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Efficacy of antiseptic mouthrinses against SARS-CoV-2: A prospective randomized placebo-controlled pilot study

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ABSTRACT

Objectives: Coronavirus-disease-19 (COVID-19) continues to affect millions of individuals worldwide. Antiviral activity of mouthrinses remains an important research area as the oral cavity is a site of SARS-CoV-2 initial replication. The aim of this study was to assess the effectiveness of three different mouthrinses in reducing the oral/oropharyngeal SARS-CoV-2 viral load.

Methods: Adult patients, hospitalized with confirmed COVID-19 were recruited for the study. Oral/oropharyngeal baseline SARS-CoV-2 samples were collected and analyzed by Real-Time-PCR. Subsequently, patients were instructed to rinse with 1 % hydrogen peroxide (H_2O_2), 0.12 % chlorhexidine (CHX), 1 % povidone-iodine (PVP-I) or Sodium Chloride 0.9 % (placebo). Viral loads were measured right after (T1), and at 45 min (T2) from the rinse.

Results: In the PVP-I 1 % group, 5/8 (62.5 %) patients at T1, and 3/8 (37.5 %) patients at T2, SARS-CoV-2 was not detectable in the swab specimens. In the H_2O_2 1 % group, 2/11 (18.2 %) patients at T1, and 2/11 (18.2 %) other patients at T2 showed no SARS-CoV-2 loads. One (12.5 %) patient in the CHX 0.12 % group showed SARS-CoV-2 negativity at T2. One (9.1 %) patient at T1, and another (9.1 %) patient at T2 showed no SARS-CoV-2 loads in the placebo group.

Conclusions: Oral SARS-CoV-2 loads were reduced at T1 in the PVP-I 1 % and H_2O_2 1 % groups.

Clinical relevance: PVP-I 1 % was the most effective rinse especially in patients with low viral copy numbers at baseline.

1. Background

The coronavirus disease 2019 (COVID-19) pandemic caused by SARS-CoV-2 infection continues to affect millions of individuals worldwide [1]. SARS-CoV-2 infection is mainly transmitted via respiratory route, either directly via physical contact between individuals, indirectly by contact with fomites (although less common, and controversially discussed), or directly through the air by inhaling droplets or aerosol via the oral, or nasal mucosa [2–4]. Non-pharmaceutical preventive measures, as well as mass vaccinations represent an effective strategy for disease control [5,6].

Aerosol generating procedures may pose health care providers working closely to the orofacial region at a higher risk of infection when exposed to patients' respiratory and salivary aerosols [7,8]. The oral environment represents a major reservoir of SARS-CoV-2, with a recent study showing oral epithelial and salivary glands (SGs) cells being a

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major niche for infection and replication of the virus. Interestingly, angiotensin converting enzyme-2 (ACE2) and transmembrane protease serine subtype-2 (TMPRSS2; the two main host entry factors) exhibited tissue-specific expression patterns in a regional fashion, with most of them expressed in the minor salivary glands (SGs; over major), in the dorsal tongue, tonsils and uvula, and microscopically in the suprabasal cells over the basal ones [9–11]. Although constant shedding of epithelial cells might serve as protection against oral mucosal infections, in this case, it might promote viral stability and transmissibility with saliva acting as carrier of the virus [12].

Antiviral activity of oral rinses remains an important area of research given to the close relationship between the oral cavity and SARS-CoV-2, with recent studies reporting that antiseptic mouthwashes may reduce the SARS-CoV-2 viral-loads in the mouth [13–16]. As such, the aim of this prospective randomized placebo-controlled pilot study was to assess the effectiveness of three different oral antiseptics (chlorhexidine 0.12 %, povidone-iodine 1 %, hydrogen peroxide 1 %) in reducing the oral and oropharyngeal SARS-CoV-2 viral loads. If proven effective, this could represent a simple, yet cost-effective preventive strategy that could be easily adopted among patients prior to an aerosol generating procedure in dental and medical settings [17].

2. Materials and methods

2.1. Study design

This was a prospective randomized placebo-controlled pilot study of adult patients (≥18 years) who were hospitalized in the Department of Infectious Diseases of the Umberto I Polyclinic Hospital, Rome, Italy between December 2020 and May 2021 with a confirmed diagnosis of SARS-CoV-2 infection, and symptomatic COVID-19 [18]. All participants signed a written informed consent. The study was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines and was approved by the Sapienza University/Umberto I Polyclinic Hospital Institutional Review Board (IRB N.001392). This research was registered on the Clinical Trials website ISRCTN (#40398).

