, could capture use of mouthwashes to test for correlation with symptoms and severity, alongside wider purchasing sales behavior of those who are asymptomatic. Modeling approaches should

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also consider population usage of mouthwash preparations and viral spread.

Underpinning research. Not all enveloped viruses are the same, and herpes, influenza, and measles viruses are considered more unstable than human coronaviruses, which may persist for up to 5 days on inanimate surfaces.[19,36,96] Thus, research needs to focus on coronaviruses in particular. The exact composition of the SARS-CoV-2 lipidome needs to be determined using lipidomics mass spectrometry. Research should determine the impact of ethanol or other agents on the infective activity of the spike protein itself, in vitro, and in vivo. A useful virus to test in vitro would be the human respiratory coronavirus 229E which is used extensively as a surrogate for human coronaviruses but only requires Category (Cat2) procedures, and its replication and propagation conditions are well established already. This would be a good representative for pathogenic coronaviruses, prior to narrowing down to SARS-CoV-2 which requires Cat3 biosecurity.

The impact of temperature and soil load needs to be considered for in vitro studies, applying the ASTM or EU standard protocols (https://www.astm.org/Standards/E2197.htm). Here, the impact of not only dose/composition but also the critical issue of contact time with agents, which is a known modifiable parameter of virucidal activity can be easily tested. The virucidal mechanisms can be determined, conducting lipidomics analysis of the envelope along with assays that determine spike protein conformation and activity. While a particular ethanol concentration may achieve full inactivation, lower amounts could either help to remove virus or lead to membrane damage (permeability/leakage) that may impact throat cell virus infectivity, for example, through potential modification of the ability of the spike glycoprotein to interact with receptors on host cells. In this regard, for HIV-1, the lipid membrane stabilizes membrane glycoproteins, regulating their sensitivity to antibody neutralization.[62] This type of action could be further enhanced if membrane disrupting agents were also included in a mouthwash. Indeed, lipidomimetic compounds have already been developed that can dampen viral infection through affecting lipid membrane structure or curvature (discussed in Section "Membrane Perturbation Without Lysis can Dampen Enveloped Virus Infectivity").

Most virucidal research uses in vitro models, where the response to the agent will be different, and also does not take into account the impact of host immunity. There is an absence of animal model studies on coronavirus respiratory illness, although macaques and mice transgenic for humanized ACE2 are beginning to be studied with early indications being that both develop mild illnesses in response to SARS-CoV-2 virus (https://www.nature.com/articles/d41586-020-00698-x).

Clinical studies. Robustly designed, appropriately powered in vivo clinical studies are needed, including determination of the most effective composition. Self-reported, nonrandomized, unblinded studies are not reliable and need to be avoided. An important sequela of these over the counter medicines is that individuals may use them prior to providing diagnostic nasopharyngeal/throat swabs. This could increase the number of false-negative tests and facilitate transmission. Currently, there is no specific advice to avoid these preparations prior to testing.

Population-based interventions could be considered, although panic buying or dangerous consumption of ethanol or methanol has to be avoided. As high-risk groups come out of self-isolation, they could represent a population to evaluate clinical outcomes resulting from real-world use of available mouthwashes. The current social restrictions will reduce a number of transmission risk factor variables and alter clinical outcomes in terms of SARS-CoV-2 infection, other respiratory infections, and adverse effects, but monitoring outcomes could provide useful data. Users could be given general advice on product use, recording timings, and duration of gargling for later analysis, or act as controls. Similarly, health workers at high risk of infection could be provided products and asked to record their use and report outcomes. Ideally, throat swabs and blood samples would be obtained for testing. There would be logistical, ethical, and regulatory issues involved in setting up investigations. However, given the theoretical plausibility and data we have reviewed plus the readily available products and urgent need to reduce SARS-CoV-2 infection, measures could be considered and action taken to instigate clinical investigation in the population during the outbreak.

Host inflammation. Mouthwashes widely utilized in daily oral and dental hygiene for cosmetic and medical reasons and have demonstrated acceptable tolerability when used multiple times daily for durations of 6 months and longer. Despite this, the impact of rinsing with these agents on throat tissue health needs to be seriously considered, since the viral lipid membrane is effectively the same as that of the host. Some of these agents, such as ethanol and hydrogen peroxide may, if used several times a day over a period of 2–3 months, induce mucosal inflammation. This was observed in a study on corneal epithelial cells, where inflammatory cytokines (IL-1b, IL-6), chemokines (IL-8/CXCL8, CCL2), and matrix metalloproteases (MMP9) were all upregulated at the mRNA levels 1–3 days after a 30-s exposure to 20% (v/v) ethanol.[48] Here, it will be important to ascertain whether a repeated daily rinse with mouthwash would have any detrimental impact on the stromal tissue lining. Alternatively, host innate immune responses in early infection could also represent a strategy to remove virus, and this has not been considered in any studies to date. Significant advances have been made into the molecular basis of alcohol-induced tissue injury. However, these studies tend to be confined to studies of acute and chronic alcohol consumption where the metabolism of alcohol into acetaldehyde and reactive oxygen intermediates modify various physiological processes linked with the maintenance of tissue homeostasis.[97] Currently, there is a lack of research into the potential impact of mouthwash on local inflammation within the throat and consideration needs be given to both its impact on antiviral immunity and the disruption of tissue integrity.

Additional Reading

We highlight a list of excellent review articles that were consulted as part of preparing this review and were a source of primary research cited herein:

Budding of viral lipid membranes: Simons and Garoff[98]

Impact of virus on lipid metabolism: Sanchez and Lagunoff[99]

Review of inactivation of coronaviruses on surfaces: Wolff et al.[100]

Emergence of SARS-CoV in 2003: Peiris et al.[101]

Two papers on the impact of ethanol on interdigitation of membranes: Slatter and Huang[102,103]

Control of infection using Povidone-Iodine: Eggers[104]

Summary of the composition of lipid membranes in cells: van Meer[105]

Search strategy

Since the review covers many areas from basic biochemistry, virology, and microbicidal research, as well as clinical

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information both in medicine and dentistry, multiple sources were consulted. Most references were identified from PubMed, ResearchGate, or Google, using search terms including "virus," "coronavirus," "lipid envelope," "alcohol," "membrane," "chlorhexidine," and others, alone and in combination. Many references that were first identified, were then investigated further to find additional source material and the original primary research which was then included. The idea for drafting the review was initiated by V.B.O. on March 21, 2020, through reaching out in person to various international experts, to get their views and input directly, via phone calls and then follow-up emails. Boots UK was approached initially to discuss the ideas and for information on formulation of available mouthwashes. Boots researchers and scientists contacted Johnson & Johnson, who provided proprietary information. Boots researchers (Kirkdale, Thornley, Povey, Inchley, and O'Shea) then conducted further searches of PubMed, Google, and ResearchGate, using the same terms. Academic and clinical expertise was consulted for virology (Stanton, Humphreys, and Bosch), clinical/ICU (Fegan and Wise), dental practice (Thomas), immunology (Jones), lipid biochemistry (Wakelem, Murphy, Simons, and O'Donnell), and microbicides (Sattar and Maillard). Phone/email correspondence with experts generated input, opinions, and identified additional references. No timeline for references was used and no languages were excluded.

Acknowledgments

The lead author gratefully acknowledges significant input from colleagues employed at Boots UK, Nottingham (Drs Andrew Inchley, Tracey Thornley, Janet Povey, Charlotte Kinchley, and Ged O'Shea), who provided formulation information on existing mouthwashes, that is not available in the public domain. Boots UK staff also linked directly with colleagues at Johnson & Johnson to obtain formulation information, and conducted literature searches, providing information to the lead author, who had full independent editorial control over drafting and editing the manuscript. The authors gratefully acknowledge expert advice on viral lipidomes from Prof. Kai Simons, Emeritus Director, Max Planck Institute of Molecular Cell Biology and Genetics, Dresden. Johnson & Johnson Ltd provided technical information on formulations. No financial agreements or other forms of payment were provided to any parties involved in preparing this review.

Funding

V.B.O. is a Royal Society Wolfson Research Merit Award Holder and acknowledges funding for LIPID MAPS from Wellcome Trust (203014/Z/16/Z).

Conflict of interest statement

None declared.

References

1. Wrapp D, Wang N, Corbett KS, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* 2020;367(6483):1260–1263.
2. Nieto-Garai JA, Glass B, Bunn C, et al. Lipidomimetic compounds act as HIV-1 entry inhibitors by altering viral membrane structure. *Front Immunol* 2018;9:1983.
3. Gerl MJ, Sampaio JL, Urban S, et al. Quantitative analysis of the lipidomes of the influenza virus envelope and MDCK cell apical membrane. *J Cell Biol* 2012;196(2):213–221.
4. Garoff H, Simons K, Dobberstein B. Assembly of the Semliki Forest virus membrane glycoproteins in the membrane of the endoplasmic reticulum in vitro. *J Mol Biol* 1978;124(4): 587–600.
5. Kalvodova L, Sampaio JL, Cordo S, Ejsing CS, Shevchenko A, Simons K. The lipidomes of vesicular stomatitis virus, Semliki Forest virus, and the host plasma membrane analyzed by quantitative shotgun mass spectrometry. *J Virol* 2009;83(16):7996–8003.
6. Cluett EB, Machamer CE. The envelope of vaccinia virus reveals an unusual phospholipid in Golgi complex membranes. *J Cell Sci* 1996;109(Pt 8):2121–2131.
7. Fleischer B, Zambrano F, Fleischer S. Biochemical characterization of the Golgi complex of mammalian cells. *J Supramol Struct* 1974;2(5–6):737–750.
8. Hornick CA, Hamilton RL, Spaziani E, Enders GH, Havel RJ. Isolation and characterization of multivesicular bodies from rat hepatocytes: an organelle distinct from secretory vesicles of the Golgi apparatus. *J Cell Biol* 1985;100(5):1558–1569.
9. Howell KE, Palade GE. Hepatic Golgi fractions resolved into membrane and content subfractions. *J Cell Biol* 1982;92(3): 822–832.
10. Keenan TW, Morre DJ. Phospholipid class and fatty acid composition of Golgi apparatus isolated from rat liver and comparison with other cell fractions. *Biochemistry* 1970;9(1): 19–25.
11. Moreau P, Cassagne C. Phospholipid trafficking and membrane biogenesis. *Biochim Biophys Acta* 1994;1197(3): 257–290.
12. Kean LS, Grant AM, Angeletti C, et al. Plasma membrane translocation of fluorescent-labeled phosphatidylethanolamine is controlled by transcription regulators, PDR1 and PDR3. *J Cell Biol* 1997;138(2):255–270.
13. Krijnse-Locker J, Ericsson M, Rottier PJ, Griffiths G. Characterization of the budding compartment of mouse hepatitis virus: evidence that transport from the RER to the Golgi complex requires only one vesicular transport step. *J Cell Biol* 1994;124(1–2):55–70.
14. Tooze J, Tooze SA. Infection of AtT20 murine pituitary tumour cells by mouse hepatitis virus strain A59: virus budding is restricted to the Golgi region. *Eur J Cell Biol* 1985;37: 203–212.
15. Yan B, Chu H, Yang D, et al. Characterization of the lipidomic profile of human coronavirus-infected cells: implications for lipid metabolism remodeling upon coronavirus replication. *Viruses* 2019;11(1):73.
16. Kapadia SB, Chisari FV. Hepatitis C virus RNA replication is regulated by host geranylgeranylation and fatty acids. *Proc Natl Acad Sci USA* 2005;102(7):2561–2566.
17. Munger J, Bajad SU, Coller HA, Shenk T, Rabinowitz JD. Dynamics of the cellular metabolome during human cytomegalovirus infection. *PLoS Pathog* 2006;2(12):e132.
18. Pratelli A, Colao V. Role of the lipid rafts in the life cycle of canine coronavirus. *J Gen Virol* 2015;96(Pt 2):331–337.
19. Kampf G, Todt D, Pfaender S, Steinmann E. Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents. *J Hosp Infect* 2020;104(3): 246–251.

