

Use of an antiviral mouthwash as an additional barrier measure in the SARS-CoV-2 transmission in adults with asymptomatic to mild COVID-19: A multicenter, randomized, double-blind controlled trial

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Abstract

Background The research hypothesis is that commercially available mouthwash with β -cyclodextrin and citrox (bioflavonoids) could decrease the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) salivary viral load in adults with asymptomatic to mild COVID-19.

Methods In this RCT, SARS-CoV-2 PCR-positive patients aged 18-85 years with asymptomatic to mild COVID-19 symptoms <8 days were recruited. A total of 154 eligible patients were randomly assigned (1:1) to antiviral β -cyclodextrin and citrox mouthwash (CDCM) or placebo. Three rinses daily were performed for 7 days. Saliva sampling was performed on day 1 at 9 a.m. (T1), 1 p.m. (T2) and 6 p.m. (T3). On the following 6 days, one sample was taken at 3 p.m. Quantitative RT-PCR was used to detect SARS-CoV-2. The trial is registered at ClinicalTrials.gov (NCT04349592).

Findings CDCM was significantly more effective than placebo 4 hours after the first intake ($p < 0.001$), with a median percentage decrease T1-T2 of -14.25% [95% CI; $-32.68\% - 0.06\%$]. In patients with an initial salivary load $> 2.95 \log_{10}$ copies/mL, there was a significant difference in the reduction in viral load at T2. Over the course of one day, the first mouthwash rinse significantly reduced the viral load, and the second dose maintained this low value, compared to placebo. At day 7, there was still a greater decrease in salivary viral load over time in the CDCM group. In individuals with an initial viral SARS-CoV-2 load higher than $4.12 \log_{10}$ copies/mL or $5.16 \log_{10}$ copies/mL, CDCM reduced the salivary viral load more quickly than placebo (MLM p -value = 0.03 ; 0.029).

Interpretation This trial supports the relevance of using mouthwash with β -cyclodextrin and citrox as an additional barrier measure on day 1 to reduce the dissemination of SARS-CoV-2. Over 7 days, the use of this mouthwash showed a benefit of viral load reduction for patients with the highest initial loads.

Introduction

The global pandemic due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has led to numerous changes and restrictions that affect people's daily lives as well as many professional activities, especially for those caring for symptomatic and infected patients.¹ This COVID-19 pandemic has served as an entry point for the medical research and healthcare communities to emphasize the importance of the oral sphere in the transmission of viruses and infectious diseases.

Saliva, composed of 99% water, has a significant role in human-to-human transmission during the COVID-19 pandemic. Every day, the human salivary glands secrete 600 mL of serous and mucinous saliva containing minerals, buffers, electrolytes, enzymes and enzyme inhibitors, growth factors and cytokines,

immunoglobulins (e.g., secretory immunoglobulin A), mucins and other glycoproteins.² In addition to saliva secreted by the major and minor salivary glands, saliva samples also contain secretions from the nasopharynx or from the lungs through the action of cilia lining the airway.³ Indeed, SARS-CoV-2 is found in nasopharyngeal secretions, and the viral load is consistently high in the saliva, mainly in the early stage of the disease.⁴ Saliva samples have previously been shown to be highly consistent, over 90%, with nasopharyngeal samples in the detection of respiratory viruses, including coronaviruses.⁵ SARS-CoV-2 is detected in 91.7% of saliva samples from COVID-19 patients, and the number can reach up to 1.2×10^8 infective copies per mL.⁶

Knowing well the persistence, transmission, and spread of SARS-CoV-2 through proximity, the oral route is now emerging as a major environmental concern for community transmission.⁷ SARS-CoV-2 may be transmitted via saliva directly or indirectly, even in patients who do not cough or have other respiratory symptoms.⁸ When a person coughs, sneezes, breathes, or converses, he or she produces saliva droplets containing microorganisms.⁹ The quantity and the size of saliva droplets differ between individuals; therefore, the risk of transmission also varies. The quantity and size of saliva droplets differ between individuals; therefore, the risk of transmission also varies. One cough or 5 min of conversation produces approximately 3000 saliva droplets. One sneeze produces approximately 40000 saliva droplets that can be disseminated several meters in the air.⁹ Saliva droplets ($> 60 \mu\text{m}$) allow the transmission of SARS-CoV-2 when persons are in close contact (1 m and 3 m).¹⁰ Moreover, although it is not yet clearly established, virus-laden aerosols (droplets $< 60 \mu\text{m}$) can contribute to the spread of SARS-CoV-2 and allow contamination at a distance of up to 7 to 8 m.¹¹ Droplets have the ability to fall to the ground. They have a diameter larger than $5 \mu\text{m}$. Through contact, all the personal items of the infected individual and the immediate environment can be considered a potential medium for the transmission of the virus even by indirect contact with those items.