Demographic data, tobacco and alcohol consumption, co-morbidities (i.e., cardiovascular diseases, hypertension, diabetes mellitus, chronic obstructive pulmonary disease, and cancer), as well as patients' past medical history and COVID-19 related symptoms were recorded and entered in a de-identified electronic spreadsheet. Exclusion criteria included patients admitted in the Intensive Care Unit (ICU), patients with a confirmed allergy to povidone iodine (PVP-I), or chlorhexidine (CHX) and its excipients, patients with thyroid disease or current radioactive iodine treatment, patients receiving treatment with Lithium, pregnant women, and patients with a history of renal failure.

2.2. Randomization

Eligible patients underwent treatment allocation through a simple randomization process from the department database of COVID-19 patients and were randomly assigned in a 3:1 ratio, (four groups) to rinse with either povidone-iodine 1 % (PVP-I) oral solution (Betadine®Antiseptic Oral Rinse, Avrio Health L.P. Stamford, Connecticut, USA), chlorhexidine 0.12 % (CHX) oral solution (Curasept® S.p.A., Saronno, Varese, Italy), hydrogen peroxide 1 % (H_2O_2) (Curasept® S.p.A., Saronno, Varese, Italy) topical solution, or placebo (Sodium Chloride 0.9 %).

2.3. Study protocol

All patients were asked to refrain from drinking, eating and perform oral care for at least 30 min before the first, and until the last sample collection. To start, an oral and oropharyngeal swab was performed prior to the oral rinse to assess patient SARS-CoV-2 viral-load at baseline (T0). Patients were then asked to rinse and gargle with 15 mL (one tablespoon) of the assigned antiseptic mouthwash for 60 s. Immediately after the rinse, a second sample was collected to evaluate SARS-CoV-2 viral-load (T1); a third, and last swab was then performed at 45 min after the rinse (T2), considered as the median time of a routinary dental and medical encounter.

Oral swabs were performed within 24 h of hospital admission following the Centers for Disease Control and Prevention (CDC) Specimen Collection Guidelines [19], using a sterile swab with a plastic shaft (eSwab™, Copan Diagnostics INC, Murrieta, California, USA) and applying a gentle rotating pressure upon the oral cavity mucosa (buccal mucosa, labial mucosa, dorsal and ventral tongue, floor of the mouth, and hard palate), and the oropharyngeal mucosa (soft palate, tonsillar pillars, palatine tonsils and pharyngeal wall). The swab was then placed into a sterile vial containing 2 mL of viral transport media (UTM®, Copan Diagnostics INC, Murrieta, California, USA) and transported to the Laboratory of Microbiology and Virology, Department of Molecular Medicine, Sapienza University of Rome, Rome, Italy, for the molecular analysis.

2.4. Molecular detection and quantification of SARS-CoV-2 RNA

Within 2 h from the sample collection, 200 μL of the specimens obtained by sampling both the oral and oropharyngeal mucosa were subjected to total RNA purification using RNA-extraction kits (Norgen Biotek Corporation, Thorold, Canada); subsequently, 2 μL of purified RNA was quantified on a NanoDrop Spectrophotometer (Thermo Fisher Scientific, Rome, Italy) to determine concentration and purity. Reverse transcription was performed on 300 ng of purified RNA using the High-Capacity cDNA Archive Kit (Applied Biosystems, Monza, Italy) followed by reverse-transcription reactions. cDNA samples were then analyzed with in-house quantitative real-time Polymerase Chain Reaction (rtPCR) targeting the N-gene of SARS-CoV-2 using the primers and the hydrolysis probe specific for the SARS-CoV-2 N gene described by Corman et al. [20]. The standards were obtained by cloning the 128 bp of viral N gene into the pCR2.1 plasmid using a TOPO TA cloning kit (In Vitrogen Corporation, San Diego, CA, USA). A linear distribution ($r = 0.99$) was obtained between 10^1 and 10^8 copies of SARS-CoV-2-DNA.

