In vitro inactivation of SARS-CoV-2 using a povidone-iodine oral rinse

Manjunath Shet¹, Jonna Westover², Rosa Hong³, David Igo¹, Marc Cataldo⁴* and Sailaja Bhaskar¹

Abstract

Background: Healthcare professionals, especially dentists and dental hygienists, are at increased risk for contracting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) through air-borne particles and splatter. This study assessed the in vitro virucidal activity of 0.5% (w/v) povidone-iodine (PVP-I) oral rinse against SARS-CoV-2 to demonstrate its utility as a professional oral rinse.

Methods: A 0.5% (w/v) PVP-I oral rinse formulation, placebo oral rinse, and positive (70% [v/v] ethanol and water) and negative (water) controls were assessed using the time-kill method. SARS-CoV-2 was propagated in Vero 76 host cells. Following neutralization validation, triplicate tests were performed for each test formulation and virucidal activity measured at 15, 30, and 60 s and 5 min.

Results: The 0.5% (w/v) PVP-I oral rinse demonstrated effective in vitro virucidal activity against SARS-CoV-2 as early as 15 s after exposure; viral titer was reduced to < 0.67 log10 50% cell culture infectious dose (CCID50)/0.1 mL (log10 reduction of > 4.0) at 30 s, whereas the placebo oral rinse reduced the SARS-CoV-2 viral titer to 4.67 and 4.5 log10 CCID50/0.1 mL at the 15- and 30-s time points, with a log10 reduction of 0.63 and 0.17, respectively. No toxicity or cytotoxic effects against Vero 76 host cells were observed with the 0.5% (w/v) PVP-I oral rinse; positive and negative controls performed as expected.

Conclusions: In vitro virucidal activity of 0.5% (w/v) PVP-I oral rinse against SARS-CoV-2 was demonstrated. Rapid inactivation of SARS-CoV-2 was observed with 0.5% (w/v) formulation with a contact duration of 15 s. Clinical investigations are needed to assess the effectiveness of PVP-I oral rinse against SARS-CoV-2 in dental practice.

Keywords: Anti-infective agents, Coronavirus, Povidone-iodine, SARS-CoV-2, Virus inactivation

Background

Since the emergence of the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) toward the end of 2019, global daily cases peaked at almost 1.5 million in December 2020, and almost 18,000 daily deaths were reported globally in January 2021 [1]. This new and highly transmissible SARS-CoV-2 has impacted all levels of society; however, healthcare professionals are at a higher risk of contracting the virus as a result of their prolonged and repeated exposure to infected and highly contagious patients.

The principal route of transmission of SARS-CoV-2 is via respiratory droplets and the upper respiratory tract. However, an emerging hypothesis suggests a vascular route of transfer of SARS-CoV-2 from the oral cavity to the respiratory system [2]. The virus can also spread through the conjunctiva [3], and high viral loads of SARS-CoV-2 have been detected in the nasopharynx and oropharynx of symptomatic as well as asymptomatic individuals [4].

Despite the use of personal protective equipment (PPE), certain healthcare professionals such as dentists and dental hygienists may be at a higher risk of exposure...
to the virus than others because aerosols and splatter released during dental procedures present an environment with a high risk of contamination through air-borne particles [5]. Consequently, the American Dental Association (ADA) interim guidelines (October 2020) and the Centers for Disease Control and Prevention (CDC) have proposed changes to dental procedures and pre-procedural oral rinse [6, 7]. Although there is currently no published evidence regarding the clinical effectiveness of preprocedural mouth rinses (PPMRs) to prevent SARS-CoV-2 transmission, PPMRs with an antimicrobial product such as chlorhexidine gluconate, essential oils, povidone-iodine (PVP-I), or cetlypyridinium chloride may reduce the level of oral microorganisms in aerosols and spatter generated during dental procedures [7]. Furthermore, while there currently seems to be insufficient scientific evidence to support the use of hydrogen peroxide as an oral rinse, in vitro studies and small clinical studies or case reports conducted with ex-US formulations have demonstrated effective antiviral activity of PVP-I solutions against SARSCoV-2 [8–10]. A small-scale randomized study in SARS-CoV-2-positive patients assessing the efficacy of PVP-I, chlorhexidine gluconate, and cetlypyridinium chloride in reducing salivary SARS-CoV-2 viral load found that viral load was reduced for up to 6 h with cetlypyridinium chloride or PVP-I, whereas another in vivo test demonstrated a significant reduction in viral load for at least 3 h after PVP-I oral rinse in 50% of patients [9, 10]. In addition, the use of PVP-I formulations has been evaluated for high-risk clinical procedures involving the oropharynx and nasopharynx and in surgical practice [11–13].