The use of cosmetic mouthwashes is an "adjuvant" treatment part of the usual treatment or individual prophylaxis, especially in oral health. Considering mouthwashes as agents that can reduce the viral load of SARS-CoV-2 in the fight against the COVID-19 pandemic is an extremely attractive concept¹²⁻¹⁴, especially in adults who are asymptomatic or have mild COVID-19. Among a nonexhaustive list of marketed mouthwashes containing antiviral molecules based on *in vitro* and *in silico* studies, compounds that could be of interest in the oral viral load fight against SARS-CoV-2, such as hydrogen peroxide, β -cyclodextrin, flavonoids, essential oils, cetylpyridinium chloride or povidone-iodine, were noticed.^{12,14,15} The concept put forward is that some commercially available mouthwash formulations may play a role in decreasing the risk of transmission of SARS-CoV-2.¹²⁻¹⁴ However, given the limited number of *in vitro* and *in silico* studies that have been conducted, there is no scientific evidence to recommend anti-SARS-CoV-2 mouthwashes to control viral load in the oral cavity. At this time, no randomized controlled trial (RCT) studies have been conducted or completed.

The research hypothesis is that commercially available mouthwashes with β -cyclodextrin and citrox (bioflavonoid) antivirals could decrease the SARS-CoV-2 viral load and thus represent an additional

barrier measure to fight against the COVID-19 pandemic. The specific question, formulated according to PICO principles, was as follows: In COVID-19 outpatients (population), does β -cyclodextrin-citrox rinsing as a posttreatment protocol (intervention) have a beneficial effect on SARS-CoV-2 saliva load parameters (outcome) compared to rinsing with placebo solution (control)?

Methods

Study design

This trial was a multicenter, double-blind RCT with two parallel arms (1:1 ratio). The clinical trial was conducted with the collaboration of four French hospital centers: Emile Roux Hospital Center (Le Puy-en-Velay, France), Protestant Infirmary (Lyon, France), St Joseph St Luc Hospital (Lyon, France) and Hospital Center (Mont de Marsan, France). Participants were enrolled at these centers, and monitoring occurred at home. Written informed consent was obtained from each participant before enrollment.

The “Committee for the Protection of Persons South Mediterranean III” (University Hospital Center of Nîmes, France) reviewed and approved the clinical trial protocol. The study followed CONSORT guidelines for trials. The study was conducted in compliance with the principles of the Declaration of Helsinki, the Good Clinical Practice guidelines, and local regulatory requirements.

Participants

The study’s planned population consisted of SARS-CoV-2 PCR-positive adult outpatients, asymptomatic patients and patients with mild clinical symptoms. Asymptomatic patients were defined as individuals without clinical signs, whereas patients with mild cases were defined as outpatients and patients with mild clinical symptoms or lower or upper respiratory tract infections.¹⁶

Eligibility was restricted to adults aged 18-85 years old with a clinical diagnosis of COVID-19 infection, clinical signs that had been present for less than 8 days, virological confirmation, an understanding and acceptance of the trial and written agreement to participate in the trial.

The exclusion criteria were pregnancy, breastfeeding, an inability to comply with the protocol, a lack of written agreement, mouthwash use on a regular basis (more than once a week), an inability to answer questions and a lack of cooperation.

Randomization and masking

Eligible patients were randomly assigned (1:1) to either the β -cyclodextrin-citrox mouthwash (CDCM) group or the placebo group. Randomization was stratified by center. Randomization was integrated into the inclusion process by generating a random number after automatic analysis of the center’s prior randomizations. The randomization codes for each allocation schedule cohort were generated individually. The randomization code was allocated to each participant in sequence in the order of registration, and then participants received experimental materials labeled with the same code. A

randomization sequence was prepared by a clinical senior investigator not involved in the trial using e-CRF Voozalyon 1.3 (Voozadoo, Caluire, France).

