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Lowering the transmission and spread of human coronavirus

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Abstract

The emergence of the severe acute respiratory syndrome coronavirus 2 pandemic has created an unprecedented healthcare, social, and economic disaster. Wearing of masks and social distancing can significantly decrease transmission and spread, however, due to circumstances such as medical or dental intervention and personal choice these practices have not been universally adopted. Additional strategies are required to lessen transmission. Nasal rinses and mouthwashes, which directly impact the major sites of reception and transmission of human coronaviruses (HCoV), may provide an additional level of protection against the virus. Common over-the-counter nasal rinses and mouthwashes/gargles were tested for their ability to inactivate high concentrations of HCoV using contact times of 30 s, 1 min, and 2 min. Reductions in titers were measured by using the tissue culture infectious dose 50 ($TCID_{50}$) assay. A 1% baby shampoo nasal rinse solution inactivated HCoV greater than 99.9% with a 2-min contact time. Several over-the-counter mouthwash/gargle products including Listerine and Listerine-like products were highly effective at inactivating infectious virus with greater than 99.9% even with a 30-s contact time. In the current manuscript we have demonstrated that several commonly available healthcare products have significant virucidal properties with respect to HCoV.

KEYWORDS

antiviral agents, coronavirus, dissemination, epidemiology, horizontal transmission, pathogenesis, shedding, virus classification

1 | INTRODUCTION

Coronaviruses are a large family of positive-stranded RNA viruses that cause minor and major infectious diseases in mammals, including humans. For decades common human coronaviruses (HCoV) have circulated in the human population without any significant mortality.[1–6] In less than 20 years, three new HCoV have emerged causing severe respiratory syndromes and significant mortality.[7–15] The newest is named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and is associated with coronavirus disease 2019 or COVID-19. Unlike its predecessors, SARS-CoV-2 spread rapidly across the world, reaching pandemic levels within 2 months. At the time of this writing the total confirmed infected was over 11 million with over 500,000 deaths reported.[16]

Currently, specific therapies for early containment and prevention of transmission and spread of SARS-CoV-2 are lacking. The major method of transmission of SARS-CoV-2 is through aerosolized respiratory droplets. Virus on surfaces (fomites) can remain viable for hours or even days and may represent an important secondary mode of transmission.[14,15,17–20] While there is a potential for other mechanisms, aerosolization and fomites are considered the most probable means of transmission and spread.

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Some of the most common symptoms of SARS-CoV-2 disease, such as coughing and sneezing, are associated with the formation of aerosols.[14,18] Persons infected but showing only mild or no symptoms can also readily spread the virus by aerosols.[14,18–20] The nasal and oral cavities are the major entry points for HCoV. This puts not only physicians, nurses, respiratory therapists, dentists, dental assistants, and others who need to be in close proximity to the face of another person to do their jobs at risk, but also families or anyone else who may come in contact with an asymptomatic infected person. Although vaccine developments are currently underway, it is estimated that the final design and testing will likely take up to 1 year or longer. In the interim it will be critical to develop methods to reduce transmission rates

Detergents are known virucides, and the use of intranasal surfactants, including 1% baby shampoo has been demonstrated to be safe and effective as a treatment for chronic rhinosinusitis.[21–25] This led us to question whether a 1% baby shampoo could inactivate HCoV. Over-the-counter mouthwash/gargles generally claim to speed the wound healing process, have antiseptic properties, prevent gingivitis, and kill germs that cause bad breath. However, there is limited evidence that indicates that they inactivate viruses, including HCoV.[26] Therefore, we decided to investigate the virucidal properties of several oral and nasopharyngeal rinses in vitro. These included common over-the-counter mouth wash/gargling products, a saline nasal rinse, and a 1% dilute solution of Johnson's baby shampoo to be used as a nasal rinse. Surprisingly, we found that several of these common products had strong virucidal properties, inactivating from 2 $\log_{10}$ (or 99%) to greater than 4 $\log_{10}$ (or 99.99%) of infectious HCoV. Our studies indicate that these rinses could serve as a complement to other healthcare and public antiviral precautions.