PVP-I is an antiseptic agent with broad-spectrum antiviral activity against a variety of pathogenic microorganisms, including viruses [14–17], with demonstrated in vitro virucidal activity against enveloped and non-enveloped viruses over short contact times relative to other commercially available antiseptic agents [18–21]. The efficacy and tolerability profiles of PVP-I compared with that of other agents, such as chlorhexidine gluconate, polyhexanide, and octenidine, have been well established [13, 15, 16, 22], and no resistance or cross-resistance with PVP-I has been documented in the past [13–15].

SARS-CoV-2 has profoundly altered the fundamental dynamics of clinical dentistry worldwide and there is a great need to raise awareness among dental practitioners regarding the virucidal activity of commercially available oral rinses. Strikingly, a recent study assessing practitioners’ knowledge, attitude, and practices for oral rinse use amidst the pandemic revealed that only 38.9% of participants surveyed were aware that PVP-I had substantial virucidal activity against SARS-CoV, MERS-CoV, influenza virus, and rotavirus [23, 24].

The aim of this study was to investigate the in vitro virucidal activity of 0.5% (w/v) PVP-I oral rinse against SARS-CoV-2 at four different contact times to demonstrate its utility as a professional oral rinse.

Methods
Test formulations
A 0.5% (w/v) PVP-I oral rinse formulation (Betadine® Oral Rinse, Avrio Health L.P.) was assessed for its in vitro virucidal activity against SARS-CoV-2 and compared against a placebo oral rinse, a positive control comprising 70% (v/v) ethanol, and a negative control comprising water.

Virus strains and host cells
The SARS-CoV-2, strain USA-WA1/2020, was kindly provided by the World Reference Center for Emerging Viruses and Arboviruses at The University of Texas Medical Branch. The virus was passaged twice in Vero 76 cells (ATCC CRL-1587) to create the working stock.

Facilities
The assays were performed at the Institute for Antiviral Research, Utah State University, Logan, Utah, USA. Standard equipment and supplies were used; calibration was performed in accordance with the standard operating procedures of the facility. This study did not include animal experiments or human subject research.

Preparation of virus suspensions and host cells
The method has been described previously [25]. Briefly, virus strains were propagated and stored per standard procedure for the production of high-titer virus stock. The culture medium used for the virucidal assay (test medium) was Minimum Essential Medium (MEM) with 2% fetal bovine serum (FBS) and 50 μg/mL gentamicin. Host cells were maintained as monolayers in disposable cell culture labware. Before testing, these cultures were seeded onto multiwell cell culture–treated plates. For virucidal suspension testing, Vero 76 cell monolayers were grown to 80%–90% confluence.