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20. Hulkower RL, Casanova LM, Rutala WA, Weber DJ, Sobsey MD. Inactivation of surrogate coronaviruses on hard surfaces by health care germicides. *Am J Infect Control* 2011;39(5): 401–407.
21. Sattar SA, Springthorpe VS, Karim Y, Loro P. Chemical disinfection of non-porous inanimate surfaces experimentally contaminated with four human pathogenic viruses. *Epidemiol Infect* 1989;102(3):493–505.
22. Kratzel A, Todt D, V'kovski, et al. Efficient inactivation of SARS-CoV-2 by WHO-recommended hand rub formulations and alcohols. *BioRxiv*. 2020. doi: 10.1101/2020.03.10.986711.
23. Brown TT Jr. Laboratory evaluation of selected disinfectants as virucidal agents against porcine parvovirus, pseudorabies virus, and transmissible gastroenteritis virus. *Am J Vet Res* 1981;42(6):1033–1036.
24. Saknimit M, Inatsuki I, Sugiyama Y, Yagami K. Virucidal efficacy of physico-chemical treatments against coronaviruses and parvoviruses of laboratory animals. *Jikken Dobutsu* 1988; 37(3):341–345.
25. Swanson DA. A state-based regression model for estimating substate life expectancy. *Demography* 1989;26(1):161–170.
26. Wood A, Payne D. The action of three antiseptics/disinfectants against enveloped and non-enveloped viruses. *J Hosp Infect* 1998;38(4):283–295.
27. Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. *Nature* 2020. doi: 10.1038/s41586-020-2196-x.
28. Pan Y, Zhang D, Yang P, Poon LLM, Wang Q. Viral load of SARS-CoV-2 in clinical samples. *Lancet Infect Dis* 2020. doi: 10.1016/S1473-3099(20)30113-4.
29. To KK, Tsang OT, Leung WS, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. *Lancet Infect Dis* 2020.
30. Liu Y, Yan LM, Wan L, et al. Viral dynamics in mild and severe cases of COVID-19. *Lancet Infect Dis* 2020. doi: 10.1016/S1473-3099(20)30196-1.
31. Liu Y, Yang Y, Zhang C, et al. Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. *Sci China Life Sci* 2020;63(3):364–374.
32. Zou L, Ruan F, Huang M, et al. SARS-CoV-2 viral load in upper respiratory specimens of infected patients. *N Engl J Med* 2020;382(12):1177–1179.
33. Xu J, Li Y, Gan F, Du Y, Yao Y. Salivary glands: potential reservoirs for COVID-19 asymptomatic infection. *J Dent Res* 2020:22034520918518. doi: 10.1177/0022034520918518.
34. Li R, Pei S, Chen B, et al. Substantial undocumented infection facilitates the rapid dissemination of novel coronavirus (SARS-CoV2). *Science* 2020.
35. Varia M, Wilson S, Sarwal S, et al. Investigation of a nosocomial outbreak of severe acute respiratory syndrome (SARS) in Toronto, Canada. *CMAJ* 2003;169(4):285–292.
36. van Doremalen N, Bushmaker T, Morris DH, et al. Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. *N Engl J Med* 2020;382:1564–1567.
37. Tamin A, Queen K, Paden CR, et al. Isolation and growth characterization of novel full length and deletion mutant human MERS-CoV strains from clinical specimens collected during 2015. *J Gen Virol* 2019;100(11):1523–1529.
38. Harcourt J, Tamin A, Lu X, et al. Severe acute respiratory syndrome coronavirus 2 from patient with 2019 novel coronavirus disease, United States. *Emerg Infect Dis* 2020;26(6). doi: 10.3201/eid2606.200516
39. Ly HV, Longo ML. The influence of short-chain alcohols on interfacial tension, mechanical properties, area/molecule, and permeability of fluid lipid bilayers. *Biophys J* 2004;87(2): 1013–1033.
40. Ahl PL, Chen L, Perkins WR, et al. Interdigitation-fusion: a new method for producing lipid vesicles of high internal volume. *Biochim Biophys Acta* 1994;1195(2):237–244.
41. Hunt GR, Jones IC. A 1H-NMR investigation of the effects of ethanol and general anaesthetics on ion channels and membrane fusion using unilamellar phospholipid membranes. *Biochim Biophys Acta* 1983;736(1):1–10.
42. Komatsu H, Guy PT, Rowe ES. Effect of unilamellar vesicle size on ethanol-induced interdigitation in dipalmitoylphosphatidylcholine. *Chem Phys Lipids* 1993;65(1):11–21.
43. Komatsu H, Okada, S. Effects of ethanol on permeability of phosphatidylcholine/cholesterol mixed liposomal membranes. *Chem Phys Lipids* 1997;85:67–74.
44. Komatsu H, Okada S. Increased permeability of phase-separated liposomal membranes with mixtures of ethanol-induced interdigitated and non-interdigitated structures. *Biochim Biophys Acta* 1995;1237(2):169–175.
45. Dennison DK, Meredith GM, Shillitoe EJ, Caffesse RG. The antiviral spectrum of Listerine antiseptic. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1995;79(4):442–448.
46. Roberts PL, Lloyd D. Virus inactivation by protein denaturants used in affinity chromatography. *Biologicals* 2007;35(4): 343–347.
47. Siddharta A, Pfaender S, Vielle NJ, et al. Virucidal activity of World Health Organization-recommended formulations against enveloped viruses, including zika, ebola, and emerging coronaviruses. *J Infect Dis* 2017;215(6):902–906.
48. Oh JY, Yu JM, Ko JH. Analysis of ethanol effects on corneal epithelium. *Invest Ophthalmol Vis Sci* 2013;54(6):3852–3856.
49. Sonmez M, Ince HY, Yalcin O, et al. The effect of alcohols on red blood cell mechanical properties and membrane fluidity depends on their molecular size. *PLoS One* 2013;8(9):e76579.
50. Chi LM, Wu WG. Mechanism of hemolysis of red blood cell mediated by ethanol. *Biochim Biophys Acta* 1991;1062(1): 46–50.
51. Tyulina OV, Huentelman MJ, Prokopieva VD, Boldyrev AA, Johnson P. Does ethanol metabolism affect erythrocyte hemolysis? *Biochim Biophys Acta* 2000;1535(1):69–77.
52. Tyulina OV, Prokopieva VD, Dodd RD, et al. In vitro effects of ethanol, acetaldehyde and fatty acid ethyl esters on human erythrocytes. *Alcohol Alcohol* 2002;37(2):179–186.
53. Wang Y, Tong J, Chang B, Wang B, Zhang D, Wang B. Effects of alcohol on intestinal epithelial barrier permeability and expression of tight junction-associated proteins. *Mol Med Rep* 2014;9(6):2352–2356.
54. 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):341–346.
55. Komatsu H, Okada S. Ethanol-induced aggregation and fusion of small phosphatidylcholine liposome: participation of interdigitated membrane formation in their processes. *Biochim Biophys Acta* 1995;1235(2):270–280.
56. Nambi P, Rowe ES, McIntosh TJ. Studies of the ethanol-induced interdigitated gel phase in phosphatidylcholines using the fluorophore 1,6-diphenyl-1,3,5-hexatriene. *Biochemistry* 1988;27(26):9175–9182.
57. Rowe ES. Thermodynamic reversibility of phase transitions. Specific effects of alcohols on phosphatidylcholines. *Biochim Biophys Acta* 1985;813(2):321–330.

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58. McConnell DS, Schullery, SE. Phospholipidvesiclefusion and drug loading: temperature, solute and cholesterol effects, and, a rapid preparationfor solute-loadedvesicles. *Biochimica et Biophysica Acta* 1985;818:13–22.
59. Boni LT, Minchey SR, Perkins WR, et al. Curvature dependent induction of the interdigitated gel phase in DPPC vesicles. *Biochim Biophys Acta* 1993;1146(2):247–257.
60. Barry JA, Gawrisch K. Effects of ethanol on lipid bilayers containing cholesterol, gangliosides, and sphingomyelin. *Biochemistry* 1995;34(27):8852–8860.
61. Trandum C, Westh P, Jorgensen K, Mouritsen OG. Association of ethanol with lipid membranes containing cholesterol, sphingomyelin and ganglioside: a titration calorimetry study. *Biochim Biophys Acta* 1999;1420(1–2):179–188.
62. Salimi H, Johnson J, Flores MG, et al. The lipid membrane of HIV-1 stabilizes the viral envelope glycoproteins and modulates their sensitivity to antibody neutralization. *J Biol Chem* 2020;295(2):348–362.
63. Sands J, Auperin D, Snipes W. Extreme sensitivity of enveloped viruses, including herpes simplex, to long-chain unsaturated monoglycerides and alcohols. *Antimicrob Agents Chemother* 1979;15(1):67–73.
64. Astani A, Reichling J, Schnitzler P. Comparative study on the antiviral activity of selected monoterpenes derived from essential oils. *Phytother Res* 2010;24(5):673–679.
65. Cheung HY, Wong MM, Cheung SH, Liang LY, Lam YW, Chiu SK. Differential actions of chlorhexidine on the cell wall of *Bacillus subtilis* and *Escherichia coli*. *PLoS One* 2012;7(5):e36659.
66. 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* 1990;69(3):874–876.
67. Bonesvoll P, Lokken P, Rolla G, Paus PN. Retention of chlorhexidine in the human oral cavity after mouth rinses. *Arch Oral Biol* 1974;19(3):209–212.
68. Ferretti GA, Raybould TP, Brown AT, et al. Chlorhexidine prophylaxis for chemotherapy- and radiotherapy-induced stomatitis: a randomized double-blind trial. *Oral Surg Oral Med Oral Pathol* 1990;69(3):331–338.
69. Kuo CC, Wang RH, Wang HH, Li CH. Meta-analysis of randomized controlled trials of the efficacy of propolis mouthwash in cancer therapy-induced oral mucositis. *Support Care Cancer* 2018;26(12):4001–4009.
70. Sorensen JB, Skovsgaard T, Bork E, Damstrup L, Ingeberg S. Double-blind, placebo-controlled, randomized study of chlorhexidine prophylaxis for 5-fluorouracil-based chemotherapy-induced oral mucositis with nonblinded randomized comparison to oral cooling (cryotherapy) in gastrointestinal malignancies. *Cancer* 2008;112(7):1600–1606.
71. Veitz-Keenan A, Ferraiolo DM. Oral care with chlorhexidine seems effective for reducing the incidence of ventilator-associated pneumonia. *Evid Based Dent* 2017;18(4):113–114.
72. 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* 2018;7(2):249–259.
73. 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):119–123.
74. Japan Ministry of Health LaW. Pandemic influenza preparedness action plan of the Japanese Government; 2007.
75. Nagatake T, Ahmed K, Oishi K. Prevention of respiratory infections by povidone-iodine gargle. *Dermatology* 2002;204(Suppl 1):32–36.
76. Shiraishi T, Nakagawa Y. Evaluation of the bactericidal activity of povidone-iodine and commercially available gargle preparations. *Dermatology* 2002;204(Suppl 1):37–41.
77. Rahimi S, Lazarou G. Late-onset allergic reaction to povidone-iodine resulting in vulvar edema and urinary retention. *Obstet Gynecol* 2010;116(Suppl 2):562–564.
78. Satomura K, Kitamura T, Kawamura T, et al. Prevention of upper respiratory tract infections by gargling: a randomized trial. *Am J Prev Med* 2005;29(4):302–307.
79. Shirai J, Kanno T, Tsuchiya Y, Mitsubayashi S, Seki R. Effects of chlorine, iodine, and quaternary ammonium compound disinfectants on several exotic disease viruses. *J Vet Med Sci* 2000;62(1):85–92.
80. Sakai M, Shimbo T, Omata K, et al. Cost-effectiveness of gargling for the prevention of upper respiratory tract infections. *BMC Health Serv Res* 2008;8:258.
81. Ramalingam S, Graham C, Dove J, Morrice L, Sheikh A. A pilot, open labelled, randomised controlled trial of hypertonic saline nasal irrigation and gargling for the common cold. *Sci Rep* 2019;9(1):1015.
82. Ramalingam S, Cai B, Wong J, et al. Antiviral innate immune response in non-myeloid cells is augmented by chloride ions via an increase in intracellular hypochlorous acid levels. *Sci Rep* 2018;8(1):13630.
83. Omidbakhsh N, Sattar SA. Broad-spectrum microbicidal activity, toxicologic assessment, and materials compatibility of a new generation of accelerated hydrogen peroxide-based environmental surface disinfectant. *Am J Infect Control* 2006;34(5):251–257.
84. Walsh LJ. Safety issues relating to the use of hydrogen peroxide in dentistry. *Aust Dent J* 2000;45(4):257–269.
85. Kraus FW, Perry WI, Nickerson JF. Salivary catalase and peroxidase values in normal subjects and in persons with periodontal disease. *Oral Surg Oral Med Oral Pathol* 1958;11(1):95–102.
86. Cai J, Chen Y, Seth S, Furukawa S, Compans RW, Jones DP. Inhibition of influenza infection by glutathione. *Free Radic Biol Med* 2003;34(7):928–936.
87. Staal FJ, Roederer M, Herzenberg LA, Herzenberg LA. Intracellular thiols regulate activation of nuclear factor kappa B and transcription of human immunodeficiency virus. *Proc Natl Acad Sci USA* 1990;87(24):9943–9947.
88. Xiao J, Deng J, Lv L, et al. Hydrogen peroxide induce human cytomegalovirus replication through the activation of p38-MAPK signaling pathway. *Viruses* 2015;7(6):2816–2833.
89. Popkin DL, Zilka S, Dimaano M, et al. Cetylpyridinium chloride (CPC) exhibits potent, rapid activity against influenza viruses in vitro and in vivo. *Pathog Immun* 2017;2(2):252–269.
90. Kohn WG, Collins AS, Cleveland JL, et al. Guidelines for infection control in dental health-care settings–2003. *MMWR Recomm Rep* 2003;52(RR-17):1–61.
91. Feres M, Figueiredo LC, Faveri M, Stewart B, de Vizio W. The effectiveness of a preprocedural mouthrinse containing cetylpyridinium chloride in reducing bacteria in the dental office. *J Am Dent Assoc* 2010;141(4):415–422.
92. Retamal-Valdes B, Soares GM, Stewart B, et al. Effectiveness of a pre-procedural mouthwash in reducing bacteria in dental aerosols: randomized clinical trial. *Braz Oral Res* 2017;31:e21.
93. Meng L, Hua F, Bian Z. Coronavirus disease 2019 (COVID-19): emerging and future challenges for dental and oral medicine. *J Dent Res* 2020;99(5):481–487.
94. Ather A, Patel B, Ruparel NB, Diogenes A, Hargreaves KM. Coronavirus disease 19 (COVID-19): implications for clinical dental care. *J Endod* 2020;46(5):584–595.