2 | MATERIALS AND METHODS

2.1 | Cell lines, cell culture, and virus

Huh7 cells (courtesy of Dr. Jianming Hu) were grown in Dulbecco modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS; DMEM10) and 100 U/ml pen/strep, in 5% CO_2 at 37°C. Infectious stocks of human coronavirus 229e (HCoV-229e) were prepared by seeding T75 flasks with 7×10^6 Huh7 cells, which were incubated overnight. HCoV-229e was used as a surrogate for SARS-CoV-2. While there are clear differences in the pathogenicity of these viruses, they are in the same virus family, have very similar structures, and are both human respiratory pathogens. On the following day, the media was changed to DMEM with 2% FBS (DMEM2) and the cells were infected with virus using a multiplicity of infection of 0.01. The infected flasks were incubated for 2 days in 5% CO_2 at 35°C. On the second day, the flasks were frozen at −80°C for at least 1 h, then thawed in a 37°C water bath taking care to remove them from the water bath before they were completely thawed. Thawing was then completed at room temperature. The cell suspensions were transferred to a 15 ml polypropylene tube and sonicated on ice in a cup sonicator at 100 W peak envelope power, three bursts of 20 s each. The lysates were clarified by centrifugation at 3000 rpm for 10 min at 4°C, and the supernatant poured into a fresh 15 ml tube. Virus solutions were aliquoted into eight 0.5 ml portions, and several smaller aliquots were then frozen for long term storage at −80°C. One of the smaller aliquots was used to determine the titer of the stock by the tissue culture infectious dose 50 ($TCID_{50}$) assay.

2.2 | $TCID_{50}$ assay

Huh7 cells were harvested, counted, and resuspended into DMEM2 to a concentration of 1.5×10^6 cells/ml. Then 100 µl of the cell suspension was added to each well of the 96-well plate. Plates were incubated overnight in 5% CO_2 at 37°C. Serial 10-fold dilutions of virus were added to each column of wells containing cells. An extra row of mock-infected cells was included across the bottom as a control. The plates were then incubated for 3 days in 5% CO_2 at 35°C. On the third day, the wells were examined for the presence of cytopathic effects (CPE) and the $TCID_{50}$ calculation was done using the Reed-Muench method,[27] based off the number of wells positive for CPE at each dilution. Figure 1 shows the development of CPE over the 3-day incubation.

2.3 | Nasal rinse and mouthwash gargling products testings

The nasal rinses tested in the study were Neti Pot (CVS Health) and Johnson's Baby Shampoo (Johnson & Johnson Consumer, Inc). The Neti Pot solution was made according to the manufacturer's instructions. Johnson's Baby Shampoo was diluted to 1% in PBS (116 mMol NaCl, 12 mMol Na_2HPO_4, 1.5 mMol KH_2PO_4 [pH 7.4]) for testing.

The mouthwash gargling products tested in the study were Peroxide Sore Mouth Cleanser (CVS Health), H_2O_2 solution diluted to 1.5% in PBS (Cumberland-Swan, Inc), Orajel Antiseptic Rinse (Church & Dwight Co, Inc), Betadine 5% (Alcon Laboratories, Inc), Crest Pro-Health (Proctor & Gamble), Listerine Antiseptic (Johnson & Johnson Consumer, Inc), Listerine Ultra (Johnson & Johnson Consumer, Inc), Equate (Wal-Mart), and Antiseptic Mouthwash (CVS Health). The manufacturers' list of active and inactive ingredients is shown in Table 1.