Once enrolled, participants each received three 200 mL medication vials. The contents of the medication vials were unknown to participants, investigators and staff. Each vial contained either a mouthwash containing the antiviral components (β -cyclodextrin (0.1%) and citrox (0.01%)) or placebo with similar appearance and content without the above-mentioned antiviral components; the labels on the vials were identical. All participants, investigators, statisticians, and laboratory staff were masked to treatment allocation.

Procedures

Participants were instructed to use three mouthwashes per day (at 9 a.m., 2 p.m., and 7 p.m.), with either 30 mL of CDCM or placebo, both provided by Curaden AG (Kriens, Switzerland) for 1 min (Appendix Fig. S1). Participants were instructed to collect their saliva by trained nurses using the “Saliva Collection System” kit (Greiner Bio-one, Kremsmünster, Austria). Saliva sampling was performed on the first day at T1 (9 a.m., before the first mouthwash) and then at T2 (1 p.m.) and T3 (6 p.m.). On the following 6 days, only one sample was taken at 3 p.m.

All samples were refrigerated and shipped by similar means of transport to laboratories of the National Reference Center for Respiratory Viruses (Hospices Civils de Lyon, Lyon, France) with PCR testing equipment, where procedures for SARS-CoV2 RNA detection and quantification were performed. RNA extraction was realized using the NucliSens easyMAG instrument (bioMérieux, Marcy-l’Etoile, France) following the manufacturer’s instructions. RdRp-IP2 and RdRp-IP4 quantitative RT-PCR was used to detect SARS-CoV-2 with the Invitrogen Superscript™ III Platinum One-Step qRT-PCR system (Invitrogen, Illkirch, France). When a sample was positive for RdRp-IP4, the quantification of the number of RNA copies was performed according to a scale ranging from 10^2 to 10^6 copies per μL . The viral load in saliva was calculated as the number of RNA copies per mL of saliva. Primer and probe sequences (Eurofins, Genomics, Germany; Appendix Table S1) correspond to the RdRp-IP2, the RdRp-IP4 assays designed at The Institut Pasteur to target a section of the RdRp gene based on the sequences of SARS-CoV-2 made available on the Global Initiative on Sharing All Influenza Data database on January 11, 2020. For further details see the appendix (pp 3-4).

Outcomes

The general objective of the study was to describe the evolution of salivary SARS-CoV-2 viral load (copies/mL) in COVID-19 outpatients receiving mouthwashes with or without antivirals.

Primary outcome measures included changes from baseline SARS-CoV-2 in salivary samples at two time points, 4 and 9 hours, within 1 day after the first intake.

Secondary outcome measures included changes from baseline SARS-CoV-2 in salivary samples at 6 days after the first dose.

Exploratory outcomes were i) the virological efficacy of the experimental therapy (CDCM) compared to the placebo as evaluated by the quantity of SARS-CoV-2 in salivary samples at T1 and 4 hours after the first and second intakes and ii) the virological efficacy of the experimental therapy compared to the placebo as evaluated by the quantity of SARS-CoV-2 in salivary samples on day 1 at T2 and T3 and day 7.

Statistical analysis

Sample-size calculations were estimated using the freeware STPLAN (Version 4.5, Department of Biomathematics, University of Texas M.D. Anderson Cancer Center, Houston, Texas, USA). The sample size was based on a minimal viral load difference of 1 log₁₀ copies/mL between control and experimental groups, a common standard deviation of 2 log₁₀ copies/mL, a power of 0.9 and a type I error of 5%. It was calculated at least 70 subjects per group. With an estimated drop-out rate of 25%, 88 subjects per group were required (unilateral test).

The primary efficacy analysis was performed on an intention-to-treat (ITT) basis with all randomly assigned patients. In the whole sample at day 1, we have performed a paired nonparametric Wilcoxon signed rank test with Bonferroni correction comparing the decrease of viral load over time: T1 vs T2, T1 vs T3 and T1 vs day 7 (i.e., secondary endpoint) in both groups. Then the two groups were compared at each time thanks to a non-parametric Mann-Whitney U test. Finally, a mixed effect linear model (viral load repeated data along time from day 1 T1 to day 7) was performed with group (CDCM/placebo) as fixed effect and individuals as random effect.