Neutralization validation
Neutralization validation was performed to confirm the effectiveness of the procedure in neutralizing the active virucidal component; this was performed for the positive control, negative control, the active test formulation, and the respective placebo. Neutralization was validated when virus recovery in the positive control matched that in the neutralized formulation.
Virucidal suspension test

Triplicate tests were set up for each test formulation. Each test tube contained 50 μL of the test formulation mixed with 50 μL of the high-titer virus suspension. Samples were incubated at 22 ± 2 °C for designated exposure times of 15 s, 30 s, 60 s, and 5 min. At the designated exposure time, samples were neutralized by performing a 1/10 dilution in MEM + 2% FBS + 50 μg/mL gentamicin. Subsequently, samples were pooled and serially diluted 1/10 using eight log_{10} dilutions in the test medium before being added to quadruplicate columns of 96-well plates seeded with monolayers of 80%–90% confluent Vero 76 cells. The plates were incubated until a maximum cytotoxic effect was observed in the control wells, and cytotoxicity was recorded as a binary result. Negative controls were run, which were identical to the test formulation runs, except that water was substituted for the 0.5% (w/v) PVP-I oral rinse. Toxicity controls were run in four additional wells of Vero 76 cells, with two wells being infected with the virus at each dilution to serve as neutralization controls, ensuring that the residual sample in the titer assay plate did not inhibit growth and detection of the surviving viruses. The plates were incubated at 37 ± 2 °C in a 5% carbon dioxide (CO₂) atmosphere for 5 days. Each well was then scored for the presence or absence of infectious virus.

Analytical methods

Viral titers were reported as log_{10} of the 50% titration end point for infectivity, expressed as the 50% cell culture infectious dose (CCID\textsubscript{50}), and were calculated using the Reed-Muench method [26]. The log_{10} of infectivity reduction, or log_{10} reduction value (LRV), was calculated using the following formula: LRV = (log_{10} CCID\textsubscript{50} of the negative control) – (log_{10} CCID\textsubscript{50} of the virucidal suspension test).

Viral titer was calculated as the difference in log_{10} CCID\textsubscript{50}/0.1 mL between the negative control and the test formulation; this was done at the designated exposure times (i.e., 15 s, 30 s, 60 s, and 5 min).

Results

All test formulations were validated for neutralization across triplicates (data not shown). The 0.5% (w/v) PVP-I oral rinse demonstrated effective in vitro virucidal activity against SARS-CoV-2 as early as 15 s after exposure, with a reduction of viral titer to 2.5 and < 0.67 log_{10} CCID\textsubscript{50}/0.1 mL (log_{10} reduction of 2.8 and > 4.0) at 15 and 30 s, respectively, under test conditions, whereas the placebo oral rinse reduced the SARS-CoV-2 viral titer to 4.67 and 4.5 log_{10} CCID\textsubscript{50}/0.1 mL at the 15- and 30-s time points, with a log_{10} reduction of 0.63 and 0.17, respectively (Table 1). The efficacy of the positive and negative controls was also evaluated at exposure times

| Test formulation                        | Incubation time | Virus titer\textsuperscript{a} | LRV\textsuperscript{b} |
|-----------------------------------------|-----------------|---------------------------------|------------------------|
| 0.5% (w/v) PVP-I oral rinse             | 50              | 15 s                            | 2.5                    | 2.8                    |
| Placebo oral rinse                      | 50              |                                 | 4.67                   | 0.63                   |
| Positive control                        | 50              |                                 | 1.3                    | 4.0                    |
| Negative control                        | NA              |                                 | 5.3                    | –                      |
| 0.5% (w/v) PVP-I oral rinse             | 50              | 30 s                            | <0.67                  | > 4.0                  |
| Placebo oral rinse                      | 50              |                                 | 4.5                    | 0.17                   |
| Positive control                        | 50              |                                 | <0.67                  | > 4.0                  |
| Negative control                        | NA              |                                 | 4.67                   | –                      |
| 0.5% (w/v) PVP-I oral rinse             | 50              | 60 s                            | 1.0                    | 3.67                   |
| Placebo oral rinse                      | 50              |                                 | 5.0                    | 0                      |
| Positive control                        | 50              |                                 | <0.67                  | > 4.0                  |
| Negative control                        | NA              |                                 | 4.67                   | –                      |
| 0.5% (w/v) PVP-I oral rinse             | 50              | 5 min                           | <0.67                  | > 4.0                  |
| Placebo oral rinse                      | 50              |                                 | 4.67                   | 0                      |
| Positive control                        | 50              |                                 | <0.67                  | > 4.0                  |
| Negative control                        | NA              |                                 | 4.67                   | –                      |

\textsuperscript{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

\textsuperscript{b} LRV is the reduction of the virus compared with that of the negative control.

