Disinfection Effect of Hexadecyl Pyridinium Chloride on SARS-CoV-2 in vitro

Ke-da Chen, Fei-ke Ma, Qing-jing Wang, Ying Wang, Xin-yi Zhuang, Xu-ning Zhang, Hai-yan Mao, Yan-jun Zhang

Keywords
Hexadecyl pyridinium chloride · Severe acute respiratory syndrome coronavirus · Disinfection effect · Cytopathic effect · Oral disinfectant

Abstract
The novel coronavirus (COVID-19 or 2019-nCoV) is a respiratory virus that can exist in the mouth and saliva of patients and spreads through aerosol dispersion. Therefore, stomatological hospitals and departments have become high-infection-risk environments. Accordingly, oral disinfectants that can effectively inactivate the virus have become a highly active area of research. Hexadecyl pyridinium chloride, povidone-iodine, and other common oral disinfectants are the natural primary choices for stomatological hospitals. Therefore, this study investigated the inhibitory effect of hexadecyl pyridinium chloride on severe acute respiratory syndrome coronavirus (SARS-CoV-2) in vitro. Vero cells infected with SARS-CoV-2 were used to determine the disinfection effect; the CCK-8 method was used to determine cytotoxicity, and viral load was determined by real-time PCR. The results showed that hexadecyl pyridinium chloride has no obvious cytotoxic effect on Vero cells in the concentration range of 0.0125–0.05 mg/mL. The in vitro experiments showed that hexadecyl pyridinium chloride significantly inhibits the virus at concentrations of 0.1 mg/mL or above at 2 min of action. Thus, the results provide experimental support for the use of hexadecyl pyridinium chloride in stomatological hospitals.

Introduction
The outbreak of coronavirus disease 2019 (COVID-19) began in Wuhan, China in December 2019 [1, 2], and the total number of confirmed COVID-19 cases has exceeded 40 million in 211 countries around the world [3, 4]. The most severe epidemic areas are mainly located in Asia and the Americas [5]. The impact on children has so far been relatively small compared to that on adults. The novel coronavirus (severe acute respiratory syndrome coronavirus; SARS-CoV-2) spread rapidly, sweeping across China in just a few days, causing varying degrees of respiratory disease and, in severe cases, death [6]. Accordingly, Ke-da Chen, Fei-ke Ma, and Qing-jing Wang contributed equally to this work.
the SARS-CoV-2 outbreak poses a massive threat to public safety, and the World Health Organization (WHO) has declared a public health emergency.

Since the pandemic outbreak, we have reached a deeper understanding of SARS-CoV-2. The genome of SARS-CoV-2 has a high similarity with the coronavirus in bats [7], and the spike protein of the coronavirus is the key target of antibodies [8]. The virus can bind to target cells via angiotensin-converting enzyme II (ACE).

Yao et al. [9] reported the first full three-dimensional fine structure of SARS-CoV-2, while Zhou et al. [7] reported the full-length genome of 2019-nCoV. It has the characteristics of a high infection rate, a wide range of transmission modes, and a high mortality rate. Therefore, people must find effective treatment and prevention methods. With the development of the epidemic, there are also many virus mutants. Scientists all over the world are analyzing the mutation genes of the virus to develop targeted vaccines and drugs [10]. The Department of public health’s weekly case data for the COVID-19 variant shows that since June 14, 2021, the number of delta variants in the UK has increased by 33,630 to 75,953, an increase of 79%. Recent data show that 99% of sequencing and genotyping across the country are delta variants [11].

SARS-CoV-2 is a respiratory disease that can be spread by direct transmission, aerosol transmission, or contact transmission. SARS-CoV-2 has a typical incubation period of 3–7 days and rarely more than 14 days. After infection, patients often develop fever, cough, fatigue, and other symptoms [12].

The virus can be present in the oral cavity and saliva of patients, presenting a high risk of infection and cross-infection to the medical staff in stomatological hospitals [13]. Thus, killing the virus in the mouth is extremely important [14] if we are trying to minimize the chance of iatrogenic infections, which can have serious consequences. Accordingly, the virucidal effects of oral disinfectants on SARS-CoV-2 to reduce the possibility of oral cavity transmission of SARS-CoV-2 is an active area of research at present [15].

Traditional disinfectors can have virucidal effects [5], and the oral disinfectant hexadecyl pyridinium chloride is effective for oral sterilization [16], as well as having activity against hepatitis B virus [17] and bacteria that cause periodontitis [18]. Hexadecyl pyridinium chloride is a widely used personal care product that has been listed by the FDA as Generally Regarded as Safe (GRAS). More importantly, it exhibits a certain action against SARS-CoV-2 that can destroy the structure of the virus surface [19].