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95. Peng X, Xu X, Li Y, Cheng L, Zhou X, Ren B. Transmission routes of 2019-nCoV and controls in dental practice. *Int J Oral Sci* 2020;12(1):9.
96. Firquet S, Beaujard S, Lobert PE, et al. Survival of enveloped and non-enveloped viruses on inanimate surfaces. *Microbes Environ* 2015;30(2):140–144.
97. Jung MK, Callaci JJ, Lauing KL, et al. Alcohol exposure and mechanisms of tissue injury and repair. *Alcohol Clin Exp Res* 2011;35(3):392–399.
98. Simons K, Garoff H. The budding mechanisms of enveloped animal viruses. *J Gen Virol* 1980;50(1):1–21.
99. Sanchez EL, Lagunoff M. Viral activation of cellular metabolism. *Virology* 2015;479–480:609–618.
100. Wolff MH, Sattar SA, Adegbunrin O, Tetro J. Environmental survival and microbicide inactivation of coronaviruses. In: A Schmidt, O Weber, MH Wolff, eds. *Coronaviruses with Special Emphasis on First Insights Concerning SARS*. Basel: Birkhäuser Basel; 2005:201–212.
101. Peiris JS, Yuen KY, Osterhaus AD, Stohr K. The severe acute respiratory syndrome. *N Engl J Med* 2003;349(25):2431–2441.
102. Slater JL, Huang, CH. Structure of biological membranes. In: P Yeagle, ed. *Structure of Biological Membranes*. Boca Raton, FL: CRC Press; 1992.
103. Slater JL, Huang CH. Interdigitated bilayer membranes. *Prog Lipid Res* 1988;27(4):325–359.
104. Eggers M. Infectious disease management and control with povidone iodine. *Infect Dis Ther* 2019;8(4):581–593.
105. van Meer G. Lipids of the Golgi membrane. *Trends Cell Biol* 1998;8(1):29–33.

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INTERNATIONAL
ENDODONTIC JOURNAL

doi:10.1111/iej.13291

Letter to the Editor

COVID-19: its impact on dental schools in Italy, clinical problems in endodontic therapy and general considerations

Dear Editor

COVID-19 is a novel disease caused by a member of the coronavirus family that originated in Wuhan, Hubei, China in December 2019. Over the last few months, the infection has spread to several countries worldwide. Coronaviruses are described by the World Health Organisation (WHO) as a large family of viruses, which may cause illness in animals or humans (https://www.who.int/news-room/q-a-detail/q-a-coronaviruses). Although COVID-19 infections in humans most often present with mild symptoms, in a variable percentage of cases, it can cause an acute respiratory syndrome, which has led to patient fatalities (Phan 2020, Lu *et al.* 2020). COVID-19 appears to be a particular risk for patient with pre-existing medical conditions (such as high blood pressure, heart disease, lung disease, cancer or diabetes; https://www.who.int/news-room/q-a-detail/q-a-coronaviruses).

Recently, a letter has been published, detailing the potential impact of COVID-19 in Dentistry (Sabino-Silva *et al.* 2020), whilst other articles have set out the routes of transmission, implications and controls required in dental practice (Peng *et al.* 2020). In addition, various world, national and dental organizations have issued guidance, for example American Dental Association (http://success.ada.org/en/practice-management/patients/~/media/32D2D1F13B3D4D919E67CFBC00AF71B0.pdf), British Dental Association (https://bda.org/news-centre/latest-news-articles/Pages/Wuhan-novel-coronavirus-advice-for-dentists.aspx) and the WHO (https://www.who.int/news-room/q-a-detail/q-a-coronaviruses).

At the present time, the principle challenge is that the population has no innate protection against COVID-19 and a vaccine has not been developed. It is also of note that no specific antiviral therapy has currently proven to be effective against SARS CoV-2 (the virus causing the disease COVID-19). This combination of no vaccine as well as no effective therapeutic countermeasure is potentially leading to an unknown but possibly challenging scenario with millions of people becoming infected globally. This would inevitably have a major impact on daily life, with the potential to drive even the healthcare systems in developed countries to the brink of collapse.

Although the exact transmission routes are yet to be determined with certainty, as with influenza and many other viruses affecting the upper and lower respiratory airways, aerosol transmission between humans has been confirmed. The mean incubation period is estimated to be around 5 days (but probably ranges from 1 to 19 days), which allows travel to spread the disease globally (https://www.who.int/news-room/q-a-detail/q-a-coronaviruses). Interestingly, only a few paediatric cases have been reported, whilst in contrast, adult and older patients are frequently involved with some requiring hospitalization and even intubation within intensive care units (Rothan & Byrareddy 2020).

The clinical and virological evidence to date is not sufficiently robust to exclude the possibility that virus transmission can occur during the incubation period from infected but asymptomatic patients. Symptoms of COVID-19 are very similar to those of human influenza and include a fever, cough, rhinitis, myalgia, conjunctivitis and fatigue. From a European perspective, at least two separate clusters of infection have been identified in Northern Italy. The entire area of Italy is now a 'red zone' with multiple limitations imposed on education, work and transport. However, considering the rapid spread of the virus, additional restrictions on the movement of the population are being considered in an attempt to reduce the number of patients.

All the Italian Schools and Universities suspended teaching activity on 24th February, 2020. The rationale for this decision was to minimize the transmission of the virus between persons of different areas in the hope of reducing the spread of the infection.

Considering that SARS CoV-2 has been identified in the saliva of infected patients and that screening to determine the presence of the virus in Italy is carried out by sampling nasopharyngeal secretions, all Medical Doctors, Nurses and Dentists exposed to potential contact with this biological material are, at least in theory, exposed to an unknown risk of acquiring the SARS CoV-23 agent. Notably, the spread of the

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disease is increasing in other European countries including France, Germany, Austria and the UK.

It is important to emphasize that prolonged close *face-to-face* contact between patients and operators during endodontic treatment creates a high risk for cross-infection. The contact between the mouth of the patients and the production of aerosols through the use of high-speed handpieces would appear to create a substantial risk for contamination and spread of the virus within dental clinics. Thus, routine endodontic therapies and emergency treatments are high risk for virus contamination. Furthermore, it is likely that University Dental Clinics and Dental Hospitals, where patients, students and teachers share the same spaces, may potentially create a reservoir and 'hub' for the spread of the virus. To confirm this potential within medicine, the virus has infected a large number of hospital healthcare doctors and nursing staff.

The prevention protocol being adopted in Italy includes:

1. Triaging patients to detect by the history any with respiratory infection, flu, acute respiratory illness, conjunctivitis and cardiovascular abnormalities;
2. Separation of patients with respiratory symptoms to limit their contact with dental staff, students and patients;
3. Avoiding dental treatment if at all possible;
4. Regular, meticulous and effective hand washing with no touching of face, eyes etc; use of face masks;
5. Decontamination of all surfaces within clinics with 0.1% sodium hypochlorite or 70% ethanol or 0.5% hydrogen peroxide, which are disinfectants already available in dental clinics (Kampf *et al.* 2020);
6. Respiratory hygiene/cough etiquette – use of tissues (Catch, Bin, Kill; https://www.england.nhs.uk/south/wp-content/uploads/sites/6/2017/09/catch-bin-kill.pdf);
7. Special precautions by dentists and assistants to prevent contamination from air droplets:
    a. Wear a disposable surgical mask and isolate the patient in a dedicated single-patient room (with closed door) separated from other staff/patients;
    b. When possible, use a rubber dam that must cover the nose of patient during the entire endodontic procedure;
    c. Application of powerful air/water surgical suction pump (aspirator) close to the tooth and a second suction close to the nose to prevent aerosol and saliva droplet diffusion;
    d. If possible, use high-speed handpiece with no exhaust;
    e. Decontamination of equipment, surgeries/operatories after reach patient.

General advice issued by many national authorities includes staying aware of the latest information on the COVID-19 outbreak. Take care of your health and protect others by doing the following:

- Regularly wash your hands with soap and water – for at least 20 s;
- Always wash your hands when you get home or into work;
- Use hand alcohol-based sanitizer gel if soap and water are not available;
- Cover your mouth and nose with a tissue or your sleeve (not your hands) when you cough or sneeze;
- Put used tissues in the bin straight away and wash your hands afterwards;
- Try to avoid close contact with people who are unwell;
- Do not touch your eyes, nose or mouth if your hands are not clean;
- If you have fever, cough and difficulty breathing, seek medical care early;
- Stay informed and follow advice given by your healthcare provider.