2.5. Statistical analysis

Median viral loads, expressed as the number of copies per mL of oral and oropharyngeal sample, as well as Cycle threshold (Ct) values were analyzed among T0, T1, T2 samples. Proportions were calculated for qualitative variables and were compared among treatment groups and placebo using the Chi square, or Fisher exact test. Median values and interquartile ranges (IQR) were calculated for quantitative variables and were compared using the Kruskall-Wallis, and the Wilcoxon tests. Statistical analysis was performed using the IBM SPSS 27.0 package (Statistical Package for Social Sciences, IBM SPSS Inc., Armonk, New York, USA).

3. Results

3.1. Sociodemographic and clinical characteristics of the study population

A total of 40 patients were screened and 38 were enrolled in the study between December 2020 and August 2021. Two patients were excluded due to absence of viral copies in the oral cavity at the baseline. Most of the patients were males ($n = 34$; 89.5 %) with a median age of 54 years (interquartile range (IQR): 45–64) and never smokers ($n = 28$; 73.7 %); thirteen (34.2 %) patients had at least one comorbidity at the time of the hospital admission. Overall, 29 (76.3 %) patients had a median of three (IQR: 1–5) COVID-19 signs and symptoms at the start of the trial, with the most common being dyspnea ($n = 19$; 50.0 %), fever ($n = 17$; 44.7 %) and cough ($n = 16$; 42.1 %) (Table 1).

Table 1
Patients' characteristics.

	$N = 38$ (%)
Age (years)	
Median age (range)	54 (45–64)
Gender	
Male	34 (89.5)
Female	4 (10.5)
Tobacco use	
Never	28 (73.7)
Current	2 (5.3)
Former	8 (21.0)
COVID-19 related signs/symptoms[a]	
Dyspnea	19 (50.0)
Fever	17 (44.7)
Cough	16 (42.1)
Headache	5 (13.2)
Diarrhea	4 (10.5)
Fatigue	3 (7.9)
Anosmia	2 (5.3)
Comorbidities[b]	
No	18 (47.4)
Yes	13 (34.2)
Unknown	7 (18.4)

[a] Numbers do not add to 38 as patients had more than one COVID-19 related sign/symptom.
[b] Includes: hypertension, cardiovascular diseases, diabetes mellitus II, chronic obstructive pulmonary diseases, obesity and any cancer.

3.2. SARS-CoV-2 viral load at T0, T1, and T2 by treatment group

3.2.1. PVP-I 1 % group

The median viral load of the PVP-I 1 % group at the baseline (T0) was 67.4 (IQR: 13.3–1597.2) copies/mL; after the rinse (T1) the median viral load was 0 (IQR: 0–9223.3) copies/mL ($p = 0.26$) and increased to a median of 9.9 (IQR: 0–3237.1) copies/mL at T2 ($p = 0.42$). Five out of eight (62.5 %) patients had a complete viral load reduction and three of them (3/8; 37.5 %) maintained SARS-CoV-2 negativity at 45 min following the rinse (T2). Overall, the median viral load reduction between T0 and T1 was 19.4 (67.3–8.3) copies/mL, and 33.2 (77.6–5.4) copies/mL between T1 and T2. The median SARS-CoV-2 Ct value in the PVP-I 1 % group at T0 was 36.3 (IQR: 32.0–38.5), followed by 40.0 (IQR: 29.8–40.0) at T1 ($p = 0.002$), and 38.8 (IQR: 30.9–40.0) at T2 ($p = 0.09$).

3.2.2. H_2O_2 1 % group

In the H_2O_2 1 % group, the median SARS-CoV-2 viral load at the baseline (T0) was 192.9 (IQR: 9.6–2841.5). After the rinse (T1), the median viral load was 153.9 (IQR: 4.5–1171.4) copies/mL ($p = 0.59$), which changed to 223.3 (IQR: 17.8–1009.4) copies/mL ($p = 0.79$) at T2. Two (18.2 %) patients at T1, and two (18.2 %) other patients at T2 showed no SARS-CoV-2 viral loads. When all the patients that had any viral load reduction were considered (T1 = 6 patients; T2 = 8 patients), the median load reduction between T0 and T1 was 275.9 (4709.8–5.5) copies/mL, and 148.1 (4331.8–26.7) between T1 and T2. The median SARS-CoV-2 Ct value in the H_2O_2 1 % group at T0 was 34.7 (IQR: 31.1–38.9), 35.7 (IQR: 32.7–35.9) at T1 ($p = 0.27$), and 34.6 (IQR: 32.5–36.7) at T2 ($p = 0.47$).