For each of the nasal rinse and oral rinse products, 200 µl of an organic load or soil of 5% BSA was added to the virus suspensions to more closely simulate physiologic conditions in the nasopharynx. Virus and product were mixed thoroughly and incubated for 30 s, 1 min, of 2 min at room temperature, then 2 ml of an appropriate neutralizer was added to the virus/disinfectant solutions. The neutralizer used for the H_2O_2 solution was catalase. The neutralizer used for the Crest Pro-Health and Orajel Antiseptic Rinse was 7% glycine. The neutralizer used for everything else was DMEM2. The solutions were then centrifuged in Amicon Ultra centrifugal filters 100,000 molecular weight cut-off (MWCO; Millipore) at 4000 rpm for 10 min. The filters were washed a total of 4× with DMEM2 and

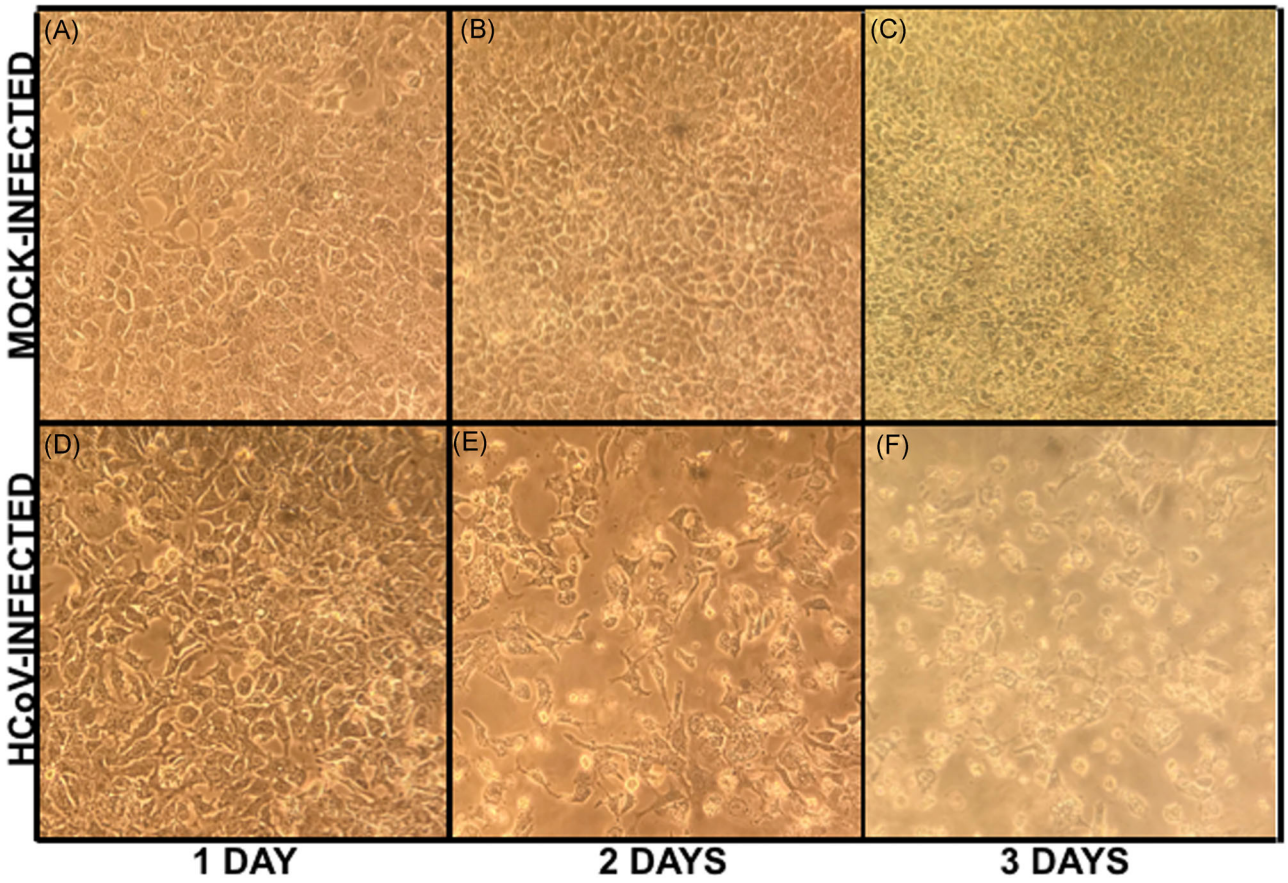

FIGURE 1 HCoV induced cytopathic effects (CPE) over 3 days. Huh7 cells were incubated overnight in 5% CO_2 at 37°C. Cells were then mock-infected (A,B, and C) or infected with HCoV-229e (D,E, and F) and then incubated at 5% CO_2 at 35°C. Panels show cells at 1 day postinfection (A and D), 2 days postinfection (B and E), and 3 days postinfection (C and F). All pictures are ×20 magnification. HCoV, human coronaviruses