Table 1: In vitro virucidal activity of 0.5% (w/v) PVP-I oral rinse formulation against SARS-CoV-2

Samples were incubated at 22 ± 2 °C for designated exposure times of 15 s, 30 s, 60 s, and 5 min.

Abbreviations: CCID\textsubscript{50}, 50% cell culture infectious dose; LRV, log_{10} reduction value; NA, not applicable; PVP-I, povidone-iodine; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; w/v, weight per volume.
cells were observed with the 0.5% (w/v) PVP-I oral rinse. As expected, no cytotoxic effects against the Vero 76 host cells were observed with the 0.5% (w/v) PVP-I oral rinse, and the positive and negative controls performed as expected. No toxicity was observed with the 0.5% (w/v) PVP-I oral rinse, and the positive and negative controls performed as expected. No cytotoxic effects against the Vero 76 host cells were observed with the 0.5% (w/v) PVP-I oral rinse.

**Discussion**

Several in vitro studies suggest that antiseptics may reduce the viral load of SARS-CoV-2 or other coronaviruses; however, limited in vivo evidence for oral antiseptics currently exists. Furthermore, available in vivo studies have several limitations such as small sample sizes and lack of suitable control groups, which leads to conflicting or inconclusive efficacy results [27]. In addition, accurate collection and measurement/quantification of SARS-CoV-2 viral load through cycle count amplification can lead to imprecise results. The current study thus set out to demonstrate in vitro, the virucidal activity of the PVP-I oral rinse at a concentration of 0.5% (w/v) PVP-I and as early as 15 s after being challenged with SARS-CoV-2. The observed log_{10} reduction in the viral titer of > 4.0 at 30 s after the contact time and lasting up to 5 min is indicative of rapid and prolonged viral inactivation. Intrinsic variance of the assay due to visual determination of the cytopathic effect is the likely cause for the LRV of 3.67 observed at 60 s. In agreement with these findings, a recent study has also shown that oral rinses containing povidone-iodine as the active compound reduce viral infectivity to up to three orders of magnitude to background levels [28].

Physical intervention, as an adjunct to supplement the use of PPE, to reduce viral transmission during the current SARS-CoV-2 pandemic and beyond may be a necessary step to ensure a safe environment for patients and healthcare workers alike. Oral rinses that can reduce the viral load in preprocedural settings, especially those where patients’ mouths and noses are exposed, may be a critical component in the prevention of transmission. Indeed, an observational study on the tolerability and usability of 0.5% (w/v) PVP-I gargles and nasal drops in 6,692 patients attending ENT consultations as a prerequisite examination reported these formulations to be feasible and useable and that they provide a needed benefit in preventing transmission between patients and healthcare workers [29]. In addition, the in vivo application of PVP-I has also been proposed to reduce viral load in otorhinolaryngology surgical practices. The use of 0.5% (w/v) PVP-I has several advantages such as its ease of preparation, cost-effectiveness, safe use, and its potential to reduce viral titers of SARS-CoV-2 [30]. Currently available alternatives to the PVP-I oral rinse comprise chlorhexidine gluconate rinse, which has been shown to have weak virucidal activity [31], and hydrogen peroxide, which, although recommended by the ADA, has insufficient supporting scientific evidence to be classified as effective for the prophylaxis of SARS-CoV-2 infection [6, 7, 31]. In addition, a systematic review reported that there is currently no available clinical evidence to support the use of nasal sprays or oral rinses to protect against SARS-CoV-2 transmission among healthcare workers but indicated that three studies (including two phase 2 randomized controlled trials) are underway to assess the level of protection that can be expected from these modes of antiseptic administration [32]. Indeed, results from a recent randomized clinical trial assessing the nasopharyngeal application of PVP-I solutions to reduce the viral load of patients with non-severe COVID-19 symptoms revealed that the use of PVP-I did not influence changes of viral ribonucleic acid (RNA) quantification over time. In addition, the study also reported a greater mean relative difference in viral titers between baseline and day 1 for participants in the intervention group (75%) compared with those in the control group (32%) [33]. This study adds to the emerging evidence that demonstrates the in vitro antiviral effectiveness of PVP-I oral rinse as early as 15 s against SARS-CoV-2 at a concentration of 0.5% (w/v). These findings suggest that the use of preprocedural PVP-I oral rinses as an adjunct to PPE for patients and healthcare providers is a viable option [6, 7], which is supported by the inclusion of PVP-I oral rinse into the World Health Organization (WHO) R&D Blueprint for COVID-19 Experimental Treatments [34].