Nevertheless, there is no significant research on SARS-CoV-2 disinfection using mouthwash disinfectant. Therefore, in the present study, we evaluated the inhibitive effect of hexadecyl pyridinium chloride against SARS-CoV-2 in the vero cell model. We hope that this study will provide invaluable information for the use of disinfectants in oral clinical treatment during the epidemic.

Materials and Methods

Virus and Cells

SARS-CoV-2 (hCoV-19/Zhejiang/OS2/2020, GISAID, ID: 455692) was isolated from a patient at the Zhejiang Provincial Center for Disease Control and Prevention. The Vero cells (ATCC CCL-81) were cultured in modified Eagle medium (MEM) (Life Technologies, USA) supplemented with 2% fetal bovine serum (Life Technologies). The cells were cultured in a 5% CO2 incubator at 35°C. The virus was propagated in Vero cells and was cultured under standard conditions. The virus titer was determined by 50% tissue culture infectious dose assay (TCID50). At 48 h post-infection (hpi), we collected the culture supernatants, centrifuged the fluid at 4856 g, 2,500 rpm for 8 min at 24°C in a Velocity 18R centrifuge (Dynamica, Livingston, UK) to purify it, and stored it at −80°C to establish virus stocks. All the experiments involving infectious viruses were conducted in the approved biosafety level III laboratory (CNAs BL0026, Zhejiang Provincial Center for Disease Control and Prevention).

CCK-8 Cell Proliferation Toxicity Test

Vero cells were seeded on 96-well plates (5,000 per well) and incubated for 24 h at 35°C under 5% CO2. The cells were then treated with 1:500 diluted hexadecyl pyridinium chloride (0.2, 0.1, 0.05, 0.025, or 0.0125 mg/mL). The cells were incubated for 24 h at 35°C. The virus was propagated in Vero cells and was cultured under standard conditions. The virus titer was determined by 50% tissue culture infectious dose assay (TCID50). At 48 h post-infection (hpi), we collected the culture supernatants, centrifuged the fluid at 4856 g, 2,500 rpm for 8 min at 24°C in a Velocity 18R centrifuge (Dynamica, Livingston, UK) to purify it, and stored it at −80°C to establish virus stocks. All the experiments involving infectious viruses were conducted in the approved biosafety level III laboratory (CNAs BL0026, Zhejiang Provincial Center for Disease Control and Prevention).

Evaluation of SARS-CoV-2 Disinfection Effect

Vero cells were inoculated in a 48-well plate with 1 × 10^5 cells/well and incubated under 5% CO2 at 35°C, incubated to the most active 75–90% slice cells in the logarithmic growth phase. Three microlitres cell solutions were mixed with SARS-CoV-2 (10^6 U/mL) and 3 μL different concentrations of hexadecyl pyridinium chloride (0.2, 0.1, 0.05, 0.025, or 0.0125 mg/mL). The cells were exposed to different concentrations of disinfectant for 30 s, 1 min, 2 min, or 5 min. The culture medium was then added to each test tube for 500-times dilution, which was divided into six equal parts (500 μL × 6). Add 500 μL of the corresponding thinner to the repeating well-containing Vero cells (Fig. 1a). For the control group, only a 500 μL medium was added alone to Vero cells. All the cells were then incubated at 35°C under 5% CO2 for 48 h again and were observed under a microscope (EVOS M7000 Imaging System; Thermo Fisher, Shanghai, China). Cell death or cell rounding, ab-
scission, aggregation, etc. is called cytopathic effect (CPE), which is observable under a microscope (see Fig. 2). We also performed ultrafiltration with centrifuge tubes (AMI-CON ULTRA 50K; Millipore, USA). Vero cells were inoculated into 48 well plates (10,000 per well) incubated at 35°C under 5% CO₂ for 48 h. First, 500 µL of virus (7.0 LogTCID₅₀/mL) and 500 µL hexadecyl pyridinium chloride of different concentrations (0.2, 0.1, 0.05, 0.025, 0.0125 mg/mL) were mixed for 2 min, then the mixture was transferred to an ultrafiltration centrifuge tube and centrifuged at 4,972.86 g at 24°C in a Velocity 18R centrifuge (Dynamica) for 5 min to concentrate virus particles. Then resuspend the virus particles in a 1 mL medium. Taking 6 µL from it, we diluted 1:500 with an extra 3 mL culture medium and added to a 48-well plate (three replicates for each solution). CPE was observed at 48 hpi. TCID₅₀ was determined by the Reed-Muench method [20].