3.2.3. CHX 0.12 % group

In the CHX 0.12 % group, the median viral load at the baseline was 218.3 (IQR: 52.8–2659.3). After the rinse (T1), the median viral load was 219.8 (IQR: 71.5–946.5) copies/mL ($p = 0.87$), and 512.9 (IQR: 35.1–1114.6) copies/mL ($p = 0.91$) at T2. Only one patient (12.5 %) showed complete absence of SARS-CoV-2 at 45 min (T2), whereas four (50.0 %) patients at T1, and three (37.5 %) patients at T2 showed a median viral copies reduction of 212.3 (IQR: 9223.4–39.1) and 148.2 (IQR: 9223.4–30.6) copies/mL, respectively. The median SARS-CoV-2 Ct value in the CHX 0.12 % group at T0 was 34.9 (IQR: 31.5–36.6), 35.0 (IQR: 32.6–36.1) at T1 ($p = 0.88$), and 33.9 (IQR: 32.4–37.6) at T2 ($p = 0.34$).

3.2.4. Placebo group

In the placebo group, the median SARS-CoV-2 viral load at the baseline was 279.9 (IQR: 32.1–909.1); after the rinse (T1), the median viral load was 71.1 (IQR: 24.9–613.2) copies/mL ($p = 0.61$), and 96.6 (IQR: 24.9–378.5) copies/mL at T2 ($p = 0.40$). One patient (9.1 %) showed no SARS-CoV-2 viral loads at T1, and another patient (9.1 %) had no SARS-CoV-2 viral loads detected at 45 min (T2). When all the patients that had viral load reduction were considered (T1 = 7 patients; T2 = 7 patients), the median load reduction between T0-T1 was 670.5 (1699.4–66.6) copies/mL, whereas between T1-T2 was 372.4 (882.9–113.1) copies/mL. The median SARS-CoV-2 Ct value in the placebo group at the baseline was 34.2 (IQR: 32.6–37.3), 36.1 at T1 (IQR: 32.7–37.1; $p = 0.91$), and 35.7 at T2 (IQR: 33.5–36.9; $p = 0.66$).

3.3. Efficacy of the rinses among the four groups

When all antiseptic mouthrinses were considered, PVP-I 1 % was found to be more effective in reducing the Ct Values at T1 compared to CHX 0.12 % ($p = 0.001$), the H_2O_2 1 % ($p = 0.027$) and the placebo ($p = 0.001$). In addition, PVP-I 1 % was found to be more effective in terms of viral load reduction both at T1 ($p = 0.03$) and at T2 ($p = 0.024$) when compared to the placebo. No other statistically significant differences were found among the other rinses (Table 3). Interestingly, when all the negative patients were considered (at T1 and T2), the median SARS-CoV-2 viral load was 21.5 copies/mL (IQR: 4.9–294.5), and the median Ct value was 37.8 (IQR: 34.1–39.8).

4. Discussion

This single-blinded randomized controlled pilot study reported on the efficacy of three oral antiseptics on the reduction of oral SARS-CoV-2 viral load in the oral and oropharyngeal region. PVP-I 1 % had the highest efficacy with five patients (62.5 %) at T1 and three patients (37.5 %) at T2 having undetectable SARS-CoV-2 viral load after the rinse, with an overall median viral load reduction of 19.4 (IQR: 67.3–8.2) viral loads/mL at T1 ($p = 0.26$), and 33.2 (IQR: 77.6–5.4) viral loads/mL at T2 ($p = 0.42$). The median Ct value in the PVP-I 1 % group at T0 was 36.3 (IQR: 32.0–38.5), followed by 40.0 (IQR: 29.8–40.0) at T1 ($p = 0.002$), and 38.8 (IQR: 30.9–40.0) at T2 ($p = 0.09$). H_2O_2 1 % showed the second highest efficacy, with six patients (54.5 %) at T1, and eight patients (72.7 %) at T2 having a median of 275.9 (IQR: 4709.8 – 5.5; $p = 0.59$) and 148.1 (IQR: 4331.8–26.7; $p = 0.79$) viral copies/mL reduction at T1 and T2, respectively, and four patients (T1 + T2) showing undetectable viral loads. Patients in the placebo group showed a median viral load reduction of 670.5 copies/mL (IQR: 1699.4 - 123.3) at T1 ($p = 0.61$) and 372.3 copies/mL (IQR: 882.9–113.1) at T2 ($p = 0.40$), but overall, only two patients (T1 + T2) showed undetectable viral loads. CHX 0.12 % was the least effective oral rinse with only one patient showing SARS-CoV-2 negativity, and seven patients (T1 + T2) had a median viral reduction of 212.2 (IQR: 9223.3 – 39.1) copies/mL at T1 and 148.2 (IQR: 9223.3 – 30.6) copies/mL at T2; of note, CHX 0.12 % was the only group where the overall median viral load did not reduce at T1 (Table 2).