In conclusion:

1. The risk for dental personnel, dental nursing staff and for dental students is high and must be managed;
2. University students are a population with a substantial number of 'potential contacts' that must be limited;
3. Universities in high-risk areas should consider using online web-based teaching;
4. The development of a simple laboratory test to detect and monitor COVID-19 in Medical and Dental personal is necessary;
5. The role of the dentist in the prevention and monitoring of viral infections should be redefined.

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References

Kampf G, Todt D, Pfaender S, Steinmann E (2020) Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents. *Journal of Hospital Infection* **104**, 246–51.

Lu R, Zhao X, Li J *et al*. (2020) Genomic characterisation and epidemiology of 2019 novel coronavirus: implication for virus origins and receptor binding. *The Lancet* **395**, 565–75.

Peng X, Xu X, Li Y, Cheng L, Zhou X, Ren B (2020) Transmission routes of 2019-nCoV and controls in dental practice. *International Journal of Oral Science* **12**, 9–11.

Phan T (2020) Novel coronavirus: from discovery to clinical diagnosis. *Infection, Genetics and Evolution* **79**, 104211. https://doi.org/10.1016/j.meegid.2020.104211

Rothan HA, Byrareddy SN (2020) The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. *Journal of Autoimmunity*. https://doi.org/10.1016/j.jaut.2020.102434

Sabino-Silva R, Jardim ACG, Siqueira WL (2020) Coronavirus COVID-19 impacts to dentistry and potential salivary diagnosis. *Clinical Oral Investigations* **12**. https://doi.org/10.1007/s00784-020-03248-x

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J Clin Periodontol 2005; 32: 341–346 doi: 10.1111/j.1600-051X.2005.00673.x

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Journal of Clinical Periodontology

Efficacy of Listerine® Antiseptic in reducing viral contamination of saliva

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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: 341–346. doi: 10.1111/j.1600-051X.2005.00673.x. © Blackwell Munksgaard, 2005.

Abstract

Aim: The anti-viral efficacy of oral antimicrobial rinses has not been adequately studied in terms of potential clinical significance. As a follow-up to an in vitro study on the effect of oral antiseptics on *Herpes simplex* virus, Type 1, this study was undertaken to evaluate the in vivo effect of an essential oil containing oral antiseptic on the reduction of viral titer in saliva during active viral infection.

Method: Patients were recruited and evaluated in a single visit protocol at the onset of a perioral outbreak, consistent historically and clinically with recurrent *Herpes* labialis. Direct immunofluorescence of cytological smears of the lesions/oral fluids was used to confirm *Herpes simplex* virus types I or II. Patients were randomly assigned to one of two treatment groups: (1) active ingredient and (2) sterile water control. The viral lesion was evaluated as to clinical stage according to standard protocol. Salivary fluid samples were taken: (1) at baseline; (2) immediately following a 30 s rinse; (3) 30 min. after the 30 s rinse; and (4) on the repeat trial, also at 60 min. after the 30 s rinse. All samples were evaluated for viral titer and results compared.

Results: In Trial 1, the sample population consisted of 19 males and 21 females with an average age of 29.2 and in Trial 2, 21 males, 19 females with an average age of 28. In both Trials 1 and 2, recoverable infectious virions were reduced to zero after a 30 s experimental rinse; whereas, the control rinse resulted in a non-significant ($p > 0.05$) reduction. The experimental group also demonstrated a continued significant ($p < 0.05$) reduction 30 min. post rinse when compared with baseline while the control group returned to baseline levels. In Trial 2, the 60 min. post rinse follow-up demonstrated a 1–2 log residual reduction from baseline in the experimental group; however, this was not significant.

Conclusions: There is clinical efficacy in utilizing an oral rinse with the antimicrobial agent Listerine® Antiseptic in reducing the presence of viral contamination in oral fluids for at least 30 min. after oral rinse. The risk of viral cross contamination generated from these oral fluids in person to person contact or during dental treatment may be reduced.

Key words: anti-microbial oral rinse; essential oils; perioral recurrent *Herpes simplex* virus; saliva

Accepted for publication 21 July 2004

There are numerous sources of microbial contamination in the dental operatory. Dental unit water dispersed by three-way syringes, water coolant for ultrasonic instruments and aerosols from a patient's oral fluids produced by high-speed handpieces, represent initial sources of potentially infectious agents to the operatory. There are various methods currently being developed and in place for reduction of these sources of microbial contamination. Standard autoclaving procedures and ''standard precautions'' should preclude the transmission of potentially infectious microbial agents from these sources to the patient or to other individuals within the dental operatory. Concerning patients themselves, oral microorganisms, including numerous viral and bacterial pathogens, can potentially be mobilized into the bioaerosal by way of the high-speed manipulations of air and water in handpiece activity. This source of cross contamination has not been adequately studied to date. The anti-microbial efficacy of

oral rinses, although assumed in many instances, has also not been adequately studied in terms of potential clinical significance to reduce or eliminate this risk of such cross contamination.

Most studies have been completed on bacterial pathogens leaving the in vivo anti-viral effectiveness of commercial mouthrinses less well documented. This project was undertaken to evaluate the efficacy of Listerine® Antiseptic (LA, Morris Plains, NJ, USA) mouthrinse *versus* a sterile water control in the reduction of infectious viral levels in saliva during an active viral infection. The human *Herpes simplex* virus (HSV-1 or -2) was selected as the test virus in order to establish a model for showing reduction of infectious virions in saliva following a 30 s rinse as recommended by the manufacturer with an antiseptic mouthrinse containing essential oils. It is well established that viral shedding and hence recovery occurs at variable levels in human saliva from the prodromal period through at least the full vesicular stages of recurrent herpetic outbreaks (Arvin & Prober 1999).

This is primarily because of variations in clinical protocol in determining the stage of the lesion and in recovery techniques, which have not been standardized in every study. In previous work in our laboratories, five stages of clinical variation have been defined, see Table 1. Other researchers have determined that early recurrent *Herpes* labialis lesions (Stage 1 and 2 in our clinical evaluation) represent the time when the greatest viral shedding will likely be occurring (Arvin & Prober 1999). Since Stage 3 is a variable period with some new vesicle formation potentially still occurring, the degree to which infectious virions can be recovered from saliva or from lesions themselves is also variable. This study was undertaken to determine if, in patients known to suffer with *Herpes*, a reduction in salivary viral titer might be accomplished by an oral rinse during clinical Stages 1–3.

Material and Methods

Two complimentary trials testing the efficacy of LA were completed. In each trial, 40 adult patients, age 18–65 years, and known by history to suffer with viral recurrences, were recruited and advised to report to our clinical facility at the first sign of prodrome, or if a perioral viral lesion occurred. The advertisement stated that the patient should have an established history of viral recurrences consistent with *Herpes*. The clinicians examining the patients and referring to the clinical site utilized standard criteria for determining the likelihood of a herpetic infection. Since the study design was a one-visit protocol, testing of the virus to determine its consistency with HSV-1 or -2 by direct immunofluorescence (DIF) microscopy was performed at the first outbreak and used to determine if further analysis of samples was warranted.

For each of the two trials, following informed consent, each patient was examined, a medical history was obtained and soft-tissue examination was performed. Patients were randomly assigned to either the experimental or the control group until a 20 patient sample in each group was met. The viral lesion, usually on the patient's perioral tissues, was staged according to standard procedures (see Table 1).

Table 1. The five stages of clinical variation during a *Herpes* outbreak

Stage	Perioral tissue sensation and visual display
1	Lesions are not visibly present; patients report as much as a 24–48 h period of sensation, usually including tingling and sometimes burning or itching. Mild erythema may be present at the site of usual recurrence. Clinicians usually call this period the prodrome.
2	Increasing edema and redness is generally present at the site that the patient suffers normal recurrences with vesicle formation. These vesicles generally continue to form for a variable period of time, usually 2–5 days and, as the vesicles continue to develop older areas will begin to burst.
3	A combination of vesicles and crusted areas with an exudate usually resembling serum is present.
4	New vesicles do not appear. However, the lesions are crusted and often coalesced into a larger scabbed area.
5	The last scabbed area will be sloughed and the tissue begins to return to normal with only minor erythema left.

Following examination and the determination that the patient's history corresponded with clear evidence of likely recurrent *Herpes* labialis, a sterile swab was rolled against the labial tissue adjacent to the area of the lesion. In instances where the lesion was Stage 3 with evidence of vesicular rupture, the swab touched this area as well. The swab was then immediately rolled onto a slide and transported to the laboratory for DIF viral identification. In addition, a second sample was obtained from the patient and consisted of 1–2 ml of pooled saliva. The sample was collected in a sterile 15 ml centrifuge tube. The samples were immediately transported to the laboratory. The volume was determined and the sample was aliquoted for later testing.

The general procedure included sampling for viral identification at baseline, followed by the patients being instructed to rinse for 30 s with either 20 cm^3 of the experimental mouthrinse (Cool Mint LA), or in the case of the control patients with 20 cm^3 of sterile distilled water. Following the 30 s rinse, the patient expectorated and then a second sample of saliva was collected 30 s post rinse. The patient was then instructed to wait for 30 min. and a third sample of pooled saliva was collected. The second trial, carried out on 40 additional patients, was identical to the first trial with the exception that a fourth oral sample was taken 60 min. post rinse. All samples were transported to the laboratory for testing.

DIF for identification of HSV-1

While complement fixation, haemagglutination and other immunological tests have been developed to assist in the diagnosis of active herpetic infections, the fluorescent antibody test also called the DIF antibody test, for HSV is generally considered to be an extremely reliable assay available for the detection of viral antigens in patient samples (Arvin & Prober 1999). The approach is more sensitive and specific than the visualization of TZANCK cells and a specific diagnosis can be made earlier in the infection (Arvin & Prober 1999). In this test, the smears taken and applied to clinical laboratory slides, after fixation in 95% ethanol, were stained with a 1–2000 dilution in phosphate buffered saline (PBS) of monoclonal antibodies to HSV-1or HSV-2 (DAKO Corp, Carpinteria, CA, USA) that had been

conjugated with fluorescein. Staining was for 20 min. in a moist chamber at room temperature. The viral antigen was evaluated for evidence of the HSV-1 or -2 antigens following several washings with PBS using fluorescence microscopy.

Cell culture systems and stock viral cultures

Primary African monkey kidney cell cultures (Vero) are excellent indicator cells for evaluation of cytopathic effects (CPE) for HSV (Crane et al. 1980). Vero cells (American Type Culture Collection, ATCC) were grown in monolayer in 75 cm^2 plastic tissue culture flasks (Corning, NY) in RPMI-1640 complete medium (RPMI-1640 CM) with the addition of the antibiotics penicillin G sodium (100 U/ml) and streptomycin sulfate (100 µg/ml) and supplemented with 10% heat-inactivated fetal bovine serum, L-glutamine (2 mM), HEPES buffer (10 mM) and MEM sodium pyruvate (1 mM). Cells were incubated at 37°C in a humidified atmosphere consisting of 5% CO_2. As a positive control of the test system, Vero cells were inoculated with 1×10^6/ml of HSV-1 McIntyre strain (ATCC) (titer previously determined by standard methods). In the case of the experimental patient samples, the Vero cells were allowed to adsorb with 1 ml of 10-fold serial dilutions of the sample (10^{-1}–10^{-7}) for 1 h at 37°C with gentle rocking and supernatant fluids were then discarded.