All analyses were reperformed on the datasets with T1 values starting at the first quartile (Q1), the second quartile (Q2) and the third quartile (Q3). No correction of p-values was made for multiple tests. Except for the Mann-Whitney test, the other tests were based on the unilateral hypothesis (H1: CDCM < placebo).

The statistical methods are indicated in the table footnotes. All analyses other than sample-size calculations and graphic illustrations were performed using R (version 3.6.0, The R Foundation for Statistical Computing Platform). This article analyzed the first outcomes of the protocol registered at ClinicalTrials.gov (NCT04349592).

Role of the funding source

The funder of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. All the authors have full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between June 9 and December 11, 2020, out of 1195 selected patients, 176 met the criteria for inclusion with COVID-19 symptom onset less than 8 days and were enrolled in this trial and then randomized (Figure 1). The baseline characteristics of the two study groups for which data were available (N=154) were similar (Appendix Table S2). The mean age of the two groups was 43.89 ± 15.55 years, ranging from 18 to 77 years; 45.75% of patients were male. A total of 95.21% were outpatients, and 78.32% of participants had no medical antecedents. Among the participants, 9.27% were asymptomatic (no clinical signs) and 90.73% had mild symptoms, with 3.68 ± 2.29 of the symptoms on the COVID-19 report forms. Adults with laboratory-confirmed COVID-19 were randomized within 4 days (95% CI; 3-5 days) of symptom onset to receive CDCM or placebo. The first saliva specimens were collected at a median time of 4 days (95% CI; 3-5 days) after nasopharyngeal PCR-positive results. The first saliva sample was reported to be in the range of 0 - $10^{1.19}$ log₁₀ SARS-CoV-2 copies per milliliter of saliva, with a median of 4.12 log₁₀ copies/mL. The first quartile (Q1) corresponded to a viral load starting at 2.95 log₁₀ copies/mL, whereas the second (Q2) corresponded to a viral load starting at 4.12 log₁₀ copies/mL, and, the third (Q3) corresponded to a viral load starting at 5.16 log₁₀ copies/mL. No serious adverse events - allergy; nasal, oral or oropharyngeal mucosa (e.g., erosions, ulcers, bleeding) irritation/burning; long-term staining of mucous membranes or teeth; or accidental ingestion - were determined to be related to the study treatment.

The primary outcome, a change in viral load during day 1, was measured in 76 of 154 patients (49.35%) in the CDCM group versus 78 (50.65%) in the placebo group. Descriptive data on the distribution of values between the CDCM group and the placebo group suggest a continuous decrease in SARS-CoV-2 salivary load with a continued reduction between T1-T2 and T2-T3 for the both groups (Figure 2). The observed data of the evolution of the median over time support this trend. The observed data of the evolution specifically for patients with high initial loads (6 log₁₀ copies/mL) at T1 are interesting. For the CDCM group, these data disappeared between T1 and T2, a potential sign of an impact on the high values, before reappearing with a lower quantity and a higher dispersion between T2 and T3. On the other hand, the data of the placebo group appeared stable between T1-T2 and T2-T3, except for 2 extreme values (10.19 and 9.54 log₁₀ copies/mL), which did not appear anymore.

The quantitative results are presented in Table 1. A significant difference was observed in viral load reduction in the before-after comparison of the same patients receiving CDCM versus no difference for the placebo group from T1 to T2 ($p < 0.001$). The percentage median decrease in salivary viral load was -14.25% [-32.68% - 0.06%] (CDCM) versus -7.31% [-22.37% - 12.63%] (placebo). It can be concluded that at T2, 4 hours after the first intake of the mouthwash, CDCM had an effect of reducing the SARS-CoV-2 viral load in adults with COVID-19 who were asymptomatic or had a mild clinical form, while the placebo did not. At T3, the salivary viral load decreases were significant for both groups compared to T1 (CDCM: $p < 0.001$; placebo: $p = 0.007$). However, no significant difference between the 2 groups was detected.

The time-by-time outcomes on day 1 according to the initial salivary load were analyzed in 3 subgroups based on the quartiles of the distribution (Q1, Q2, Q3). The descriptive results and the quantitative results

are presented in Figure 2 and Table 2.