centrifuged at 4000 rpm for 10 min. The virus-containing eluents were then assayed for infectivity using the $TCID_{50}$ method. At least three replicate assays were done for each product and contact time. Untreated controls were included for every set of assays performed.

3 | RESULTS

3.1 | Nasal rinses

The ability of 1% baby shampoo to inactivate high numbers of virus after various contact times is shown in Table 2. With contact times of 1 and 2 min, the 1% baby shampoo solution was able to inactivate more than 99% and more than 99.9% or more of the virus, respectively. A contact time of 30 s had a variable effect. The assay was performed four times using this contact time on different days with results ranging from less than a 90% reduction in infectious virus to between 99% and 99.9% reduction in infectious virus. In comparison, the over-the-counter saline nasal rinse, Neti Pot, had no effect on the infectivity of the virus at any incubation time tested.

3.2 | Oral rinses

We initially tested Peroxide Sore Mouth (CVS), Orajel Antiseptic Rinse (Church & Dwight Co, Inc), 1.5% H_2O_2 (Cumberland-Swan, Inc), Crest Pro-Health (Proctor & Gamble), and Listerine Antiseptic (Johnson & Johnson Consumer Inc). The first three, Peroxide Sore Mouth, Orajel Antiseptic Rinse, and 1.5% H_2O_2, all have H_2O_2 as their active ingredient (Table 1). Crest Pro-Health lists cetylpyridium chloride as its active ingredient (Table 1). Listerine lists four active ingredients (Table 1), eucalyptol, menthol, methyl salicylate, and thymol. Similar to the nasal rinses, we tested contact times of 30 s, 1 min, and 2 min. The three products with H_2O_2 as their active ingredient all demonstrated similar abilities to inactivate HCoV (Table 3), replicate assays showed some variability but overall the reduction of infectious virus ranged from lower than a 1 $\log_{10}$ reduction to a 2 $\log_{10}$ reduction or less than 90% to 99%. Crest Pro-Health decreased infectious virus by at least 3 $\log_{10}$ to greater than 4 $\log_{10}$, or 99.9% to more than 99.99%; again, the contact times used made little difference. Listerine Antiseptic was able to decreases the infectious virus levels by greater than 4 $\log_{10}$, or greater than