Estimation of HSV-1 concentration using plaque assay

A standardized procedure was used for the plaque assays using Vero cells. Three-day grown monolayers of Vero cells, in triplicate on Falcon Petri dishes (60 × 15 mm) were overlayed with either 1 ml of the dilutions (10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}) of the HSV-1 McIntyre strain aliquot to be titrated or the diluted salivary samples from the patients. A Petri dish with a 3-day monolayer of uninfected Vero cells was included as a negative control. Following adsorption and aspiration of supernatant fluids, the plates were overlayed with RPMI-1640 CM (3 × concentration) in 1% methyl cellulose in a 1:2 v/v solution. The plates were incubated undisturbed at 37°C with 5% CO_2 and examined daily for 5 days. Supernatant fluids were aspirated from the plates at the end of 5 days and plaques were counted as clear zones within the monolayer of cells and expressed as plaque forming units per ml (PFU/ml).

Statistical analysis

Statistical analyses were performed on the data using a paired Student's *t* test to compare differences between the control and experimental groups at the pre-treatment, post treatment, 30 min. post treatment and, in the case of trial 2, 60 min. post treatment time periods. A *p*-value of less than 0.05 was considered significant.

Results

Ninety-two patients demonstrating clinical evidence of recurrent *Herpes* labialis and/or reporting a history consistent with recurrent herpetic perioral lesions were evaluated. In Trial 1, four of these patients and in Trial 2, eight of these patients were non-reactive by DIF and, therefore, no further evaluation of their samples was performed. In Trial 1, the sample population consisted of 19 males and 21 females with an average age of 29.2 years. Lesions were clinically evaluated according the staging scale described in Tables 1 and 2. Twenty-five of the patients demonstrated lesions consistent with the Stage 2, seven of the patients were in Stage 3 with some early rupture of vesicles and eight of the

Table 2. Trial #1 in vivo efficacy of LA against *Herpes simplex* virus *(PFU/ml)*

Pt #	Sex	Age	Stage	Pre	Post	30 min. post	DIF
1	F	24	3	1.6 × 10 (3)	0	1.0 × 10 (1)	HSV-1
2	F	36	2	3.8 × 10 (5)	0	6.7 × 10 (2)	HSV-1
3	M	27	1	6.1 × 10 (4)	0	5.8 × 10 (1)	HSV-1
4	F	41	1	4.0 × 10 (5)	0	0	HSV-1
5	M	29	2	5.4 × 10 (4)	0	1.6 × 10 (1)	HSV-1
6	M	21	2	7.1 × 10 (5)	0	8.9 × 10 (2)	HSV-1
7	F	29	2	9.0 × 10 (5)	1.0 × 10 (1)	5.7 × 10 (1)	HSV-1
8	F	40	3	4.7 × 10 (4)	0	0	HSV-1
9	M	38	2	9.8 × 10 (4)	0	0	HSV-1
10	M	32	1	5.1 × 10 (6)	3.7 × 10 (1)	3.6 × 10 (2)	HSV-1/2
11	F	20	3	8.7 × 10 (5)	0	0	HSV-1
12	M	28	3	4.6 × 10 (3)	0	0	HSV-1
13	M	34	1	7.4 × 10 (4)	0	0	HSV-1
14	F	29	2	2.6 × 10 (4)	0	0	HSV-1
15	M	44	2	3.7 × 10 (4)	0	1.9 × 10 (1)	HSV-1
16	F	21	2	6.1 × 10 (5)	0	8.4 × 10 (1)	HSV-1
17	F	20	1	1.8 × 10 (5)	0	0	HSV-1
18	M	39	2	3.1 × 10 (3)	0	0	HSV-1
19	M	22	2	4.6 × 10 (5)	0	2.6 × 10 (1)	HSV-1
20	F	35	2	5.5 × 10 (6)	0	1.0 × 10 (2)	HSV-1
21	F	26	1	2.2 × 10 (5)	7.4 × 10 (3)	9.4 × 10 (4)	HSV-1
22	M	38	2	1.9 × 10 (4)	1.0 × 10 (4)	1.0 × 10 (4)	HSV-1
23	M	33	2	2.7 × 10 (4)	9.1 × 10 (3)	3.1 × 10 (5)	HSV-1
24	M	25	1	8.6 × 10 (4)	6.4 × 10 (3)	4.6 × 10 (3)	HSV-1
25	F	44	2	7.4 × 10 (5)	6.1 × 10 (4)	9.1 × 10 (4)	HSV-1
26	M	23	3	6.8 × 10 (4)	7.8 × 10 (4)	5.4 × 10 (4)	HSV-1
27	M	37	3	6.8 × 10 (4)	7.8 × 10 (4)	5.4 × 10 (4)	HSV-1
28	F	29	2	7.8 × 10 (5)	3.7 × 10 (4)	8.1 × 10 (4)	HSV-1
29	F	25	2	6.1 × 10 (5)	2.9 × 10 (4)	5.1 × 10 (5)	HSV-1/2
30	F	30	2	1.8 × 10 (6)	1.0 × 10 (6)	1.0 × 10 (6)	HSV-1
31	F	23	2	1.7 × 10 (5)	9.1 × 10 (4)	7.0 × 10 (5)	HSV-1
32	M	24	2	2.9 × 10 (4)	8.4 × 10 (4)	3.0 × 10 (4)	HSV-1
33	F	32	2	3.8 × 10 (4)	2.6 × 10 (4)	6.4 × 10 (4)	HSV-1
34	F	19	1	9.6 × 10 (5)	3.1 × 10 (4)	5.0 × 10 (5)	HSV-1
35	M	28	3	4.1 × 10 (4)	3.7 × 10 (4)	1.9 × 10 (4)	HSV-1
36	M	20	2	3.7 × 10 (5)	7.8 × 10 (4)	9.0 × 10 (4)	HSV-1
37	F	31	2	6.7 × 10 (5)	5.5 × 10 (4)	4.1 × 10 (5)	HSV-1
38	F	18	2	4.0 × 10 (4)	2.8 × 10 (4)	4.9 × 10 (4)	HSV-1
39	F	26	2	6.8 × 10 (5)	1.2 × 10 (4)	6.1 × 10 (5)	HSV-1
40	M	23	2	7.3 × 10 (5)	6.0 × 10 (4)	9.8 × 10 (4)	HSV-1

DIF, direct immunofluorescence assay; LA, Listerine® Antiseptic; HSV, *Herpes simplex* virus; M, male; F, female; PFU, plaque forming units.

patients demonstrated mild erythema only with evidence of prodrome (Stage 1). The patients were randomly assigned to either the control or the experimental group and samples were taken.

The results of Trial 1 experimentation are illustrated in Table 2 and Fig. 2. Thirty-eight of the patients, by DIF, reacted with HSV-1 monoclonal antibodies; two of the patients reacted with HSV-1 and -2, a male and a female patient. A clinical photograph of a typical Stage 2/3 lesion is shown in Fig. 1. Although patients were accrued randomly, the tables are presented with those patients receiving the experimental rinse listed as numbers 1–20 and controls as 21–40. The data indicate that 18 of the experimental patients demonstrated zero recoverable virions following the 30 s rinse and nine of those patients remained at zero following the 30 min. delay. The other 11 patients returned to a 1–2 log recoverable sample. The pre-evaluation of recoverable virions was consistent with the literature (Arvin & Prober 1999) utilizing viral shedding in saliva.



Fig. 1. A typical stage 2/3 recurrent *Herpes* labialis lesion.

In Trial 2, the average age was 28 years with 19 females and 21 males. Twenty-nine stage 2, three stage 1 and eight stage 3 lesions were evaluated. Two HSV-1 and HSV-2 reactions were noted on DIF. Eighteen patients had zero recoverable virions post rinse, 12 remained zero after 30 min. and the log reductions were comparable with Trial 1. At 60 min. post rinse, all 20 patients were shedding 1–2 logs lower than their pre-rinse levels. In Trial 2, control patient data were comparable with Trial 1.(Fig. 3, Table 3)

Our recovery rate was consistent between the control sample and the experimental sample. The control patients were well distributed in gender and age distribution with the experimental patients. The rinsing with sterile distilled water resulted in a 1–2 log reduction in recoverable virions at the 30 s sampling, however, the return to numbers comparable with the pre-rinse state were found at 30 min. These data indicate a direct effect of LA on viral kill and some residual effect at 30 min. generally to the 3–4 log level of reduction.

Following statistical analysis, no significant differences were found between the control and the experimental groups at baseline. A significant difference ($p < 0.05$) was found between the control group and the experimental group following a 30 s rinse. A significant difference ($p < 0.05$) was found between the control group and the experimental group at 30 min. post rinse. When the controls were compared over the course of both Trials of the experiment, there were no significant changes noted from the post rinse time period to the 30 s time period as compared with baseline. However, there was a trend of a 1–2 log reductions using the sterile distilled water rinse. When the experimental subjects were compared over the course of the experiment, significant reductions were found at the $p < 0.001$ between baseline and the post rinse and between baseline and the post 30 min. rinse.

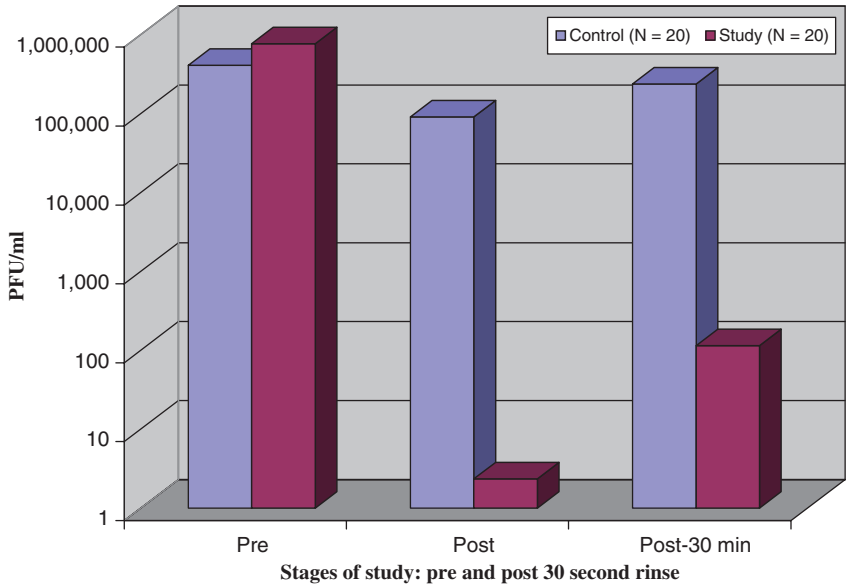


Fig. 2. Trial #1 mean recovered virions illustrating in vivo efficacy of Listerine® Antiseptic.

Conclusions

In a study investigating anti-microbial efficacy of mouthrinses, LA was found to completely kill microorganisms including methicillin-resistant *Staphylococcus aureus*, *Streptococcus pyogenes*, *Helicobacter pylori*, *Candida albicans*, *Streptococcus mutans*, *Actinomyces viscosus*, *Porphyromonas gingivalis*, *Prevotella intermedia* and *Actinobacillus actinomycetemcomitans* within 30 s when exposed to LA (Okuda et al. 1998). In the same study, LA was also reported to weakly inactivate HIV-1 viruses (Okuda et al. 1998).