The efficacy of common oral antiseptic solutions for SARS-CoV-2 inactivation has been previously described in vitro with several studies showing a considerable virucidal effect of PVP-I, CHX, Cetylpyridinium Chloride (CPC) and H_2O_2 at different concentrations, and within 15, 20 and 30 s of contact time with the virus [21–23]. Nevertheless, only few studies have clinically assessed their capability to reduce SARS-CoV-2 viral loads in the oral and oropharyngeal region in patients with COVID-19. Martinez Lamas et al. [24] explored the effectiveness of PVP-I 1 % oral solution in four patients with SARS-CoV-2

Table 2
Changes in SARS-CoV-2 viral loads at admission (T0), right after rising with antiseptics (T1) and after 45 min (T2).

	CHX 0.12 % (N = 8)	PVP-I 1 % (N = 8)	H_2O_2 1 % (N = 11)	Placebo (N = 11)
T1				
SARS-CoV-2 negative[a]	0 (0.0)	5 (62.5)	2 (18.2)	1 (9.1)
SARS-CoV-2 positive	8 (100)	3 (37.5)	7 (63.6)	10 (90.9)
Unknown	0 (0.0)	0 (0.0)	2 (18.2)	0 (0.0)
T2				
SARS-CoV-2 negative[a]	1 (12.5)	3 (37.5)	2 (18.2)	1 (9.1)
SARS-CoV-2 positive	7 (87.5)	5 (62.5)	9 (81.8)	10 (90.9)
SARS-CoV-2 viral loads/mL				
T0 median (range)	218.3 (52.9–2659.3)	67.4 (13.3–1597.2)	192.9 (9.6–2841.5)	279.8 (32.1–909.1)
p-value[1]	0.87	0.26	0.59	0.61
T1 median (range)	219.8 (71.5–946.5)	0 (0–9223.3)	153.9 (4.5–1171.4)	71.1 (24.9–613.2)
p-Value[2]	0.91	0.42	0.79	0.40
T2 median (range)	512.9 (35.1–1114.6)	9.9 (0–3237.1)	223.3 (17.8–1009.4)	96.6 (24.9–378.5)
Real time PCR Ct value				
T0 median (range)	34.9 (31.5–36.6)	36.3 (32.0–38.5)	34.7 (31.1–38.9)	34.2 (32.6–37.3)
p-Value[1]	0.88	0.002[c]	0.27	0.91
T1 median (range)	35.0 (32.6–36.1)	40.0 (29.8–40.0)	35.1 (32.7–39.5)	36.1 (32.7–37.1)
p-Value[2]	0.34	0.09	0.47	0.66
T2 median (range)	33.9 (32.4–37.6)	38.8 (30.9–40.0)	34.6 (32.5–38.0)	35.7 (33.5–36.9)
Variation of viral loads/1 mL T1-T0				
Reduction[b]	4 (50.0)	5 (62.5)	6 (54.5)	7 (63.6)
Median (range)[b]	212.3 (9223.4–39.1)	19.4 (67.3–8.3)	275.9 (4709.8–5.5)	670.5 (1699.4–66.6)
Increase	4 (50.0)	3 (37.5)	3 (27.3)	4 (36.4)
Median (range)	508.5 (124.8–6905.9)	9223.4 (379.0–9223.4)	65.5 (43.7–163.9)	159.7 (24.3–1023.1)
Variation of viral loads/1 mL T2-T0				
Reduction[b]	4 (50.0)	4 (50.0)	8 (72.7)	7 (63.6)
Median (range)[b]	148.2 (9223.4–30.6)	33.2 (77.6–5.4)	148.1 (4331.8–26.7)	372.4 (882.9–113.1)
Increase	3 (37.5)	4 (50.0)	3 (27.3)	4 (36.4)
Median (range)	683.7 (185.1–9223.4)	1787.4 (376.3–9223.4)	65.4 (43.7–1630.1)	262.6 (85.3–1054.7)