**Conclusion**

This study demonstrated the in vitro virucidal activity of a PVP-I (0.5% w/v) oral rinse against SARS-CoV-2 using the time-kill method. Rapid inactivation of SARS-CoV-2 was observed at a concentration of 0.5% (w/v), with a contact duration of as early as 15 s. Following these promising results, clinically focused investigations are needed to assess the effectiveness of PVP-I oral rinse in the dental practice setting, perhaps including various strains or variants of SARS-CoV-2.

**Abbreviations**

- ADA: American Dental Association
- CCID_{50}: 50% Cell culture infectious dose
- CDC: Centers for Disease Control and Prevention
- CO_{2}: Carbon dioxide
- FBS: Fetal bovine serum
- LRV: Log_{10} reduction value
- MEM: Minimum essential medium
- MERS-CoV: Middle East respiratory syndrome-related coronavirus
- NA: Not applicable
- PPE: Personal protective equipment
- PPMRs: Preprocedural...
mout rinses; PVP-I: Povidone-iodine; RNA: Ribonucleic acid; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; w/v: Weight per volume; WHO: World Health Organization.

Acknowledgements
The authors thank the participating staff at the testing facility, namely the Institute for Antiviral Research in the Department of Animal, Dairy, and Veterinary Sciences, Utah State University, Logan, Utah, USA, for their contributions. Medical writing support was provided by Juliane Moloney, Ph.D., supporting Cactus Life Sciences (part of Cactus Communications) and funded by Avrio Health, L.P., a subsidiary of Purdue Pharma, L.P. The authors retained full control over the content of this manuscript and approved the final version for submission.

Authors’ contributions
MS, JW, RH, DI, and SB made substantial contributions to the conception and design of the work. All authors were involved in the acquisition, analysis, and interpretation of data; drafted the work; and substantively revised it. All authors approved the final version of this manuscript and agreed both to be personally accountable for their contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature. All authors read and approved the final manuscript.

Funding
This work was supported by Avrio Health, L.P., a subsidiary of Purdue Pharma, L.P.

Availability of data and material
All data generated and analyzed during this study are available from the corresponding author on reasonable request.

Declarations
Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
MS, DI, and SB are full-time employees of Imbrium Therapeutics, L.P, a subsidiary of Purdue Pharma, L.P. JW has no conflicts of interest to disclose. RH is a full-time employee of Avrio Health, L.P, a subsidiary of Purdue Pharma, L.P. MC is a full-time employee of Purdue Pharma, L.P.

Author details
1Imbrium Therapeutics L.P, Stamford, CT, USA. 2The Institute for Antiviral Research, Utah State University, Logan, UT, USA. 3Avrio Health L.P, New York, NY, USA. 4Purdue Pharma L.P, Stamford, CT 06901, USA.