Dennison et al. (1995) found chlorhexidine to completely inhibit HSV and influenza A virus for an exposure time of 30 s; whereas, rotavirus, a non-enveloped virus was only minimally inhibited (12.2%) (Dennison et al. 1995). Several other investigators also reported anti-HSV effects of LA and also found comparative ineffectiveness of LA and chlorhexidine in non-enveloped viruses (Bernstein et al. 1989, Croughan & Behbehani 1988, Park & Park 1989). In our in vitro study (Baqui et al. 2001), LA was found to possess slightly better anti-HSV effectiveness than 0.2% chlorhexidine. This work also confirmed the antiviral effectiveness of LA against HSV-1 McIntyre strain and

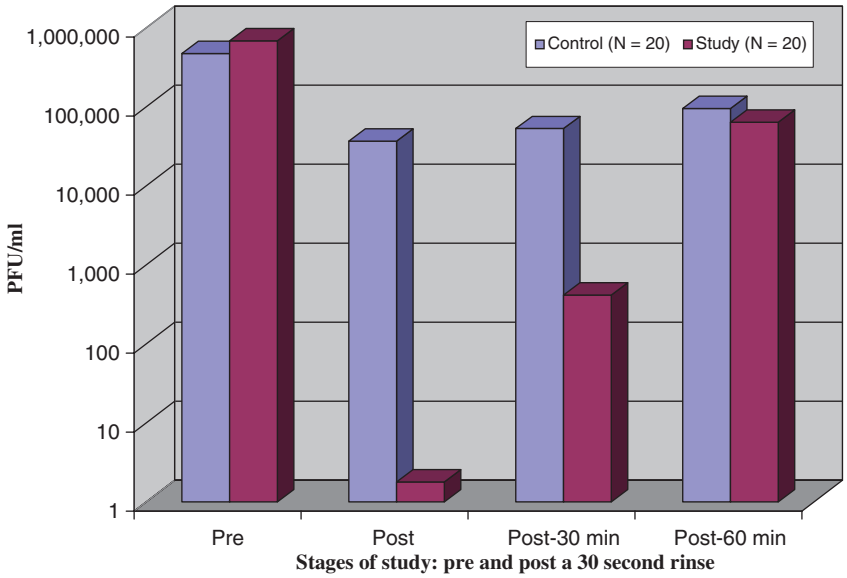


Fig. 3. Trial #2 mean recovered virions illustrating in vivo efficacy of Listerine® Antiseptic.

Table 3. Trial #2 in vivo efficacy of LA against *Herpes simplex* virus (PFU/ml)

Pt #	Sex	Age	Stage	Pre	Post	30 min. post	60 min. post	DIF
1	F	29	2	5.5 × 10 (6)	0	0	8.6 × 10 (3)	HSV-1
2	M	22	2	4.6 × 10 (5)	0	0	4.6 × 10 (1)	HSV-1
3	M	40	2	3.1 × 10 (4)	1.0 × 10 (1)	0	7.4 × 10 (3)	HSV-1
4	F	22	1	6.1 × 10 (5)	0	0	3.7 × 10 (3)	HSV-1
5	F	20	2	1.8 × 10 (5)	0	1.4 × 10 (1)	2.6 × 10 (4)	HSV1
6	M	34	2	3.7 × 10 (4)	0	6.7 × 10 (3)	6.1 × 10 (4)	HSV- 1
7	F	31	2	2.6 × 10 (3)	0	0	9.8 × 10 (3)	HSV-1
8	M	43	3	2.4 × 10 (4)	0	8.9 × 10 (2)	4.6 × 10 (5)	HSV-1
9	M	28	3	7.4 × 10 (4)	0	0	2.2 × 10 (4)	HSV-1
10	F	19	2	4.9 × 10 (3)	0	5.7 × 10 (2)	5.4 × 10 (5)	HSV-1
11	M	38	2	8.8 × 10 (4)	0	0	4.6 × 10 (3)	HSV-1/2
12	M	33	2	5.0 × 10 (5)	2.6 × 10 (1)	0	2.0 × 10 (4)	HSV-1
13	F	29	2	9.1 × 10 (4)	0	0	9.1 × 10 (4)	HSV-1
14	F	38	2	4.2 × 10 (4)	0	1.9 × 10 (1)	5.4 × 10 (3)	HSV-1
15	M	29	3	8.9 × 10 (5)	0	9.4 × 10 (1)	5.7 × 10 (2)	HSV-1
16	M	21	2	6.4 × 10 (4)	0	0	1.0 × 10 (3)	HSV-1
17	F	28	1	5.4 × 10 (4)	0	2.6 × 10 (1)	2.2 × 10 (3)	HSV-1
18	F	24	2	3.8 × 10 (5)	0	1.6 × 10 (1)	7.4 × 10 (3)	HSV-1
19	F	36	2	1.6 × 10 (3)	0	0	9.4 × 10 (2)	HSV-1
20	M	25	3	7.4 × 10 (4)	0	0	3.7 × 10 (3)	HSV-1
21	F	19	3	5.8 × 10 (6)	1.7 × 10 (5)	1.0 × 10 (5)	2.4 × 10 (5)	HSV-1
22	F	25	2	6.1 × 10 (5)	2.9 × 10 (5)	5.0 × 10 (5)	6.4 × 10 (5)	HSV-1/2
23	M	26	2	2.6 × 10 (4)	7.7 × 10 (3)	5.0 × 10 (3)	6.1 × 10 (4)	HSV-1
24	M	18	1	7.9 × 10 (4)	2.0 × 10 (5)	9.5 × 10 (4)	6.6 × 10 (4)	HSV-1
25	F	23	2	6.1 × 10 (5)	8.4 × 10 (4)	5.0 × 10 (4)	6.4 × 10 (4)	HSV-1
26	M	37	3	1.8 × 10 (4)	3.9 × 10 (3)	9.8 × 10 (3)	6.1 × 10 (3)	HSV-1
27	F	30	2	2.7 × 10 (4)	2.0 × 10 (2)	5.8 × 10 (3)	6.0 × 10 (3)	HSV-1
28	F	26	2	6.6 × 10 (5)	4.7 × 10 (4)	3.9 × 10 (3)	4.7 × 10 (4)	HSV-1
29	M	26	2	1.8 × 10 (5)	3.4 × 10 (4)	3.0 × 10 (4)	1.9 × 10 (5)	HSV-1
30	M	21	2	7.9 × 10 (5)	1.3 × 10 (3)	8.9 × 10 (3)	6.4 × 10 (4)	HSV-1
31	F	31	2	2.7 × 10 (4)	1.0 × 10 (4)	2.2 × 10 (3)	1.6 × 10 (4)	HSV-1
32	M	25	2	1.0 × 10 (5)	7.4 × 10 (3)	6.1 × 10 (4)	9.8 × 10 (3)	HSV-1
33	M	20	2	1.4 × 10 (4)	3.4 × 10 (4)	1.9 × 10 (3)	1.2 × 10 (4)	HSV-1
34	M	38	2	6.7 × 10 (4)	5.7 × 10 (3)	9.7 × 10 (3)	4.1 × 10 (1)	HSV-1
35	M	33	3	9.8 × 10 (3)	8.7 × 10 (3)	7.2 × 10 (4)	5.5 × 10 (3)	HSV-1
36	M	25	2	3.4 × 10 (5)	2.4 × 10 (4)	1.9 × 10 (4)	3.3 × 10 (5)	HSV-1
37	F	44	3	4.7 × 10 (4)	6.7 × 10 (3)	5.6 × 10 (4)	1.9 × 10 (4)	HSV-1
38	M	23	2	7.6 × 10 (3)	5.0 × 10 (3)	1.7 × 10 (4)	2.0 × 10 (4)	HSV-1
39	F	30	2	1.8 × 10 (4)	3.1 × 10 (3)	6.6 × 10 (3)	7.1 × 10 (4)	HSV-1
40	F	23	2	3.1 × 10 (4)	9.1 × 10 (3)	4.9 × 10 (3)	2.9 × 10 (4)	HSV-1

DIF, direct immunofluorescence assay; LA, Listerine® Antiseptic; HSV, *Herpes simplex* virus; M, male; F, female; PFU, plaque forming units.

extended the effectiveness to another enveloped virus, the HIV-1.

In our in vitro study, we developed a semi-quantitative tissue culture assay for measuring inactivation of HIV-1 in the presence of different dilutions of the reagents used. This utilized a sensitive CPE, which is a relatively rapid, inexpensive and simple method for determining infectivity. For in vitro and in vivo estimation of anti-HSV-1 effect, we have utilized a standard plaque formation assay on monolayers of Vero cells.

In this current in vivo study, the effects of rinsing with an essential oil containing mouthrinse (Cool Mint LA) resulted in effectively zero recoverable virions at 30 s post rinse and this reduction in viral presence in saliva remained at a significant reduction for approximately 30 min. for all experimental patients and a continued reduction at 60 min. post rinse in Trial 2. Rinsing with the control (sterile distilled water) resulted in a statistically non-significant reduction in viral presence in saliva at 30 s and no change from baseline at 30 min. These results are consistent with the previous finding of effects of LA on enveloped viruses reported by Dennison et al. (1995) and other investigators (Wood & Payne 1998). The implication of these studies is that when coupled with our in vitro studies previously published (Baqui et al. 2001) there is clinical efficacy in utilizing an oral rinse with an antimicrobial agent such as LA to reduce viral contamination of saliva.

The clinical significance may be that reduction in infectious virus levels at the level demonstrated in these experiments significantly reduces, but may not eliminate, the risk of cross contamination. The necessary level for infectivity in saliva has not been determined and may be the subject of future studies. The additional clinical implication is that the effects of antimicrobial rinses on enveloped viruses have been consistent in the literature and that other viruses such as the coronavirus recently identified in SARS (McIntosh 2002; Mufson 2000) may also be affected by rinsing with the antimicrobial agent LA. The societal value of such a rinse cannot be accurately assessed; however, any reduction in infectious virus levels should at least theoretically reduce the risk of transmission to uninfected individuals.

Acknowledgement

This research was supported in part by grants from Pfizer Consumer Healthcare, Morris Plains, NJ and from the AIDS International Training and Research Program.

References

Arvin, A. M. & Prober, C. G. (1999) *Herpes simplex* viruses. In: *Manual of Clinical Microbiology*, (eds). Murray, P. R., Baron, E. J., Pfaller, M. A., Tenover, F. C. & Yolken, R. H. pp. 878–887. Washington, DC: SM Press.

Baqui, A. A. M. A., Kelley, J. I., Jabra-Rizk, M. A., DePaola, L. G., Falkler, W. A. & Meiller, T. F. (2001) In vitro effect of oral antiseptics on human immunodeficiency virus-1 and *Herpes simplex* virus type 1. *Journal of Clinical Periodontology* **28**, 610–616.

Bernstein, D., Schiff, G., Echler, G., Prince, A., Feller, M. & Briner, W. (1989) In vitro virucidal effectiveness of a 0.12% chlorhexidine gluconate mouthrinse. *Journal of Dental Research* **69**, 874–876.

Crane, L. R., Gutterman, P. A., Chapel, T. & Lerner, A. M. (1980) Incubation of swab materials with *Herpes simplex* virus. *Journal of Infectious Diseases* **141**, 531–534.

Croughan, W. S. & Behbehani, A. M. (1988) Comparative study of inactivation of *Herpes simplex* virus type 1 and type 2 by commonly used antiseptic agents. *Journal of Clinical Microbiology* **26**, 213–215.

Dennison, D. K., Meredith, G. M., Shillitoe, E. J. & Caffesse, R. G. (1995) The antiviral spectrum of Listerine antiseptic. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics* **79**, 442–448.

McIntosh, K. (2002) Coronaviruses. In: Richman, D. D. , Whitley, R. J. & Hayden, F. G. (eds). *Clinical Virology*, 2nd edition, pp. 1087–1096. Washington, DC: ASM Press.

Mufson, M. A. (2000) Respiratory viruses. In: Specter, S.,, Hodinka, R. L. & Young, S. A. (eds). *Clinical Virology Manual*, 3rd edition, pp. 235–251. Washington, DC: ASM Press.

Okuda, K., Adachi, M. & Iijima, K. (1998) The efficacy of antimicrobial mouth rinses in oral health care. *Bulletin of Tokyo Dental College* **39**, 7–14.