[a] A sample was considered SARS-CoV-2 negative (i.e. SARS-CoV-2 not detectable) when the Ct value was >40.
[b] This variable also includes patients that had a complete reduction of the viral load.
[c] Statistically significant.
[1] This p-value refers to the differences detected between T0 and T1.
[2] This p-value refers to the differences detected between T1 and T2.

infection with a median of 37 days (range: 28–41) from the beginning of the study. A nasopharyngeal swab and a salivary sample were obtained at the baseline; all patients rinsed with 15 mL of PVP-I 1 % oral solution for 1 min and salivary samples were taken at 5 min, 1 h, 2 h, and 3 h after the rinse. SARS-CoV-2 was detected in all baseline salivary samples, whereas the nasopharyngeal PCR test was positive in one out of four patients. After the rinse, all patients had reduction of the viral load detected in the saliva samples, with 2/4 participants showing a more significant reduction of the viral copies for at least 3 h (no *p*-values were reported in this study). Similarly, Gottsauner et al. [25] assessed the efficacy of H_2O_2 1 % mouthrinse in 10 patients with SARS-CoV-2 infection. SARS-CoV-2 viral loads were detected at the baseline using 20 mL of 0.9 % NaCl for 30 s; patients were then instructed to rinse and gargle with 20 mL 1 % H_2O_2 for 30 s. SARS-CoV-2 viral load was then measured at 30 min from the oral rinse. The median SARS-CoV-2 viral load at the baseline was 1.8×10^3 (3.1×10^2; 4.7×10^4) copies/mL, whereas the median viral loads after the H_2O_2 1 % rinse was 1.5×10^3 (8.3×10^2; 3.4×10^4) copies/mL of SARS-CoV-2 RNA ($p = 0.96$). In their randomized controlled study, Elzein et al. [26] examined the virucidal activity of CHX 0.2 % and PVP-I 1 % oral solutions in COVID-19 positive symptomatic patients ($n = 61$). First, baseline viral loads were detected by sampling patients' saliva into a sterile container; then, each group rinsed for 30 s with their respective solution. Saliva collection was performed 5 min after the rinse. There was an increase of the mean Ct values of human RNaseP in the saliva, from 25.41 ± 2.5 [18.4–32.21] cycles detected before the gargle, to 26 ± 2.72 [19.49–32.5] cycles, and a statistically significant difference in terms of viral load reduction between the delta Ct of patients using the placebo solution (0.519 ± 0.519) and each of the 2 solutions PVP-I 1 % (4.72 ± 0.89) and CHX 0.2 % (6.37 ± 1.08) ($p = 0.012$ and $p = 0.0024$, respectively). No difference was detected in terms of delta Ct between the two solutions ($p = 0.332$). Another recent large study evaluated the efficacy of CHX 0.12 % oral solution, and spray (for the oropharynx) in 294 hospitalized patients with confirmed COVID-19 [27]. Patients were instructed to rinse with CHX 0.12 % oral solution (group one) or gargle with the CHX 0.12 % oral solution and topical spray (group two) for 30 s, two times a day for four days. On day 4 patients were tested again for presence of SARS-CoV-2 by rRT-PCR. Overall, 41/121 (62.1 %) patients that used the CHX 0.12 % rinse tested negative for SARS-CoV-2, whereas among patients who used a combination of oral rinse and oropharyngeal spray, 93 (86.0 %) tested negative for SARS-CoV-2. Of note, neither the Ct values, nor the viral loads were determined before, and after the study. In their recent blinded, placebo-controlled clinical trial, Chaudhary et al. evaluated the efficacy of H_2O_2 1 %, CHX 0.12 %, and PVP-I 0.5 % oral rinses, in 201 asymptomatic, pre-symptomatic, and post-symptomatic patients with COVID-19 [16]. Initially all patients were asked to collect their saliva in a sterile vial containing; then they were asked to rinse their mouth with 15 mL of the randomly assigned mouth rinse for 60 s. Ultimately, salivary samples were additionally collected at 15, and 45-min after the rinse. Overall, salivary SARS-CoV-2 was detected in 23 % of asymptomatic, 60 % of post-symptomatic, and 28 % of pre-symptomatic participants at the baseline. All four mouth rinses (including placebo)

Table 3
Rinse efficacy comparison among the CHX, H_2O_2, PVP-I and placebo groups at T0, T1 and T2.