**Fig. 1.** Experimental steps for determining the disinfection effect of hexadecyl pyridinium chloride on SARS-CoV-2. a SARS-CoV-2 (10⁶ U/mL) was exposed to different concentrations (0.2, 0.1, 0.05, 0.025, 0.0125 mg/mL) of hexadecyl pyridinium chloride for different durations (30 s, 1 min, 2 min, 5 min) then diluted 1:500 to test the disinfection effect. b The hexadecyl pyridinium chloride (0.2, 0.1, 0.05, 0.025, or 0.0125 mg/mL) and 500 µL SARS-CoV-2 were mixed for 2 min, then centrifuged at 8,000 rpm for 5 min. Subsequently, 6 µL of 1 mL solution was diluted 1:500 with 3 mL extra medium and added to 48-well plate (500 µL/well, three replicates for each solution).

**Viral RNA Extraction and RT-PCR**

The cultured cell density for total RNA extraction was 2.5 × 10⁴ cells. Virus RNA was extracted from 200 µL Vero cell culture supernatant (48 h after infection) using an automated nucleic acid extraction system (MVR01; Liferiver, Shanghai, China). Briefly, an aliquot of 200 µL Vero cell culture supernatant was added to the sample well of the preassembled plate, followed with the addition of 20 L proteinase K. Then the plate was loaded into the automatic nucleic acid extraction machine. Through a series of steps including sample lysis, nucleic acid binding, and washing, purified nucleic acids were isolated and collected. Real-time polymerase chain reaction (RT-PCR) was used to simultaneously detect the RdRP, N and E genes of the virus, and SARS-CoV-2 was quantified using a LightCycler® 480 System (Roche, Basel, Switzerland) with a OneStep RT-PCR kit (Z-RR-0479-02-50; Liferiver). The cycle procedure consists of initial denaturation at 95°C for 5 min, followed by 30 denaturation cycles at 95°C for 45 s, annealing at 60°C for 45 s, extension at 72°C for 60 s, and then the final tensile step at 72°C for 10 min. All the experiments were performed in triplicate. The 2⁻ΔΔCt method was used to determine the relative expression levels between the treatment group and the control group.
**Immunofluorescence Microscopy**

Vero cells were washed with 10 mM phosphate-buffered saline (PBS; PH 7.4) at 48 hpi, fixed in 80% precooled acetone (Sigma-Aldrich, St Louis, MO, USA) for 30 min, incubated in 1% bovine serum albumin in 10 mM PBS (PH 7.4) at room temperature, and incubated with rabbit anti-Spike RBD polyclonal antibodies (1:1,000; rabbit anti-Spike RBD polyclonal antibodies SARS-CoV-2 (2019-nCoV) Spike RBD Antibody, Rabbit PAb, Antigen Affinity Purified; cat No. 40592-T62; Sino Biological Inc., Beijing, China; Cat: 40592-T62) at 4°C overnight. Then, the cells were washed twice with 10 mM PBS (PH 7.4) and incubated at room temperature for 2 h. Cells were counterstained with 2-(4-amidinophenyl)-6-indolecarbamidine dihydrochloride to stain the nuclei. The stained cells were observed under a fluorescence microscope and photographed (EVOS M7000 Imaging System; Thermo Fisher).

**Statistical Analysis**

EC50 (concentration for 50% of maximal effect) was calculated by nonlinear regression, and Graphpad Prism 8.0.1 (GraphPad Software, San Diego, CA, USA) was used to analyze the data.