TABLE 1 Nasal rinse and mouthwash gargling product's ingredients

Product	Company	Active ingredients[a]	Inactive ingredients[a]	
Neti Pot	CVS	Sodium bicarbonate (700 mg) Sodium chloride (2300) mg	None	
Johnson's Baby Shampoo[b]	Johnson & Johnson Consumer Inc	Water Cocamidopropyl betaine Decyl glucoside Sodium cocoyl isethionate Lauryl glucoside PEG-80 Sorbitan laurate glycerin	Citric acid Sodium benzoate PEG-150 distearate Sodium methyl cocoyl taurate Fragrance Polyquaternium-10 Disodium EDTA	
Peroxide Sore Mouth	CVS	Hydrogen peroxide (1.5%)	Water Sorbitol Polypylene glycol Poloxamer 338 Polysorbate 20	Methyl salicylate Menthol Sodium saccharin Blue 1
Orajel Antiseptic Rinse	Church & Dwight Co., Inc	Hydrogen peroxide (1.5%) Menthol (0.1%)	Alcohol (4.1%) Blue 1 Disodium ethylenediaminetetraacetic acid Methyl salicylate Phosphoris acid	Poloxamer 338 Polysorbate 20 Sodium saccharin Sorbitol Water
1.5% H_2O_2	Cumberland-Swan, Inc	Hydrogen peroxide (1.5%)	Water	
Crest Pro-Health	Proctor & Gamble	Cetylpyridium chloride (0.07%)	Water Glycerin Flavor Poloxamer 407 Sodium saccharin	Methyl paraben Sucralose Propylparaben Blue 1
Listerine Antiseptic	Johnson & Johnson Consumer Inc	Eucalyptol (0.092%) Menthol (0.042%) Methyl Salicylate (0.06%) Thymol (0.064%)	Water Alcohol (21.6%) Sorbitol Poloxamer 407 Benzoic acid	Sodium saccharin Sodium benzoate Flavor Green 3
Listerine Ultra	Johnson & Johnson Consumer Inc	Eucalyptol (0.092%) Menthol (0.042%) Methyl Salicylate (0.06%) Thymol (0.064%)	Water Alcohol (21.6%) Sorbitol Poloxamer 407 Benzoic acid Zinc chloride	Flavor Sodium benzoate Sucralose Sodium saccharin Green 3
Equate	Wal-Mart Company Inc	Eucalyptol (0.092%) Menthol (0.042%) Methyl Salicylate (0.06%) Thymol (0.064%)	Water Alcohol (21.6%) Sorbitol Flavor Poloxamer 407 Benzoic acid	Zinc chloride Flavor Sodium benzoate Sucralose Sodium saccharin FD&C Blue 1

TABLE 1 (Continued)

Product	Company	Active ingredients[a]	Inactive ingredients[a]	
Antiseptic Mouthwash	CVS	Eucalyptol (0.092%) Menthol (0.042%) Methyl salicylate (0.06%) Thymol (0.064%)	Water Alcohol (21.6%) Sorbitol solution Flavor Poloxamer 407	Benzoic acid Sodium saccharin Sodium benzoate FD&C Green 3
Betadine 5%	Alcon Laboratories, Inc	Povidone-Iodine (5%)	Water Citric acid Dibasic sodium phosphate Glycerin	Nonoxynol-9 Sodium chloride Sodium hydroxide

[a]As listed by the manufacturer.

[b]Manufacturer did not differentiate between active and inactive ingredients.

99.99%. After incubation times of 1 and 2 min, we were unable to detect any remaining infectious virus (Table 3).

After observing the results of Listerine Antiseptic, we wanted to see if products with similar composition would have the same efficacy. We decided to test Listerine Ultra (Johnson & Johnson Consumer Inc), Equate (Wal-Mart Company Inc), and Antiseptic Mouthwash (CVS). While the results obtained with these three products were similar to those of Listerine Antiseptic, there were some interesting differences even though they all list exactly the same active ingredients and similar inactive ingredients (Table 1). All showed slightly lower efficacy, particularly at the shorter contact times, and Equate showed the greatest variability (Table 3). However, the Listerine-like mouthwashes/gargles decreased infectious virus titers by greater than 99%.

Povidone-Iodine (PVP-I) formulations are common antiseptics used before and after surgery. PVP-I formulations are also commonly used in over-the-counter skin cleansers and mouthwashes/gargles. Previous studies have demonstrated the efficacy of various PVP-I formulations at inactivating HCoV.[28–31] For comparison to these products we tested Betadine 5% (Alcon Laboratories, Inc). The results we obtained were similar to what others found with PVP-I formulations (Table 3).[28–31]

4 | DISCUSSION

Our results suggest that several nasal/sinus and oral rinses had potent virucidal properties and could have the potential to inactivate HCoV and decrease viral load in vivo.

Studies of chronic rhinosinusitis have shown the safe use of 1% baby shampoo formulations as a nasal rinse,[21–25] but there is no literature to date that evaluates its use against HCoV or other viruses. Our study shows that a 1% baby shampoo solution was effective at inactivating HCoV in a time-dependent manner. The dilute rinse was able to reduce the amount of infectious virus by close to 99% after a contact time of 1 min and greater than 99.9% after a contact time of 2 min. With a contact time of 30 s 1% baby shampoo showed variable results ranging from less than 90% reduction in infectious virus to up toward a 99.9% reduction. Overall the results show a clear time-dependent decrease of infectious virus. In contrast, a commonly used saline rinse formulation (Neti-Pot) had no effect on infectious viral count in our study.