Variables	p-Value					
	CHX vs H_2O_2	CHX vs PVP-I	CHX vs placebo	H_2O_2 vs PVP-I	H_2O_2 vs placebo	PVP-I vs placebo
SARS-CoV-2 Ct value T0	0.717	0.505	0.84	0.717	0.652	0.6
SARS-CoV-2 Ct value T1	0.37	0.001[a]	0.84	0.027[a]	0.456	0.001[a]
SARS-CoV-2 Ct value T2	0.968	0.234	0.442	0.442	0.478	0.152
SARS-CoV-2/mL viral load T0	0.717	0.505	0.84	0.717	0.652	0.6
SARS-CoV-2/mL viral load T1	0.606	0.328	0.395	0.606	0.882	0.395
SARS-CoV-2/mL viral load T2	0.778	0.645	0.6	0.6	>0.99	0.545
Increase T1-T0	0.229	0.229	0.486	0.1	0.629	0.114
Reduction T1-T0	0.808	0.111	0.527	0.435	0.463	0.03[a]
Increase T2-T0	0.4	0.686	0.486	>0.99	>0.99	0.686
Reduction T2-T0	>0.99	0.2	0.527	0.154	0.694	0.024[a]

Abbreviations: CHX, chlorhexidine; H_2O_2, hydrogen peroxide; PVP-I, povidone-iodine.
[a] Statistically significant.

reduced salivary carriage of SARS-CoV-2 with a median viral reduction of 61 % through 89 % (mean, 25 %–74 %) at 15 min, and a median reduction ranged from 70 % through 97 % at 45 min (mean, 30 %- 43 %). Neither the 15-min reduction in viral load nor the persistence of reduction at 45 min differed among the mouth rinses ($p > 0.05$, Dunn test). In their randomized clinical trial, Ferrer et al. evaluated the efficacy of five mouthwashes (CPC 0.07 %, CHX 0.12 %, H_2O_2 1 %, PVP-I 2 % and distillated water) in reducing the SARS-CoV-2 loads in the saliva of 84 patients. Patients were asked to provide an unstimulated sample of saliva into a sterile container; then, each patient were asked to rinse with their respective oral rinse for 60 s. Subsequently, three more saliva samples (at 30, 60 and 120 min) were collected to determine the SARS-CoV-2 viral load. Overall, none of the patients had their salivary viral loads reduced at any timepoint compared to the baseline. However, when looking at the relative changes compared to the values before the mouthwash, the highest effects on viral load reduction were observed 120 min after treatment in the PVP-I and CPC groups, with an approximately 30 % mean viral load reductions [28]. Similarly, Seneviratne et al. Explored the effectiveness of PVP-I 0.5 %, CHX 0.2 %, CPC 0.07 % and water (control) in reducing the SARS-CoV-2 viral load in 34 COVID-19 patients. Subjects were instructed to passively collect 3 mL of saliva prior to the oral rinse, and then asked to rinse with their respective antiseptic mouthwash. To evaluate the duration of the efficacy of mouthrinses, salivary samples were collected at 5 min, at the 3 h and 6 h post-rinsing, and Ct Values were evaluated at each time. Overall, a statistically significant ($p < 0.05$) increase in fold change of Ct value at 5 min (1) and 6 h (0.9) was observed post-rinsing with CPC and an increase in fold change of Ct value at 5 min (1.1) and 3 h (1.2) was also observed in patients rinsing with PVP-I, compared to the water group patients [29]. Our results showed that PVP-I 1 % was the most effective oral rinse/gargle to reduce oral SARS-CoV-2 viral loads, followed by H_2O_2 1 %, and with CHX 0.12 % being the least effective. Differently to the abovementioned studies, we also evaluated that all the patients with a negative oral viral load (T1 and T2) had a baseline median viral load largely low, with 21.5 copies/mL (IQR: 4.9–294.5), and with a median Ct value of 37.8 (IQR: 34.1–39.8) presumably indicating a higher effect of such solutions in asymptomatic or pre-symptomatic patients. Of note, both in our study and in Chaudhary et al. [16], viral loads reduced in the placebo group, which may be explained by a possible mechanical effect of the placebo rinse in the reduction of the viral load.