Park, J. B. & Park, N.-H. (1989) Effect of chlorhexidine on the in vitro and in vivo herpes simplex virus infection. *Oral Surgery, Oral Medicine and Oral Pathology* **67**, 149–153.

Wood, A. & Payne, D. (1998) The action of three antiseptics/disinfectants against enveloped and non-enveloped viruses. *Journal of Hospital Infection* **38**, 283–295.

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Title

Comparison of In Vitro Inactivation of SARS CoV-2 with Hydrogen Peroxide and Povidone-Iodine Oral Antiseptic Rinses

Running Title

Comparison of In Vitro Inactivation of SARS CoV-2 with H_2O_2 and PVP-I Rinses

Authors (First Middle Last)

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/jopr.13220.

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Conflict of interest*: The authors deny any conflicts of interest.*

Funding: Sponsorship for this study was funded by Veloce Biopharma, Florida, USA. All authors had full access to all of the data in this study and take complete responsibility for the integrity of the data and accuracy of the data analysis.

ABSTRACT

Purpose: To evaluate the in vitro inactivation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) with hydrogen peroxide (H_2O_2) and povidone-iodine (PVP-I) oral antiseptic rinses at clinically recommended concentrations and contact times.

Materials and Methods: SARS-CoV-2, USA-WA1/2020 strain virus stock was prepared prior to testing by growing in Vero 76 cells. The culture media for prepared virus stock was minimum essential medium (MEM) with 2% fetal bovine serum (FBS) and 50 µg/mL gentamicin. Test compounds consisting of PVP-I oral rinse solutions and H_2O_2 aqueous solutions were mixed directly with the virus solution so that the final concentration was 50% of the test compound and 50% of the virus solution. Thus PVP-I was tested at concentrations of 0.5%, 1.25% and 1.5%, and H_2O_2 was tested at 3% and

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1.5% concentrations to represent clinically recommended concentrations. Ethanol and water were evaluated in parallel as standard positive and negative controls. All samples were tested at contact periods of 15 seconds and 30 seconds. Surviving virus from each sample was then quantified by standard end-point dilution assay and the log reduction value of each compound compared to the negative control was calculated.

Results: After the 15-second and 30-second contact times, PVP-I oral antiseptic rinse at all 3 concentrations of 0.5%, 1.25% and 1.5% completely inactivated SARS-CoV-2. The H_2O_2 solutions at concentrations of 1.5% and 3.0% showed minimal viricidal activity after 15 seconds and 30 seconds of contact time.

Conclusions: SARS-CoV-2 virus was completely inactivated by PVP-I oral antiseptic rinse in vitro, at the lowest concentration of 0.5 % and at the lowest contact time of 15 seconds. Hydrogen peroxide at the recommended oral rinse concentrations of 1.5% and 3.0% was minimally effective as a viricidal agent after contact times as long as 30 seconds. Therefore, preprocedural rinsing with diluted PVP-I in the range of 0.5% to 1.5% may be preferred over hydrogen peroxide during the COVID-19 pandemic.

Index words: Povidone-iodine; Hydrogen peroxide; Oral rinse; SARS-CoV-2; Corona virus; Dental safety

The coronavirus disease 2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has resulted in exceptional challenges to infection control procedures in the dental office.[1,2] Human modes of transmission of SARS-CoV-2 from infected individuals can occur by simple procedures such as breathing, talking, coughing or sneezing.[2,3] It has

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been accepted that the oral cavity including oropharynx, and the nasopharynx have the highest viral loads.[2-4] Adding to the risk is the nature of dental care, which involves close physical contact of dental professionals with patients, and constant exposure to saliva which is known to have a high load of oral microbes including virus.[2-4] Therefore, any dental procedure, especially prosthodontic procedures, which can aerosolize contaminated saliva (using handpieces, air-water syringes and ultrasonic instrumentation), has the potential to significantly increase airborne transmission of the virus.[5-7] All of this places dental professionals at a significantly higher risk for infection, and be a source for further transmission of infection. This behooves dental professionals to consider additional and augmented protocols for infection control in the dental office.[6,7]

The nasal and oral cavities are considered a major portal of entry for SARS-CoV-2, and are directly associated with the evolutionary process of COVID-19. They are involved in inhalation of virus containing droplet particles and aerosols in the ambient regions, as well as in expectorations of virus containing droplets.[8,9] Therefore, a significant amount of attention has been paid during the COVID-19 pandemic towards enhancing personal protection equipment (PPE) for dental professionals.[9] Some examples include higher particulate filtration masks (N-95), respirators and face shields.[9-11] Additional equipment such as routine use of high efficiency particulate air (HEPA) filters and improved designs for high volume evacuator (HEV) have also been advocated.[10,11]

An additional component of protection that has recently begun to receive increased attention is the use of preprocedural oral rinses.[2,3,7,12] Due to the knowledge that the oropharyngeal region is a major site of viral replication during the early asymptomatic stages of COVID-19, it has been suggested that oral antiseptic rinses that target the lipid envelope of SARS- CoV-2, has the potential to reduce viral load in the oropharynx.[12] There is increasing recognition that oral antiseptic rinses with significant viricidal activity may have a role as potential therapeutic agents to inactivate

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infective particles generated in the oropharyngeal region.[7,12,13] Recent articles have discussed the importance of preprocedural oral antiseptic rinses for use on patients as well as dental and medical professionals, in order to reduce the risk of transmission associated with viral shedding from asymptomatic individuals.[6,7,14]

A variety of oral antiseptic rinses have been suggested in recent literature for preprocedural use to reduce viral transmission.[12] Oral rinses ranging from chlorhexidine gluconate, ethanol, essential oils, povidone-iodine (PVP-I), hydrogen peroxide (H_2O_2) chlorinated water, hypertonic saline, bioflavonoids, cyclodextrins, cetylpyridinium chloride have been recommended.[10-12] Presently, there are no clinical studies reported on any oral antiseptic rinses specifically against SARS-CoV-2. A recent in vitro study of PVP-I oral rinses has demonstrated complete inactivation of SARS-CoV-2 at concentrations between 0.5% and 1.5% and contact times as little as 15 seconds.[7] Though PVP-I solutions at concentrations below 2.5% have been demonstrated to be safe for routine, repeated use in the oral cavity, they are not recommended for patients with active thyroid disease, pregnancy, anaphylactic allergy and in patients undergoing radioactive iodine therapy.[15-17]

Therefore, it would be helpful to study alternatives to PVP-I to mitigate these contraindications to allow a broader population to seek the benefits of preprocedural oral rinsing. Hydrogen peroxide oral rinse is a popular rinse anecdotally used by dentists due to its long history of use in teeth whitening procedures.[18,19] It has also been recommended by the American Dental Association (ADA) as a preprocedural rinse option during the COVID-19 pandemic.[10] Some of the advantages of H_2O_2 include easy accessibility, low cost and long track record in dentistry.[18,19] However its disadvantages include its potential for toxicity under routine use by dental professionals,[18] gastric and colon disturbances,[20] inactivation in the mouth due to host catalase activity in the saliva,[21] and absence of any clinical or in vitro evidence for viricidal activity against SARS-CoV-2.[12]

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The purpose of this study was to compare in vitro inactivation of SARS CoV-2 with hydrogen peroxide (H_2O_2) and povidone-iodine (PVP-I) oral antiseptic rinses at clinically recommended concentrations and clinically convenient timescales. The null hypotheses were that there would be no difference between viricidal activity of H_2O_2 and PVP-I and that there would be no difference in viricidal activity at varied concentrations and contact times for both types of oral rinses.

Materials and methods

All laboratory procedures with SARS-CoV-2 was conducted in biosafety level 3 (BSL-3) laboratories at The Institute for Antiviral Research at Utah State University at Logan, Utah, following established standard operating procedures approved by the Utah State University Biohazards Committee. Hydrogen peroxide 6% (w/w) and 3% (w/w) oral rinse solutions were prepared from dilution of purified United States Pharmacopeia (USP) grade hydrogen peroxide 30% aqueous solutions (Sigma-Aldrich; St. Louis, MO) with sterile deionized water. Povidone-iodine (PVP-I) oral rinse antiseptic solutions (Veloce BioPharma; Fort Lauderdale, FL) at concentrations of 3.0%, 2.5% and 1.0% were prepared as well. SARS-CoV-2, USA-WA1/2020 strain, virus stock was prepared prior to testing by growing in Vero 76 cells. Culture media for the prepared stock (test media) was minimum essential medium (MEM) with 2% fetal bovine serum (FBS) and 50 µg/mL gentamicin.

The 5 test compounds were then incubated in a 1:1 ratio with the virus solution so that the final concentration of each individual test compound was 50% of the starting concentration. The test solutions were chosen to represent the recommended PVP-I and peroxide rinse concentrations after 1:1 dilution with virus stock solution, in order to evaluate the clinically significant concentrations. A single concentration of each sample was tested in triplicate. Test media without any virus was added to 2 tubes of the compounds to serve as toxicity and neutralization controls. Ethanol (70%) was tested in parallel as a positive control and water only, as a negative control. Incubation times of 15

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seconds and 30 seconds were chosen to represent reasonable and conveniently achievable rinse and gargle times for both patients and providers in clinical settings. The test solutions and virus were incubated at room temperature (22 ±2°C) for 15 seconds and for 30 seconds per standard testing protocols. The solutions were then neutralized by a 1/10 dilution in MEM with 2% FBS, 50 µg/mL gentamicin.

The surviving virus from each sample was quantified by standard end-point dilution assay. Briefly, the neutralized samples were pooled and serially diluted using eight log dilutions in test medium. Then 100 µL of each dilution was plated into quadruplicate wells of 96-well plates containing 80-90% confluent Vero 76 cells. The toxicity controls were added to an additional 4 wells of Vero 76 cells and 2 of those wells at each dilution were infected with virus to serve as neutralization controls, ensuring that residual sample in the titer assay plate did not inhibit growth and detection of surviving virus. Plates were incubated at 37 ±2°C with 5% CO_2 for 5 days. Each well was then scored for presence or absence of infectious virus by examining for any cytopathic effect (CPE) in the wells. Briefly, after incubation in wells of known dilution containing susceptible cells, if any active virus was present at the start of the incubation, the virus grew resulting in a CPE in the wells where it was plated. Using the CPE count and the dilution of that well, the concentration that allowed the virus to grow in the cells and cause the CPE, were used for calculation. The titers were then measured using a standard endpoint dilution 50% cell culture infectious dose (CCID50) assay calculated using the Reed-Muench equation.[22] Subsequently, the log reduction value (LRV) of each compound compared to the negative (water) control was calculated.

Results

The virus titers and log reduction value of SARS-CoV-2 when incubated with a single concentration of the test compounds for 15 seconds are shown in Table 1. After the 15-second contact period, all 3 of

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the PVP-I tested solutions were effective at reducing >4.33 log10 CCID50 infectious virus from 5.0 log10 CCID50/0.1 mL to <0.67 log10 CCID50/0.1 mL. The H_2O_2 solutions at the clinically recommended and commercially available concentrations of 3.0% and 1.5% had minimal viricidal activity after 15 seconds. Table 2 shows the virus titers and LRV of SARS-CoV-2 when the virus was incubated for 30 seconds with each of the 5 test compounds at 50/50 ratio. For the 30-second second contact time, the compounds had nearly the same effect as the 15 second contact time, with PVP-I showing more effective viricidal activity than H_2O_2. Both the positive control (ethanol) and neutralization controls (water) performed as anticipated.