Most of the common over-the-counter mouth washes/gargles tested demonstrated at least a 90% reduction in infectious virus at 1 min of contract time with the majority of products showing increasing virucidal activity with longer contact times. The products had varying active ingredients and formulations. Interestingly, three of the products tested (Peroxide Sore Mouth, Orajel Antiseptic Rinse, and 1.5% H_2O_2) all contained 1.5% H_2O_2 as their active ingredient (Table 1). With these three products there were variable results with a reduction of infectious virus ranged from below 90% to 99%. The similar results obtained from all three products suggest that the inactive ingredients (Table 1) that are in the Peroxide Sore Mouth and Orajel Antiseptic Rinse provide no noteworthy additional effect toward inactivating the infectious virus. These results agree with a recently published study showing that both 1.5% and 3% H_2O_2 showed between a 90% and a 99% decrease in infectious HCoV.[32]

TABLE 2 The effect of nasal rinses on HCoV

Nasal rinses	log10 Decrease contact time: 2 min (% inactivation)	log10 Decrease contact time: 1 min (% inactivation)	log10 Decrease contact time: 30 sec (% inactivation)
Neti Pot	No change (0%)	No change (0%)	No change (0%)
1% Baby Shampoo J&J	between >3 and >4 $\log_{10}$ (>99.9% to >99.99%)	between >2 and <3 $\log_{10}$ (>99% to <99.9%)	between <1 and <3 $\log_{10}$ (<90% to <99.9%)

Abbreviation: HCoV, human coronaviruses.

TABLE 3 The effect of mouth wash/gargles on HCoV

Mouth Wash/gargle	log10 Decrease contact time: 2 min (% inactivation)	log10 Decrease contact time: 1 min (% inactivation)	log10 Decrease contact time: 30 sec (% inactivation)
Peroxide Sore Mouth	between >1 and <2 log_{10} (>90% to <99%)	between >1 and <3 log_{10} (>90% to <99.9%)	between <1 and <2 log_{10} (<90% to 99%)
Orajel Antiseptic Rinse	between <1 and <2 log_{10} (<90% to <99%)	between ≥1 and <2 log_{10} (≥90% to <99%)	between >1 and <2 log_{10} (>90% to <99%)
1.5% H_2O_2	<1 log10 (<90%)	between <1 and <3 log_{10} (<90% to <99.9%)	between <1 and <2 log_{10} (<90% to <99%)
Crest Pro Health	between ≥3 and >4 log_{10} (≥99.9% to >99.99%)	>4 log_{10} (>99.99%)	between ≥3 and <4 log_{10} (≥99.9% to <99.99%)
Listerine Antiseptic	>4 log_{10}[a] (>99.99%)	>4 log_{10}[a] (>99.99%)	>4 log_{10} (>99.99%)
Listerine Ultra	≥4 log_{10} (≥99.99%)	≥4 log_{10} (≥99.99%)	between ≥3 and <4 log_{10} (≥99.9% to <99.99%)
Equate Antiseptic	between >3 and ≥4 log_{10} (>99.9% to ≥99.99%)	between >2 and <4 log_{10} (>99% to <99.99%)	between >2 and <4 log_{10} (>99% to <99.99%)
CVS Antiseptic Mouth Wash	between ≥3 and ≥4 log_{10} (≥99.9% to ≥99.99%)	between ≥3 and ≥4 log_{10} (≥99.9% to ≥99.99%)	between >3 and <4 log_{10} (>99.9% to <99.99%)
Betadine 5%	>4 log_{10} (>99.99%)	between >3 and >4 log_{10} (>99.9% to >99.99%)	between >3 and <4 log_{10} (>99.9% to <99.99%)

Abbreviation: HCoV, human coronaviruses.

[a]No detection of remaining infectious virus.