Received: 16 September 2021 Accepted: 14 February 2022

Published online: 25 February 2022

References
1. Johns Hopkins University. Coronavirus Resource Center. 2021. https://coronavirus.jhu.edu/map.html.
2. Lloyd-Jones G, Molyam S, Pntes CC, et al. The COVID-19 pathway: A proposed oral-vascular-pulmonary route of SARS-CoV-2 infection and the importance of oral healthcare measures. J Oral Med and Dent Res. 2021;2:1–25.
3. Guerres-Vilahoz N, Burgos-Blasco B, Vidal-Villegas B, et al. Novel insights into the transmission of SARS-CoV-2 through the ocular surface and its detection in tears and conjunctival secretions: a review. Adv Ther. 2020;37:4086–95.
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;135:429–37.
6. The American Dental Association (ADA). Summary of ADA guidance during the COVID-19 crisis. 30 Oct 2020.
7. The Centers of Disease Control and Prevention (CDC). Interim infection prevention and control guidance for dental settings during the coronavirus disease 2019 (COVID-19) pandemic. 4 Dec 2020.
8. Cavalcante-Leão BL, de Araujo C-M, Bassi I-B, et al. Is there scientific evidence of the mouthwashes effectiveness in reducing viral load in COVID-19? A systematic review. J Exp Dent. 2021;13:e119–89.
9. Serevičiutė C, Balan P, Ko KKK, et al. Efficacy of commercial mouth-rinse on SARS-CoV-2 viral load in saliva: randomized control trial in Singapore. Infection. 2021;49:305–11.
10. Martínez Lamas L, Díez Dios P, Pérez Rodríguez MT, et al. Is povidone iodine mouthwash effective against SARS-CoV-2? First in vivo tests. Oral Dis 2020 July 2 [Epub ahead of print].
11. Yan CH, Bleier BS. Prophylactic and therapeutic topical povidone-iodine in coronavirus disease 2019 (COVID-19): what is the evidence? Int Forum Allergy Rhinol. 2020;10:1271–3.
12. Khan MM, Parab SR, Paranjape M. Repurposing 0.5% povidone iodine solution in otorhinolaryngology practice in Covid 19 pandemic. Am J Otolaryngol. 2020;41:102618.
13. Mohamed NA, Baharom N, Wan Sulaiman WS, et al. Early viral clearance among COVID-19 patients when gargling with povidone-iodine and essential oils: a clinical trial. medRxiv. 2020. https://doi.org/10.1101/2020.09.07.20180448.
14. Barreto R, Barrois B, Lambert J, Malhotra-Kumar S, Santos-Fernandes V, Monstreux S. Addressing the challenges in antiseptic: focus on povidone iodine. Int J Antimicrob Agents. 2020;56:106064.
15. Lachapelle JM, Castel O, Casado AF, et al. Antiseptics in the era of bacterial resistance: a focus on povidone iodine. Clin Pract. 2013;10:579–92.
16. Williamson DA, Carter GP, Howden BP. Current and emerging topical antibacterials and antiseptics: agents, action, and resistance patterns. Clin Microbiol Rev. 2017;30:827–60.
17. Eggers M. Infectious disease management and control with povidone iodine. Infect Dis Ther. 2019;8:581–93.
18. Eggers M, Eickmann M, Kawalski K, Zorn J, Reimer K. Povidone-iodine hand wash and hand rub products demonstrated excellent in vitro virucidal efficacy against Ebola virus and modified vaccinia virus Ankara, the new European test virus for enveloped viruses. BMC Infect Dis. 2015;15:375.
19. Eggers M, Eickmann M, Zorn J. Rapid and effective virucidal activity of povidone-iodine products against Middle East Respiratory Syndrome Coronavirus (MERS-CoV) and Modified Vaccinia Virus Ankara (MVA). Infect Dis Ther. 2015;4:491–501.
20. Kawana R, Kitamura T, Nakagomi O, et al. Inactivation of human viruses by povidone-iodine in comparison with other antiseptics. Dermatology. 1997;195(Supp1):29–35.
21. Hassandarvish P, Tiong V, Mohamed NA, et al. In vitro virucidal activity of povidone iodine gargle and mouthwash against SARS-CoV-2: implications for dental practice. Br Dent J 2020 Dec 10;1–4 [Epub ahead of print].
22. Kampf G, Todt D, Pfänder S, Steinhämm E. Persistence of SARS-CoV-2 in different environments at room temperature and in dry powder. Euro Surveill. 2022;27:2100720.
23. Maehara K, Masubuchi T, Nishi Y, et al. Effectiveness of a povidone-iodine nasal antiseptic for rapid inactivation of SARS-CoV-2 in viral aerosols. J Antimicrob Chemother. 2022;77:219–24.
24. Imran E, Khurshid Z, Adanir N, Ahi H, Almarzouki N, Baeshen HA. Dental practitioners’ knowledge, attitude and practices for mouthwash use amidst the COVID-19 pandemic. Risk Manag Healthc Policy. 2021;14:605–18. https://doi.org/10.2147/RMHP.S287547.
25. Imran E, Khurshid Z, Al Qadi AA, Al-Quraini AA, Tariq K. Preprocedural use of povidone-iodine mouthwash during dental procedures in the COVID-19 pandemic. Eur J Dent. 2020;14(5S):15182–4. https://doi.org/10.1055-s0-0040-1717001.
26. Frank S, Brown SM, Capriotti JA, Westover JB, Pelletier JS, Tessema B. Prophylactic and therapeutic topical povidone-iodine nasal solution for dental care in the COVID-19 crisis. 30 Oct 2020.
27. Mateos-Moreno MV, Mira A, Auzina-Márquez V, Ferrer MD. Oral antiseptics against coronavirus: in-vitro and clinical evidence. J Hosp Infect. 2021;113:30–43. https://doi.org/10.1016/j.jhin.2021.04.004.
28. Meister TL, Brüggemann Y, Todt D, Conzelmann C, Müller JA, Groß R, Münch J, Krawczyk A, Steinmann J, Steinmann J, Pfaender S, Steinmann E. Virucidal efficacy of different oral rinses against severe acute respiratory syndrome coronavirus 2. J Infect Dis. 2020;222(8):1289–92. https://doi.org/10.1093/infdis/jiaa471.

