**INTRODUCTION**

Mouthwashes are widely used solutions in contemporary dental practice for rinsing the mouth, due to their ability to reduce the number of micro-organisms in the oral cavity and colony-forming units in dental aerosols. Oral cavity may act as a potential reservoir for the transmission of the highly contagious novel coronavirus as it has a high affinity to oral tissues and saliva. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been detected up to 91.7% in the saliva of COVID-19 patients, including the presence of live virus. A human cellular receptor angiotensin-converting enzyme-2 present in oral epithelial cells is an important receptor for COVID-19 infectivity as it binds to the viral attachment protein, the spike glycoprotein, which makes oral cavity a critical site for disease transmission. Asymptomatic cases, harboring high viral loads, may be potential transmitters of the disease to their contacts, which could be the treating dental practitioners and their team as well. Although the evidence to date regarding the prevention of SARS-CoV-2 transmission with the use of preprocedural rinse in dental offices is still in its infancy, yet many health regulatory agencies have recommended the use of preprocedural mouthwashes before oral procedures to keep a safe margin.

To date, the available little evidence that has recommended the use of a preprocedural mouth rinse to “reduce the polymicrobial load present in patients’ saliva” suggests the use of...
povidone-iodine 1%, hydrogen peroxide 1%, chlorhexidine 0.2%, cetlypyridinium chloride 0.1% and essential oils. However, there are quite a few conflicting reports regarding the effectiveness of chlorhexidine against SARS-CoV-2, despite the chlorhexidine being the mainstay for the dentists for decades. The current work evaluated the effectiveness of the contemporary gold-standard chlorhexidine and also povidone iodine as antiviral agents against SARS CoV 2, in an in vitro setting, by quantitative reverse-transcription polymerase chain reaction (qRT-PCR) testing.  

**Study methods**  
All the experiments involving the handling of SARS-CoV-2 virus were performed in the BSL3 facility at the Council of Scientific and Industrial Research-Institute of Microbial Technology. The SARS-CoV-2 strain used in the study was isolated from an Indian patient and cultured using the VeroE6 cell line.  

**MATERIALS AND METHODS**  
Twenty percent chlorhexidine digluconate was procured from Sigma Aldrich (Cat. Nos. C9394) as test compound and povidone iodine was procured from the market as a commercially available mouth rinse. Chlorhexidine digluconate was reconstituted in the concentrations of 0.2% and 0.12%. Povidone iodine as available commercially in 2% was diluted to achieve 1% effective concentration. The analysis was carried out at two contact time points that is 30 and 60 s.  

**STUDY METHOD**  
The SARS-CoV-2 virus stock was prepared by cultivating virus using VeroE6 cell line. For test agents, 2 µL of virus stock (pfu ~2 × 10^7/mL) was mixed with 18 µL of the test sample. All the samples were incubated for 30 s and 60 s. After incubation, the samples were collected and aseptically transferred to a 48-well tissue culture plate containing about 2.5 × 10^4 Vero E6 cells having 100 µL Dulbecco’s Modified Eagle’s medium (DMEM) supplemented with 5% fetal bovine serum (FBS) and antibiotics. The plate was incubated for 1 h at 37°C in a humidified chamber with an atmosphere of 5% CO2 to allow virus infection to the cells. After incubation, the virus suspension was discarded, and cells were washed with 100 µL phosphate-buffered saline followed by the addition of fresh DMEM supplemented with 5% FBS and antibiotics. The plate was incubated for 24 h at 37°C in a humidified chamber with an atmosphere of 5% CO2. Postincubation, 140 µL of the culture supernatants were harvested for RNA isolation and qRT-PCR (real-time quantitative reverse transcription PCR) based analysis. The RNA was isolated as per the kit protocol (Indian Council of Medical Research [ICMR] approved viral isolation kit from Manufacturing & Delivering Innovations (MDI) devices, India) and eluted in 50 µL elution buffer. The qRT-PCR was performed using 8 µL of the eluted RNA sample as a template. The assay protocol for qRT-PCR was setup following the kit manufacturer’s instructions. ICMR approved DiAGSure nCOV-19 Detection Assay kit from GCC Biotech. India was used for performing qRT-PCR-based assays. The analysis of the virus inactivation was based on the quantification of viral RNA (cycle threshold [Ct] profile) present in the culture supernatant using qRT-PCR.  

**RESULTS**  
All test compounds showed efficacy against SARS-CoV-2 at both the analyzed contact times, i.e., 30 and 60 s. Both the tested compounds showed a high level of anti-viral effectiveness in the test. Very subtle differences were observed in the activity of both the compounds in terms of percent inactivation of virus, though a differential relative change in Ct values was seen. The Ct value is the number of cycles of PCR amplification, necessary to identify a detectable amount of viral RNA in the sample. The relative change in the Ct values is documented as an alteration observed in the viral load. Greatest and smallest relative change in Ct values was detected for Chlorhexidine 0.2% at 60 s and povidone-iodine 1% at 30 s, respectively. Among the different concentrations of chlorhexidine, the smallest relative change in Ct values was observed for Chlorhexidine digluconate solution 0.12% [Table 1].  