**Results**

**Toxic Effect of Hexadecyl Pyridinium Chloride on Vero Cells**

CCK-8 cell proliferation toxicity tests and cell pathology observations were used to determine the toxicity of the disinfectant on Vero cells and SARS-CoV-2 virus. The results are shown in Figure 2. Observing the effect of time on the inhibition rate of CCI at different concentrations, it was found that the disinfection effect of CCI was the best at 2 min, so we used 2 min for the subsequent ultrafiltration experiments. Hexadecyl pyridinium chloride has an apparent toxic effect on Vero cells at a concentration of 0.05 mg/mL or above after 30 s, 1 min, 2 min, and 5 min. Upon dilution and ultrafiltration, the toxic effect of the disinfectant on cells is greatly reduced, and the disinfectant is nontoxic after ultrafiltration. The results are shown in Figure 2.
Fig. 3. Immunofluorescence microscopy of virus infection. SARS-CoV-2-infected Vero cells were treated with different concentrations (0.1, 0.025, 0.0125 mg/mL) of hexadecyl pyridinium chloride. After dilution (1:500), 200 µL was used for immunofluorescence analysis. When the concentration is 0.1 mg/mL, the virus is completely inhibited. The infected cells were fixed. Then, anti-spike RBD Rabbit PAb (1:1,000; Sino Biological) was used as the first antibody and Alexa Fluor488®-conjugated Goat Anti-rabbit IgG (1:1,500; Abcam) was used as the second antibody. The nuclei were stained with DAPI. The scale is 100 µm.
Inhibitory Effect of Hexadecyl Pyridinium Chloride on SARS-CoV-2 in vitro

The Vero cell culture medium was treated 48 hpi with hexadecyl pyridinium chloride solution (0.2, 0.1, 0.05, 0.025, or 0.0125 mg/mL) and a 1:500 dilution of hexadecyl pyridinium chloride under ultrafiltration conditions. The supernatant of the culture was collected for RT-PCR, and the inhibition% was calculated: Inhibition% = (1 − 2−ΔΔCt) × 100%.

The results shown in Figure 2 demonstrate that the disinfection effect of hexadecyl pyridinium chloride is time and concentration-dependent. When the action time is 30 s, the disinfection effect is poor, but when it is 2 min or above, the disinfection effect is better. There is no significant difference in inhibition rate after 2 min. When the concentration is 0.1 mg/mL or above, there is a clear virus-elimination effect. The results show that, in the Vero cell model, hexadecyl pyridinium chloride has the strongest virus-elimination effect at a concentration of 0.1 mg/mL for 2 min.

Immunofluorescence Microscopy and TCID50 Testing Verified the Disinfection Effect of Hexadecyl Pyridinium Chloride

The supernatants (200 μL) of 48 hpi cells were collected and treated with hexadecyl pyridinium chloride at different concentrations (0.2, 0.1, 0.05, 0.025, and 0.0125 mg/mL) for TCID50 analysis, and the results are shown in Figure 2. The titer of the virus decreases with increasing hexadecyl pyridinium chloride concentration, showing a dose-dependent relationship. The results are shown in Figure 3. Hexadecyl pyridinium chloride at 0.1 mg/mL or above exhibits a good inhibitory effect on virus proliferation when the action time is longer than 2 min. In summary, these results show that the disinfection effect had remarkable results when the concentration of hexadecyl pyridinium chloride is 0.1 mg/mL and the action time is 2 min, and that it is significantly correlated with the inhibition of the CPE.

Discussion

COVID-19 caused by SARS-CoV-2 has spread worldwide, having a huge impact on global public health [21, 22]. Accordingly, countries around the world are making every effort to cope with this severe challenge. However, SARS-CoV-2 is highly contagious, and the virus can be detected for a long time in the environment [23]. This brings a certain degree of difficulty to the prevention of COVID-19. In such a worrying situation, personal hygiene disinfection and protection of medical personnel are effective measures to prevent COVID-19 from spreading widely. On the front line of the battle against coronavirus are stomatological hospitals, where the risk of infection is particularly high [24]. Thus, effective antiviral oral disinfectants are urgently required to protect medical staff and control the spread of the virus. Oral disinfectants mainly include alcohols, aldehydes, quaternary ammonium compounds, chlorhexidine, and chlorine-based disinfectants [25]. Previous experimental studies have shown that the oral disinfectant hexadecyl pyridinium chloride exerts an inactivation effect on certain bacteria and viruses [15, 26].

In this study, we found that hexadecyl pyridinium chloride (0.1 mg/mL, 2 min) can be recommended as an oral disinfectant for the prevention of COVID-19 in stomatological hospitals. Furthermore, in the test concentration ranging from 0.0125 to 0.05 mg/mL, hexadecyl pyridinium chloride has no apparent cytotoxic effect. It is acknowledged that the clinical use concentration of hexadecyl pyridinium chloride is 0.2 mg/mL. Our study also shows that the optimal disinfection concentration of hexadecyl pyridinium chloride is 0.1 mg/mL, which is close to the previous optimal disinfection concentration [27]. Considering that the disinfection effect of hexadecyl pyridinium chloride will be affected by how long the disinfection lasts, if the disinfectant is kept in the mouth for too long, it will lead to drug resistance and may also lead to cross-resistance to antibiotics [28]. To further verify the 2 min is the optimal disinfection time, we conducted ultrafiltration centrifugation experiments.