For patients with an initially salivary SARS-CoV-2 load $>Q1$ ($2.95 \log_{10}$ copies/mL), there was a significant difference in the reduction in the viral load between T1 and T2. CDCM had an effect, while the placebo did not ($p=0.025$). The reduction in SARS-CoV-2 salivary load for T1-T3 was similar in the CDCM group and the placebo group ($p=0.053$). The salivary viral load significantly decreased over the T1-T3 period for both groups ($p<0.001$); however, there was a more positive impact for the CDCM group (-19.15% [-40.44% - -4.08%] for CDCM vs. -14.47% [-29.49% - 4.39%] for placebo).

For patients with an initially salivary SARS-CoV-2 load $>Q2$ ($4.12 \log_{10}$ copies/mL), the results did not show a significant difference between CDCM and placebo at T2 ($p=0.122$) or T3 ($p=0.121$). The median percentage decrease between T1 and T3 was -16.02% for CDCM (95% CI; -35.85% - -3.52%) and -12.34% for placebo (95% CI; -27.76% - 3.11%).

For patients with an initial SARS-CoV-2 saliva load $>Q3$ ($5.16 \log_{10}$ copies/mL), the quantitative results showed no significant difference between the 2 groups for periods T1-T2 and T1-T3. The median percentage decrease at T1-T3 was -24.14% for CDCM (95% CI; -41.05% - -4.49%) and -17.17% for placebo (95% CI; -29.26% - -3.54%).

Secondary outcomes concerned the change from baseline in the amount of SARS-CoV-2 in salivary samples at 7 days. The virological efficacy of CDCM compared to placebo was evaluated by the quantity of SARS-CoV-2 in salivary samples on days 1 and 7. Among the 154 participants, data on secondary outcomes were available for 141 (91.6%). The number of patients was 73 (51.8%) in the CDCM group vs. 68 (48.2%) in the placebo group. The distribution of values over time in the CDCM and placebo groups suggests a similar, continuous decrease in the salivary load of SARS-CoV-2 (Figure 3 and Appendix Fig. S2).

Descriptive data on the distribution of values between the CDCM group and the placebo group suggested a continuous decrease over the 7 days (Figure 3). In the CDCM group, half of the patients had a zero viral load at days 5, 6 and 7 while the same result was only observed at day 5 and day 7 in the placebo group.

Quantitative data analysis indicated that at day 7, no significant difference between patients receiving CDCM and those receiving placebo was observed ($p=0.417$) (Table 2). In both groups, the viral load was significantly lower on day 7 than on day 1 T1 ($p<0.001$). With the linear mixed model, a higher but nonsignificant ($p=0.098$) reduction in the viral load was observed in the CDCM group (mean difference -0.18 [90% CI; -0.41 - 0.05]). Detailed results of the analyses are provided in Table 1 and Appendix Table S3.

The descriptive analysis according to the initial SARS-CoV-2 load is presented in Figure 3. Regardless of the initial load, at day 7, the median salivary viral load was always lower for the CDCM group than for the placebo group. Quantitative data analysis revealed no significant differences between the two groups for the values $> Q1$, $Q2$, and $Q3$ (Table 1). However, for initial salivary loads greater than $5.16 \log_{10}$

copies/mL (>Q3), the median percentage decrease at day 7 compared with that at T1 for the CDCM group vs. the placebo group was -100% [-100% - -62.26%]. vs. -73.97% [-100% - -55.68%]. With the linear mixed model, a more significant reduction in the viral load was observed for patients with an initial viral load > Q2 in the CDCM group; mean difference (-0.28 [90% CI; -0.52 - -0.04]); p=0.03. Likewise, the analysis of patients with an initial viral load indicated a mean difference in the CDCM group versus the placebo group of -0.44 [90% CI; -0.80 - 0.07] (p=0.029).

Discussion

Our trial tested the hypothesis that a 1-min morning rinse with a β -cyclodextrin and citrox mouthwash would lead to superior short-term clinical results compared to a placebo rinse in terms of reducing salivary viral load in COVID-19 patients. A single CDCM rinse has the potential to significantly reduce the risk of SARS-CoV-2 contamination from saliva. Over the course of one day, the first CDCM rinse significantly reduced the viral load, and the second dose maintained this low value, compared to placebo. Two doses of mouthwashes at the same concentrations and using similar dosing schedules were well tolerated.