29. Khan MM, Parab SR. Tolerability and usability of 0.5% PVP-I gargles and nasal drops in 6692 patients: observational study. Am J Otolaryngol. 2021;42(1):102880. https://doi.org/10.1016/j.amjoto.2020.102880.

30. Khan MM, Parab SR. 0.5% Povidone iodine irrigation in otorhinolaryngology surgical practice during COVID-19 pandemic. Am J Otolaryngol. 2020;41(6):102687. https://doi.org/10.1016/j.amjoto.2020.102687.

31. Maurya RK, Singh H, Kapoor P, Sharma P, Srivastava D. Povidone-iodine preprocedural rinse—an evidence-based, second-line defense against severe acute respiratory coronavirus virus 2 (SARS-CoV-2) in dental healthcare. Infect Control Hosp Epidemiol 2021 March 12 [Epub ahead of print].

32. Burton MJ, Clarkson JE, Goulao B, et al. Use of antimicrobial mouthwashes (gargling) and nasal sprays by healthcare workers to protect them when treating patients with suspected or confirmed COVID-19 infection. Cochrane Database Syst Rev. 2020;9:CD013626

33. Guenezan J, Garcia M, Strasters D, et al. Povidone iodine mouthwash, gargle, and nasal spray to reduce nasopharyngeal viral load in patients with COVID-19: a randomized clinical trial. JAMA Otolaryngol Head Neck Surg. 2021;147(4):400–1. https://doi.org/10.1001/jamaoto.2020.5490.

34. World Health Organization. WHO R&D Blueprint COVID 19: Experimental Treatments. 2020. https://www.who.int/docs/default-source/coronaviruse/covid-classification-of-treatment-types-rev.pdf.

**Publisher’s Note**
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.