Our research also has some limitations. First, the Vero cell model may be a relatively simple means for the study of the disinfection effect of hexadecyl pyridinium chloride on SARS-CoV-2. We intend to study the disinfection effect of hexadecyl pyridinium chloride in Calu-3, Huh-7 [29], and other oral mucosal cell models and animal models that provide more complex virus environments. We will also further explore the disinfection mechanism of hexadecyl pyridinium chloride. Furthermore, we may also consider the use of different ways to apply the disinfectant. For example, previous studies have shown that the combined use of multiple disinfectants can increase the effectiveness of disinfection [30], so we also propose further research from the aspect of the combined use of disinfectants. In short, due to the oxidation strength of chlorinated compounds, hexadecyl pyridinium chloride has a disinfection effect on bacteria and viruses and thus application prospects as a disinfectant in stomatological hospitals.
Acknowledgments

We gratefully acknowledge Ke-da Chen, Fei-ke Ma, and Qing-jing Wang for their help on improving the manuscript. We thank Ying Wang, Xin-yi Zhuang and Xu-ning Zhang for their help in data acquisition. We thank Hai-yan Mao and Yan-jun Zhang for their helpful suggestions.

Statement of Ethics

This article does not contain any studies with human participants or animals.

Conflict of Interest Statement

All the authors declare that they have no conflict of interest.

Funding Sources

Grant support for this paper was provided by (1) The Key Technologies R&D Program of the National Ministry of Science (2018ZX10734-401), (2) General project of the Traditional Chinese Medicine Administration of Zhejiang Province (2016ZA134), and (3) The Innovation and Entrepreneurship Training Program for College students of Zhejiang Shuren University (202111842048).

Author Contributions

Ke-da Chen, Fei-ke Ma, and Qing-jing Wang contributed to conception, design, data acquisition, and analysis and drafted and critically revised the manuscript; Ying Wang, Xin-yi Zhuang, and Xu-ning Zhang assisted with data management and data analysis; Hai-yan Mao and Yan-jun Zhang conceived and designed the study. All the authors gave final approval and agree to be accountable for all aspects of the work.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author. A preprint version of this article is available on Research Square [31].

References

1. Chakraborty C, Sharma AR, Sharma G, Bhattacharya M, Lee SS. SARS-CoV-2 causing pneumonia-associated respiratory disorder (COVID-19): diagnostic and proposed therapeutic options. Eur Rev Med Pharmacol Sci. 2020;24(7):4016–26.
2. Esakandari H, Nabi-Afjadi M, Fakkari-Afjadi J, Rahamandian N, Miresmaeili S-M, Bahreini E. A comprehensive review of COVID-19 characteristics. Biol Proced Online. 2020;22(1):19.
3. Yang L, Tian D, Liu W. Strategies for vaccine development of COVID-19. Sheng Wu Gong Cheng Xue Bao. 2020;36(4):593–604.
4. Younes N, Al-Sadeq DW, Al-figeheef H, Younes S, Al-Jamal O, Daas HI, et al. Challenges in laboratory diagnosis of the novel coronavirus SARS-CoV-2. Viruses. 2020;12(6):582.
5. Rabenau HF, Kampf G, Cinatl J, Doerr HW. Efficacy of various disinfectants against SARS coronavirus. J Hosp Infect. 2005;61(2):107–11.
6. Kariwa H, Fujii N, Takashima I. Inactivating activity of reagents in mouth rinses against SARS-CoV-2 virus. Cell. 2020;183(3):730–8.e13.
7. Zhou P, Yang X-L, Wang X-G, Hu B, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579(7800):270–3.
8. Walls AC, Park Y-J, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell. 2020;181(2):281–92.e6.
9. Yao H, Song Y, Chen Y, Wu N, Xu J, Sun C, et al. Molecular architecture of the SARS-CoV-2 virus. Cell. 2020;183(3):730–8.e13.
10. Islam OK, Al-Emran HM, Hasan MS, Anwar A, Jahid MIK, Hossain MA, et al. Emergence of European and North American mutant variants of SARS-CoV-2 in South-East Asia. Transbound Emerg Dis. 2021;68(2):824–32.
11. O’Dowd A. Covid-19: Cases of delta variant rise by 79%, but rate of growth slows. BMJ. 2021;373:n1596.
12. Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. Lancet Respir Med. 2020;8(5):475–81.
13. Baghdizade Fini M. What dentists need to know about COVID-19. Oral Oncol. 2020;105:104741.
14. Carrouel F, Gonçalves LS, Conte MP, Campus G, Fisher J, Fraticelli L, et al. Antiviral activity of reagents in mouth rinses against SARS-CoV-2. J Dent Res. 2021;100(2):124–32.
15. Seneviratne CJ, Balan P, Ko KKK, Udawatte NS, Lai D, Ng DHL, et al. Efficacy of commercial mouth-rinse on SARS-CoV-2 viral load in saliva: randomized control trial in Singapore. Infection. 2021;49(2):305–11.
16. Vergara-Buenaventura A, Castro-Ruiz C. Use of mouthwash against COVID-19 in dentistry. Br J Oral Maxillofac Surg. 2020;58(8):924–7.
17. Seo HW, Seo JP, Cho Y, Ko E, Kim YJ, Jung G. Cetylpyridinium chloride interaction with the hepatitis B virus core protein inhibits capsid assembly. Virus Res. 2019;263:102–11.
18. Miranda SLF, Damaceno JT, Faveri M, Figueiredo LC, Soares GMS, Feres M, et al. In vitro antimicrobial effect of cetylpyridinium chloride on complex multispecies subgingival biofilm. Braz Dent J. 2020;31(2):103–8.
19. Baker N, Williams AJ, Tropsha A, Ekins S. Repurposing quaternary ammonium compounds as potential treatments for COVID-19. Pharm Res. 2020;37(6):104.
20. Ramakrishnan MA. Determination of 50% endpoint titer using a simple formula. World J Virol. 2016;5(2):85–6.
21. Corbett KS, Edwards DK, Leist SR, Abiona OM, Boyoglu-Barnum S, Gillespie RA, et al. SARS-CoV-2 mRNA vaccine design enabled by prototype pathogen preparedness. Nature. 2020;586(7830):567–71.
Disinfection Effect of Hexadecyl Pyridinium Chloride on SARS-CoV-2