The rationale for selecting CDCM was defined by considering its potential benefit to reduce contamination from adults who are asymptomatic or those who have mild SARS-CoV-2 infection. The primary endpoint of satisfactory clinical relevance suggests an effect of CDCM versus placebo (decrease during the T1-T2 period: -14.25% [95% CI; -32.68% - 0.06%] versus -7.31% [95% CI; -22.37% - 12.63%]). The salivary load significantly decreased over the T1-T3 period for both groups (p<0.001; p= 0.007), with a more positive impact for the CDCM group versus the placebo group. The percentage decrease in the T1-T3 period was -15.96% [95% CI; -40.3% - 3.25%] versus -12.24% [95% CI; -28.82% - 9.61%].

According to the initial salivary viral load, several observations should be noted. First, among participants with an initial load higher than $2.95 \log_{10}$ copies/mL (>Q1), a significant decrease of -15.09% [95% CI; -32.03% - -2.46%] in the CDCM group was observed in the 4 hours separating T2 from T1. Second, their salivary viral load significantly decreased over the T1-T3 period in both the CDCM group and the placebo group; however, there was a more positive impact in the CDCM group than in the placebo group (% decrease T1-T3 median: -19.15% [95% CI; -40.44% - -4.08%] vs. -14.47% [95% CI; -29.49% - 4.39%]). Third, for patients with initial loads greater than $4.12 \log_{10}$ copies/mL (>Q2) or than $5.16 \log_{10}$ copies/mL (>Q3), a higher but nonsignificant percentage of viral load reduction was observed for the CDCM group than for the placebo group. A suggested hypothesis, requiring further research, is that, for participants with the highest viral load, i.e., > Q2, the frequency of mouthwash use could be insufficient to significantly impact the reduction in the viral load within a very short period of time (4 hours; 9 hours).

A positive treatment effect was demonstrated in a clinical situation qualified as "minimal" in terms of time. Early administration of CDCM to adults with asymptomatic/mild COVID-19 reduced the salivary viral load and thus the potential spread of this virus. Our results argue in favor of an early generalized use of CDCM among the general adult population in specific events.

Our study provided, for the general population, unclear evidence for the secondary outcome, defined as antiviral load responses to mouthwashes on day 7, that CDCM led to a larger decrease in SARS-CoV-2 salivary viral load than placebo. On day 7 the median load was 0 [95% CI; 0 - 2.65] for the CDCM group versus 0 [95% CI; 0 - 3.05]. By eliminating the fluctuation effect, there was still a greater drop in salivary viral load over time in the CDCM group. For patients with an initial viral SARS-CoV-2 load higher than Q2 or Q3, CDCM reduced the salivary viral load more quickly than placebo (MLM p-value = 0.03 and 0.029).

One of the strengths of our trial is, in a planned ITT analysis, its adequate sample size and rigorous design, intended to be primarily pragmatic but with substantial emphasis on internal validity. The calculated p-values presented can support powerful statistical conclusions. Our clinical project is the first RCT available focusing on the potential impact of mouthwashes on the salivary viral load of COVID-19 patients. Additionally, the viral load profiles of our participants and our results indicate that this mouthwash treatment may reduce salivary transmission among the general population. Indeed, we have reported data on this treatment from adults in the general ambulatory population, symptomatic and asymptomatic, who tested positive for SARS-CoV-2 infection, including data from more sensitive groups, such as older individuals and individuals with comorbidities. Seventy-five percent of patients were between 30.2 and 55.0 years of age. Hospitalized patients (4.11%) had mild clinical cases. None of them had any health complications during the trial.

The use of a daily antiviral mouthwash, as an additional barrier measure, could be important in the fight against the COVID-19 pandemic because the oral cavity is an important reservoir of SARS-CoV-2. Transmission from asymptomatic individuals was estimated to account for more than half of all transmissions, and 30% of individuals with infection never develop symptoms.¹⁷ In addition, many individuals with symptomatic cases of COVID-19 did not seek medical advice despite recommendations. Collaborative surveillance data in France indicate that only 31% of people with COVID-19-like symptoms consulted a healthcare provider.¹⁸ A preventive intervention involving a twice-daily (9 a.m., 1 p.m.) CDCM rinse before scheduled events or for vulnerable communities, such as healthcare workers, could be beneficial.