Crest Pro-Health listed 0.07% cetylpyridium chloride as its only active ingredient. It was slightly more effective at similar time points. It was able to reduce the amount of infectious virus between 99.9% and greater than 99.99%.

Listerine Antiseptic is an alcohol-based eucalyptol, menthol, methyl salicylate, and thymol formulation that historically has claimed numerous antimicrobial properties. It currently lists only a claim to kill germs that cause bad breath. Our tests show that it is highly effective at inactivating HCoV in solution (Table 3). Even at the lowest contact time of 30 s it inactivated greater than 99.99% of HCoV. Interestingly, other related products (Listerine Ultra, Equate Antiseptic, and CVS Antiseptic Mouth Wash), while showing substantial reductions, were not as efficient as Listerine Antiseptic (Table 3). These three products were unable to show a reduction of greater than 99.99% with 30-s contact time. Equate required 2-min contact time to show a greater 99.99% reduction.

Preparations of PVP-I are well-established general antimicrobials, commonly used as presurgical disinfectants for skin and mucosal surfaces, as well as for wound treatment and eye applications. In many parts of the world, PVP-I formulations are also used as mouthwashes or gargles.[28–33] Our results agree with the published studies demonstrating virucidal activity against HCoV.

Chlorohexidine, another widely used antimicrobial mouthwash/gargle, was not tested in our study. However, it has been recently shown to weakly inactivate human and animal CoV.[34,35]

Several possible limitations of this work must be acknowledged. We did not use SARS-CoV-2 in this study as the virus as it was more expensive, less available, and would have required biosafety level-3 laboratory conditions. Instead, we used high numbers of infectious HCoV-229e, a common surrogate for SARS-CoV-2. This allowed us to rapidly test a multitude of products at varying contact times to identify potential rinses and optimal wash times in the mitigation efforts against COVID-19. Second, we used an in vitro suspension of the virus with soil as a surrogate for oral and nasopharyngeal debris. Although this condition has been used previously, it is possible that this does not represent the true nature of the nasopharyngeal endothelial ecosystem. Third, the in vitro suspension does not consider the potential mechanical action from the act of rinsing. It is possible that in vivo agitation during a rinse may assist in viral load reduction or alternatively decrease effect by altering contact time with viral particles. Future clinical trials will be needed to evaluate the effect of these rinses in patients. Finally, $TCID_{50}$ assays begin with a 10-fold dilution, therefore, the lowest reduction level we could measure is 90% or 1 log_{10}. A product may be able to reduce the amount of infectious virus by 50% or 80% which the $TCID_{50}$ assay would not be able to measure.

5 | CONCLUSION

The rapid spread of SARS-CoV-2 across the world has created an unprecedented healthcare, social, and economic disaster. With the most significant mode of transmission considered to be through aerosolized droplets,[14,15,17–20] wearing masks and social distancing can significantly decrease transmission and spread.[36] However, these practices have not been universally adopted. While we wait for

definitive therapies and vaccines to contain and prevent the spread of SARS-CoV-2, additional strategies are required to lessen transmission. Nasal rinses and mouthwashes, which directly treat the major sites of reception and transmission of HCoV, may provide an additional level of protection against the virus. While clinical trials will be necessary to confirm the virucidal potential of these products and assess their ability to limit transmission of HCoV within the general population, in the current manuscript we have demonstrated here that several commonly available healthcare products have significant virucidal properties with respect to HCoV.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Craig Meyers: responsible for the studies, designed studies, performed protocol, analyzed results, and wrote the manuscript. Richard Robison: assisted in designing studies, analyzing results, and reviewing and editing the manuscript. Janice Milici: assisted in performing the studies, analyzing the results, and reviewing and editing the manuscript. Samina Alam: assisted in performing the studies, analyzing the results, and reviewing and editing the manuscript. David Quillen: Assisted in designing studies, analyzing results, and reviewing and editing the manuscript. David Goldenberg: assisted in designing studies, analyzing results, and reviewing and editing the manuscript. Rena Kass: assisted in designing studies, analyzing results, and reviewing and editing the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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