**DISCUSSION**  
The use of preprocedural mouth rinses with an oxidative mechanism such as hydrogen peroxide 1% or povidone-iodine 1% has been recommended since these have proven effective against other Coronaviridae family viruses. The effectiveness of povidone-iodine has been well demonstrated through many in vitro studies against multiple viruses, including SARS-CoV, MERS-CoV, and influenza virus A. Regarding chlorhexidine, however, there is no clear evidence reported regarding its efficacy against SARS-CoV2. Few papers document its efficacy against viruses in general and specifically against enveloped viruses, even against coronaviruses, at small concentrations also. However, Peng et al. recommended to avoid chlorhexidine though it was not tested directly against SARS-CoV-2 in their study. Another study by Yoon et al. reported the viral suppression in saliva for 2 h duration. In the current investigation, 0.2% chlorhexidine gluconate appeared most effective compound to achieve the inactivation of virus.

**Table 1: Severe acute respiratory syndrome coronavirus 2 inactivation (relative cycle threshold change and percent severe acute respiratory syndrome coronavirus 2 inactivation) by test compounds at 30 and 60 s contact time**  

| Sample                  | Time of exposure | Relative Ct change | Percent SARS-CoV-2 inactivation |
|-------------------------|------------------|--------------------|--------------------------------|
| Chlorhexidine digluconate (0.12%) | 30 s             | 10.5±0.5           | 99.9                           |
| Chlorhexidine digluconate (0.12%) | 60 s             | 11±1.0             | 99.9                           |
| Chlorhexidine digluconate (0.2%)  | 30 s             | 12.5±0.5           | >99.9                          |
| Chlorhexidine digluconate (0.2%)  | 60 s             | 13±0               | >99.9                          |
| Povidone-iodine          | 30 s             | 9.5±0.5            | 99.8                           |
| Povidone-iodine          | 60 s             | 11±2               | >99.9                          |

SARS-CoV-2 – Severe acute respiratory syndrome coronavirus 2; Ct – Cycle threshold
at merely 30 s of contact time, thus achieving immediate inactivation of the virus. Further, the well-documented property of substantively exhibited by chlorhexidine makes it most appealing to be an effective strategy against SARS CoV 2 transmission in clinical dental settings.

The method employed for estimating viral load in investigation is a standardized protocol of qRT-PCR, which is a semiquantitative evaluation. Although it does not determine the viability and the precise quantity of the virus, still the method has been regarded as a surrogate measure of viral RNA load from clinical specimens, keeping in view the difficulties in culturing the SARS CoV 2 virus.\(^7\) Since all test compounds in the current study showed an increase in the Ct values, thus indicated the clearance of the virus from the sample.

**CONCLUSION**

Within the limitations of the present study, it can be concluded that the most routinely used antiseptic components in mouth rinses i.e chlorhexidine and povidone-iodine are effective against SARS CoV2. Chlorhexidine digluconate in 0.2% concentration inactivated more than 99.9% of SARS CoV2 virus, in minimal contact time of 30 seconds, and was considered as more efficacious than povidone-iodine 1% utilized for 30 and 60 seconds. The preprocedural and routine use of chlorhexidine digluconate mouth rinses seems to be a very promising and effective infection transmission and control strategy against SARS CoV2, in contemporary dental clinical settings, particularly where aerosol generation is significant. However, these interpolations are based on *in vitro* analysis only and confirmatory recommendations can only be made after clinical, *in vivo* studies.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

**REFERENCES**

1. To KK, Tsang OT, Yip CC, Chan KH, Wu TC, Chan JM, et al. Consistent detection of 2019 novel coronavirus in saliva. Clin Infect Dis 2020;71:841-3.
2. Xu H, Zhong L, Deng J, Peng J, Dan H, Zeng X, et al. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. Int J Oral Sci 2020;12:8.
3. Vergara-Buenaventura A, Castro-Ruiz C. Use of mouthwashes against COVID-19 in dentistry. Br J Oral Maxillofac Surg 2020;58:924-7.
4. Lim KS, Kam PC. Chlorhexidine—pharmacology and clinical applications. Anaesth Intensive Care 2008;36:502-12.
5. 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;12:9.
6. Yoon JG, Yoon J, Song JY, Yoon SY, Lim CS, Seong H, et al. Clinical significance of a high SARS-CoV-2 viral load in the saliva. J Korean Med Sci 2020;20:e195.
7. Joynt GM, Wu WK. Understanding COVID-19: What does viral RNA load really mean? Lancet Infect Dis 2020;20:635-6.