The antiviral activity of mouthwash is based on β -cyclodextrin and citrox (bioflavonoids).¹⁴ These molecules have demonstrated antiviral activity against several viruses, but evidence for their action against SARS-CoV-2 was based only on *in silico* studies.¹⁴ The standard formulation of the reference CDCM also contains chlorhexidine (0.09% CHX), which is used as a preservative agent to inhibit the growth of microorganisms in this product as specified in article 10a of Directive 2001/83/EC.¹⁹ A debate could be initiated on the relevance of selecting this molecule as an excipient instead of an active ingredient for higher concentration (CHX 0.12%).¹⁴ The use of this mouthwash could be associated with damage related to the toxicity of the solution itself or to alterations in the natural microbial flora of the mouth or nose. Three daily doses of 30 mL of CDCM for 1 min over a 7-day period were well tolerated in adults with COVID-19. The rate of compliance for the CDCM group was 97%. No impaired sense of taste or temporary discoloration of the teeth or tongue were related to the study treatment.

Our study had limitations related to the duration of infectivity, unclear virological levels, i.e., detection and viral load, in patients and their relationship to infectivity and disease severity. The time elapsed from the first salivary collection to the time of first symptom reporting or the time delay estimate for adults without clinical symptoms was one of these limitations. The time from symptom onset to first saliva collection was 5.61 ± 1.58 days. Since infectivity appears to peak at or before symptom onset, the initial viral load data from our trial underestimate the salivary concentration load of the general population during the incubation period.²⁰ In addition, 9.27% of the participants did not show clinical signs at baseline. In this case, it was impossible to estimate the time delay between the contamination of the individual and the salivary collection. COVID-19 patients with mild-to-moderate illness are highly unlikely to be infectious after more than 10 days of symptoms.²¹

In summary, a β -cyclodextrin and citrox mouthwash had a significant beneficial effect compared to a placebo mouthwash on reducing SARS-CoV-2 salivary viral load in adults with asymptomatic or mild COVID-19 4 hours after the initial dose. In the same context, CDCM, associated with 3 daily mouthwashes during this period, had a beneficial effect on reducing the SARS-CoV-2 salivary viral load 7 days after the initial intake in adults with high ($4.12 \log_{10}$ copies/mL) or very high ($5.16 \log_{10}$ copies/mL) salivary viral loads at baseline. CDCM accelerated the reduction in viral load and maintain this difference compared with placebo. The results of our trial support a reduction in the transmission of SARS-CoV-2 salivary viral load in the general population. Specific indications for the use of this mouthwash could be at the onset of clinical symptoms in adult outpatients and in patients diagnosed as SARS-CoV-2 positive. CDCM, in conjunction with social distancing, hand hygiene, masks, and the screening of nonill individuals in the general population, may provide an additional barrier measure to reduce the risk of contamination by lowering the droplet viral load emitted by adults with COVID-19.

Declarations

Authors' contributions

FC, DB, and CD proposed the original study idea. FC was the coordinator officer of this trial. FC, PT, CD and DB designed the trial and study protocol. MV, PT, MR, and FC contributed to the data interpretation and PT, FC, MV and HP verified the data. EGD, AE, MEL, and GI were responsible for the site work including the recruitment, follow up, and data collection. HP monitored the trial. MBD and MV were responsible to the laboratory analysis. PT, and MR did the main analysis. FC, DB wrote the first draft the manuscript and CD, MBD, MR, MV and PT contributed to the revision of the manuscript. All authors reviewed and accepted the paper before submission.

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Conflict of interest

DB reports non-financial support and other from Curaden AG Switzerland, outside the submitted work. All other authors declare no competing interests.

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Research In Context

Evidence before this study

SARS-CoV-2 may be transmitted via saliva directly or indirectly, even in patients who do not cough or have other respiratory symptoms. Considering mouthwashes as agents that can reduce the viral load

saliva of SARS-CoV-2 in the fight against the COVID-19 pandemic is an extremely attractive concept. At this time, we identified no published clinical trials of the effect of anti-SARS-CoV-2 mouthwashes to control saliva viral load in patients with COVID-19.

Added value of this study

Our study is the first randomized, double-blind, placebo controlled clinical trial assessing the effect of a commercially available mouthwash with β -cyclodextrin and citrox on the SARS-CoV-2 salivary viral load in adults with asymptomatic to mild COVID-19. In the intention-to-treat population, the primary endpoint of time to clinical improvement was significantly different between groups, active mouthwash was more effective than placebo 4 hours after the first intake. Over the course of one day, the first mouthwash rinse significantly reduced the viral load, and the second dose maintained this low value, compared to placebo. At the secondary endpoint 7 days after the first intake, there was still a greater decrease in salivary viral load over time for patients for patients who took active mouthwash. In individuals with a high initial viral SARS-CoV-2 load, active mouthwash reduced significantly the salivary viral load more quickly than placebo.