Discussion

The purpose of this study was to compare in vitro inactivation of SARS CoV-2 with hydrogen peroxide (H_2O_2) and povidone-iodine (PVP-I) oral antiseptic rinses at different clinically recommended concentrations and contact times of 15 seconds and 30 seconds. The first null hypothesis related to the comparison of the 2 tested oral rinses was rejected and the second null hypothesis related to contact times for PVP-I was not rejected. The findings from this study confirm the results of a recent study by Bidra et al[7] which also showed that PVP-I oral rinses completely inactivated SARS CoV-2 with a concentration as low as 0.5% and contact time as low as 15 seconds.

The Centers for Disease Control and Prevention (CDC) has suggested chlorhexidine gluconate, essential oils, PVP-I or cetylpyridinium chloride as options for preprocedural rinsing before dental procedures.[11] The American Dental Association (ADA) interim guidelines has suggested only 1.5% H_2O_2 or 0.2% PVP-I as options for preprocedural oral rinsing.[10] Findings from this study showed that H_2O_2 had weak viricidal activity and the log reduction value for low-concentrations of PVP-I were 3 times higher than H_2O_2 at the tested concentrations and contact times. It is possible that

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commercially available H_2O_2 oral rinses may have additional ingredients incorporated to improve the viricidal activity and that the viricidal activity may be better or worse at increased contact times. However, the 15 and 30 second contact times were chosen to represent convenient, routinely achievable and recommended time periods for oral rinsing in the clinical setting.[23] In light of the findings of this study, the recommended practice of preprocedural rinsing with hydrogen peroxide in solutions at concentrations between 1.5% and 3% may not be effective and therefore the current guidelines from the ADA may need to be updated. Additional concerns related to local toxicity of hard and soft tissues from routine use of H_2O_2 also requires further investigation. Furthermore, additional alternatives for patients unable to use PVP-I oral rinses should be investigated soon.

PVP-I oral rinses at non-toxic dilute concentrations have been shown to inactivate viruses related to severe acute respiratory syndrome (SARS), middle east respiratory syndrome (MERS) and now SARS-CoV-2 as well.[7,24,25] Randomized clinical trials on samples of COVID-19 positive patients comparing dilute PVP-I oral rinses as the standard agent against other oral rinses is the next important next step in further determination of the ideal pre-procedural rinse strategy for routine dental care. In the interim, dental professionals and patients can benefit from routine preprocedural rinsing with at least 0.5% PVP-I as it is inexpensive, safe for use in oral cavity up to 2.5%, rarely allergic, easily accessible, and has also been listed as a broad-spectrum antimicrobial agent for topical uses on the World Health Organization's List of Essential Medicines.[7,13,26,27] PVP-I at 0.23% concentration is routinely used in Japan and has also been recommended by the Japanese Ministry of Health, Labor and Welfare for daily gargling to prevent upper respiratory tract infections.[12,24,25] Despite its brown color PVP-I oral rinse has not been shown to stain teeth or cause a change in gustatory function.[28-30] One clinical trial showed significantly less staining of PVP-I and higher preference among patients compared to chlorhexidine gluconate.[30] The predictable and verifiable in vitro viricidal effect of PVP-I at very low concentrations and contact times may open up new

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possibilities not only in the field of management of viral infectious diseases in medicine, but also in improvement of dental and medical equipment and related water lines.

Conclusions

SARS-CoV-2 virus was completely inactivated by PVP-I oral antiseptic rinse in vitro, at all concentrations tested in as little as 15 seconds. Hydrogen peroxide solutions at the recommended oral rinse concentrations of 1.5% and 3.0% showed minimal viricidal effect after contact times as long as 30 seconds. Until further clinical research is available, preprocedural oral rinsing using dilute PVP-I in the range of 0.5% to 1.5% (for patients and health care providers) should be the preferred choice over hydrogen peroxide, during the COVID-19 pandemic. This additional procedure has the ability to be an important adjunctive to personal protective equipment, masks and hand hygiene procedures.

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References

1. Volgenant CMC, Persoon IF, de Ruijter RAG et al: Infection control in dental health care during and after the SARS-CoV-2 outbreak. Oral Dis 2020 . In press.
2. Peng X, Xu X, Li Y, et al: Transmission routes of 2019-nCoV and controls in dental practice. Int J Oral Sci 2020;12:9
3. Carrouel F, Conte MP, Fisher J, Gonet al: COVID-19: A Recommendation to Examine the Effect of Mouthrinses with β-Cyclodextrin Combined with Citrox in Preventing Infection and Progression. J Clin Med 2020;15;9:1126
4. Zou L, Ruan F, Huang M, et al: SARS-CoV-2 viral load in upper respiratory specimens of infected patients. N Engl J Med 2020;382:1177–9
5. Harrel SK, Molinari J: Aerosols and splatter in dentistry: a brief review of the literature and infection control implications. J Am Dent Assoc 2004;135:429-437
6. Tessema B, Frank S, Bidra A: SARS-CoV-2 Viral Inactivation Using Low Dose Povidone-Iodine Oral Rinse-Immediate Application for the Prosthodontic Practice. J Prosthodont 2020. In press
7. Bidra AS, Pelletier JS, Westover JB, et al: Rapid In vitro Inactivation of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Using Povidone-Iodine Oral Antiseptic Rinse. J Prosthodont 2020. In press.
8. To KK, Tsang OT, Leung WS, et al: Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. Lancet Infect Dis 2020;20:565-574
9. Chu DK, Akl EA, Duda S, et al: Physical distancing, face masks, and eye protection to prevent person-to-person transmission of SARS-CoV-2 and COVID-19: a systematic review and meta-analysis. Lancet 2020; S0140-6736:31142-9

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10. ADA Interim Guidance for Minimizing Risk of COVID-19 Transmission. https://www.ada.org/~/media/CPS/Files/COVID/ADA_COVID_Int_Guidance_Treat_Pts.pdf?utm_source=cpsorg&utm_medium=cpsalertbar&utm_content=cv-pm-ebd-interimresponse&utm_campaign=covid-19. Accessed May 28, 2020
11. The Centers for Disease Control and Prevention (CDC). Interim Infection Prevention and Control Guidance for Dental Settings During the COVID-19 Response. https://www.cdc.gov/coronavirus/2019-ncov/hcp/dental-settings.html Accessed May 28, 2020
12. O'Donnell VB, Thomas D, Stanton R et al: Potential role of oral rinses targeting the viral lipid envelope in SARS-CoV-2 infection. Function 2020. In press.
13. Gui D, Pepe G, Magalini S: Just one more hygiene practice in COVID-19. Eur Rev Med Pharmacol Sci 2020;24:3438-3439
14. Frank S, Capriotti J, Brown SM et al: Povidone-Iodine Use in Sinonasal and Oral Cavities: A Review of Safety in the COVID-19 Era. Ear Nose Throat J 2020. In press.
15. Foley TP Jr: The relationship between autoimmune thyroid disease and iodine intake: a review. Endokrynol Pol 1992;43 Suppl 1:53-69
16. Furudate S, Nishimaki T, Muto T: 125I uptake competing with iodine absorption by the thyroid gland following povidone-iodine skin application. Exp. Anim 1997;46:197-202
17. Gray PEA, Katelaris CH, Lipson D: Recurrent anaphylaxis caused by topical povidone iodine (Betadine). J Paediatr Child Health 2013;49:506-7
18. Walsh LJ: Safety issues relating to the use of hydrogen peroxide in dentistry. Aust Dent J. 2000;45:257-69

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19. Caruso AA, Del Prete A, Lazzarino AI: Hydrogen peroxide and viral infections: A literature review with research hypothesis definition in relation to the current covid-19 pandemic. Med Hypotheses 2020. In press.
20. Zanelli M, Ragazzi M, De Marco L: Chemical gastritis and colitis related to hydrogen peroxide mouthwash. Br J Clin Pharmacol 2017;83:427-428
21. Kraus FW, Perry WI, Nickerson JF: Salivary catalase and peroxidase values in normal subjects and in persons with periodontal disease. Oral Surg Oral Med Oral Pathol 1958;11: 95–102
22. Reed, LJ, Muench, H: A simple method of estimating fifty percent endpoints. Am J Epidemiol 1938;27:493-497
23. Osso D, Kanani N: Antiseptic mouth rinses: an update on comparative effectiveness, risks and recommendations. J Dent Hyg 2013;87:10-8
24. Kariwa H, Fujii N, Takashima I: Inactivation of SARS coronavirus by means of povidoneiodine, physical conditions and chemical reagents. Dermatology 2006;212 Suppl: 119–23
25. Eggers M, Koburger-Janssen T, Eickmann M, et al: In vitro bactericidal and virucidal efficacy of povidone-iodine gargle/mouthwash against respiratory and oral tract pathogens. Infect Dis Ther 2018;7:249-259
26. Madan PD, Sequeira PS, Shenoy K, et al: The effect of three mouthwashes on radiation-induced oral mucositis in patients with head and neck malignancies: a randomized control trial. J Cancer Res Ther 2008;4:3–8
27. World Health Organization. Essential medicines and health products. https://www.who.int/medicines/publications/essentialmedicines/en/ Accessed June 20, 2020
28. Kovesi G: The use of betadine antiseptic in the treatment of oral surgical, paradontological and oral mucosal diseases. Fogorvosi szemle 1999;92:243-250

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29. Kanagalingam J, Feliciano R, Hah JH, et al: Practical use of povidone-iodine antiseptic in the maintenance of oral health and in the prevention and treatment of common oropharyngeal infections. Version 2. Int J Clin Pract 2015;69:1247-56
30. Fine PD: A clinical trial to compare the effect of two antiseptic mouthwashes on gingival inflammation. J Hosp Infect 1985;6 Suppl A:189-93

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Table 1. Viricidal efficacy of test compounds against SARS-CoV-2 after a 15-second incubation with virus at $22 \pm 2°C$

Test Product	Percent concentration of PVP-I as tested	Percent concentration of H_2O_2 as tested	Incubation Time (in seconds)	Virus[a] Titer	LRV[b]
PVP-I 1.0% Oral Rinse	0.5	0	15	<0.67	>4.33
PVP-I 2.5% Oral Rinse	1.25	0	15	<0.67	>4.33
PVP-I 3.0% Oral rinse	1.5	0	15	<0.67	>4.33
H_2O_2 3.0%	0	1.5	15	≤3.67	1.33
H_2O_2 6.0%	0	3.0	15	≤4.0	1.00
Ethanol Control	N/A	N/A	15	<0.67	>4.33
Virus Control	N/A	N/A	15	5.0	N/A

[a]Log_{10} $CCID_{50}$ of virus per 0.1 mL. The assay lower limit of detection is 0.67 Log_{10} $CCID_{50}$/0.1 mL.

[b]LRV (log reduction value) is the reduction of virus compared to the virus control

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Table 2. Viricidal efficacy of test compounds against SARS-CoV-2 after a 30-second incubation with virus at $22 \pm 2°C$.

Test Product	Percent concentration of PVP-I as tested	Percent concentration of H_2O_2 as tested	Incubation Time (in seconds)	Virus[a] Titer	LRV[b]
PVP-I 1.0% Oral Rinse	0.5	0	30	<0.67	>3.63
PVP-I 2.5% Oral Rinse	1.25	0	30	<0.67	>3.63
PVP-I 3.0% Oral rinse	1.5	0	30	<0.67	>3.63
H_2O_2 3.0%	0	1.5	30	≤3.33	1.0
H_2O_2 6.0%	0	3.0	30	≤2.5	1.8
Ethanol Control	N/A	N/A	30	<0.67	>3.63
Virus Control	N/A	N/A	30	4.3	N/A

[a]Log_{10} $CCID_{50}$ of virus per 0.1 mL. The assay lower limit of detection is 0.67 Log_{10} $CCID_{50}$/0.1 mL.

[b]LRV (log reduction value) is the reduction of virus compared to the virus control

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