22 Gao Q, Bao L, Mao H, Wang L, Xu K, Yang M, et al. Development of an inactivated vaccine candidate for SARS-CoV-2. Science. 2020;369(6499):77–81.

23 Carratufo F, Del Giudice C, Morelli M, Cerullo V, Libralato G, Galdiero E, et al. Persistence of SARS-CoV-2 in the environment and COVID-19 transmission risk from environmental matrices and surfaces. Environ Pollut. 2020;265(Pt B):115010.

24 Amato A, Caggiano M, Amato M, Moccia G, Capunzo M, De Caro F, et al. Infection control in dental practice during the COVID-19 pandemic. Int J Environ Res Public Health. 2020;17(13):4769.

25 Khokhar M, Roy D, Purohit P, Goyal M, Setia P. Viricidal treatments for prevention of coronavirus infection. Pathog Glob Health. 2020;114(7):349–59.

26 Alvarez DM, Duarte LF, Corrales N, Smith PC, González PA. Cetylpyridinium chloride blocks herpes simplex virus replication in gingival fibroblasts. Antiviral Res. 2020;179:104818.

27 Rösing CK, Cavagni J, Gaio EJ, Muniz FWMG, Ranzan N, Oballe HJR, et al. Efficacy of two mouthwashes with cetylpyridinium chloride: a controlled randomized clinical trial. Braz Oral Res. 2017;31:e47.

28 Mao X, Auer DL, Buchalla W, Hiller K-A, Maisch T, Hellwig E, et al. Cetylpyridinium chloride: mechanism of action, antimicrobial efficacy in biofilms, and potential risks of resistance. Antimicrob Agents Chemother. 2020;64(8):e00576-20.

29 Yao H, Lu X, Chen Q, Xu K, Chen Y, Cheng M, et al. Patient-derived SARS-CoV-2 mutations impact viral replication dynamics and infectivity in vitro and with clinical implications in vivo. Cell Discov. 2020;6(1):76.

30 Jiang L, Li M, Tang J, Zhao X, Zhang J, Zhu H, et al. Effect of different disinfectants on bacterial aerosol diversity in poultry houses. Front Microbiol. 2018;9:2113.

31 Chen K, Ma F, Wang Y, Zhuang X, Zhang X, Mao H, et al. Disinfection effect of hexadecyl pyridinium chloride on SARS-CoV-2 in vitro. Forthcoming 2021 [2022 Apr 6].