One of the strengths of our work was that our samples to detect the SARS-CoV-2 viral load were obtained by trained investigators, whereas most the previous studies sampled SARS-CoV-2 viral loads from a patient self-collection of the saliva which may have introduced a bias. Also, our study compared three different oral antiseptic solutions, whereas most of the previous studies only analyzed one (PVP-I or CHX) or two (PVP-I and H_2O_2/PVP-I and CHX) oral mouth rinses and did not include the placebo group. Nevertheless, our study has several limitations. First, the sample size was relatively small with patients (although larger than some other studies) and patients with severe COVID-19 disease (i.e., admitted in SICU and ICU) were not included; therefore, the findings may not be generalizable to all COVID-19 patients. Secondly, we did not assess the long term (hours, days) effect of the antiseptic solutions, nor the efficacy of a combination of multiple oral antiseptics on SARS-CoV-2 viral loads reduction. Other limitations were related to the technical approach; the rtPCR technique is only able to detect RNA copies but cannot give any indication on the infectivity of the detected virus fragments. However, the assessment of SARS-CoV-2 infectivity would require viral isolation assays in BSL-3 facilities that are not widely available. Nevertheless, it is reasonable to hypothesize that the low viral loads we detected (about 10^2 to 10^3 RNA copies per mL; Ct values >30) do not correspond to infectious virus. As a matter of fact, oropharyngeal viral load in hospitalized patients declined with the course of the disease. Notwithstanding, we retain that the viral load reductions we observed after the rinse could be effective also if applied in cases SARS-CoV-2 recent infections in subjects with asymptomatic/pauci-symptomatic infections, that are seen for dental care.

5. Conclusions

In summary, we showed that all patients had a reduced oral SARS-CoV-2 viral loads at T1 after using any of these rinses, except for the CHX 0.12 % group where the median viral load had marginally increased; nevertheless, none of the reductions was statistically significant.

Among all groups, PVP-I 1 % was the most effective rinse against SARS-CoV-2 especially in patients with low viral copy numbers at baseline. Low viral load is usually encountered in either asymptomatic patients or in patients in the recovering stage of the condition, which, despite their mild/moderate clinical symptoms, may continue to present a certain period of viral shedding, suggesting the possibility of transmission during their asymptomatic period. The use of PVP-I 1 % could be considered as an additional prevention measure along with the recommended personal protective equipment in medical and dental settings for patients requiring procedures in the oral and oropharyngeal area. Future larger prospective studies evaluating the length of therapy and efficacy, and the combination of multiple oral antiseptics are needed to identify effective ways of reducing oral SARS-CoV-2 viral loads in patients with COVID-19.

Authors' contribution

All authors contributed to the study conception and design. Material preparation, manuscript preparation and drafting were performed by Paolo Junior Fantozzi, Pampena Emanuele, Umberto Romeo and Villa Alessandro. Data collection and analysis was performed by D'Ettorre

Gabriella, Lazzaro Alessandro, Gentilini Elio, Di Vanna Domenico, Pierangeli Alessandra, Oliveto Giuseppe and Sorrentino Leonardo; statistical analysis was performed by Pampena Riccardo. The remain authors contributed correcting and drafting the manuscript and supervised the whole study. All authors read and approved the final manuscript.

Compliance with ethical standards

We declare that this manuscript is original, has not been published before, and is not currently being considered for publication elsewhere.

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Ethical approval

The datasets generated during and/or analyzed during the current study are not publicly available due to the privacy policy of the Sapienza University of Rome and Umberto I Hospital but are available from the corresponding author on reasonable request. The study was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines and was approved by the Sapienza University/Umberto I Polyclinic Hospital Institutional Review Board (IRB N.001392). This research was registered on the Clinical Trials website ISRCTN (#40398).

Informed consent

Informed consent was obtained from all individual participants included in the study.

Declaration of competing interest

We know no conflict of interest associated with this publication.

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