Implications of all the available evidence

The robustness of the results of our trial supports, as an additional barrier measure, a significant relevance of using mouthwash with β -cyclodextrin and citrox on day 1 to reduce the dissemination of SARS-CoV-2 in the general population. Over 7 days, the use of this mouthwash showed a benefit of viral load reduction for adults with the highest initial loads.

Tables

	CDCM (N=76)	Placebo (N=78)	p-value ^a
Global			
Day 1 T1	N=76	N=78	
median [IQR]	4.14 [2.95 - 5.15]	4.04 [2.95 - 5.14]	
Day 1 T2	N=76	N=78	
median [IQR]	3.61 [1.6 - 4.37]	3.92 [1.92 - 4.94]	
median difference T1-T2 [IQR]	-0.53 [-1.47 - 0.00]	-0.07 [-1.00 - 0.39]	<0.001
p-value ^b	<0.001	0.121	
% decrease T1-T2 median [IQR]	-14.25% [-32.68% - 0.06%]	-7.31% [-22.37% - 12.63%]	
Day 1 T3	N=75	N=77	
median [IQR]	3.17 [0 - 4.31]	3.34 [0 - 4.91]	
median difference T1-T3 [IQR]	-0.46 [-1.81 - 0.01]	-0.35 [-1.23 - 0.27]	0.069
p-value ^b	<0.001	0.007	
% decrease T1-T3 median [IQR]	-15.96% [-40.3% - 3.25%]	-12.24% [-28.82% - 9.61%]	
Day 7	N=73	N=68	
median [IQR]	0 [0 - 2.65]	0 [0 - 3.05]	
median difference T1-day 7 [IQR]	-3.69 [-4.24 - -1.85]	-3.26 [-4.49 - -2.12]	0.417
p-value ^b	<0.001	<0.001	
% decrease T1-day 7 median [IQR]	-100% [-100% - -70.64%]	-100% [-100% - -72.41%]	
mean difference	-0.18 [90%CI -0.41 - 0.05]		
MLM p-value ^c	0.098		

Data are expressed in log₁₀ copies per milliliter of saliva or in % for the % of variation. ^aMann-Whitney test of the differences with a unilateral alternative hypothesis (H1 CDCM < Placebo). ^bWilcoxon rank signed test of the differences. ^cMixed linear model of the concentrations over time with a unilateral alternative hypothesis (H1 CDCM < Placebo). CDCM: β-cyclodextrin-citrox mouthwash

Table 1: Salivary SARS-CoV-2 load on the first day at 9 a.m., 1 p.m. and 6 p.m. for the CDCM and placebo groups

	CDCM	Placebo	p-value ^a
>Q1			
Day 1 T1	N=56	N=55	
median [IQR]	4.68 [4.09 - 5.74]	4.59 [3.85 - 5.67]	
Day 1 T2	N=56	N=55	
median [IQR]	4.10 [3.28 - 4.59]	4.27 [3.45 - 5.13]	
median difference T1-T2 [IQR]	-0.75 [-1.72 - -0.13]	-0.35 [-1.18 - 0.34]	0.025
p-value ^b	<0.001	0.01	
% decrease T1-T2 median [IQR]	-15.09% [-32.03% - -2.46%]	-9.28% [-23.25% - 8.37%]	
Day 1 T3	N=56	N=55	
median [IQR]	3.65 [2.71 - 4.64]	4.20 [2.98 - 5.07]	
median difference T1-T3 [IQR]	-0.97 [-1.94 - -0.23]	-0.74 [-1.51 - 0.20]	0.053
p-value ^b	<0.001	<0.001	
% decrease T1-T3 median [IQR]	-19.15% [-40.44% - -4.08%]	-14.47% [-29.49% - 4.39%]	
Day 7	N=53	N=47	
median [IQR]	0.00 [0.00 - 1.33]	0.00 [0.00 - 1.79]	
median difference T1-day 7 [IQR]	-4.02 [-4.69 - -3.48]	-3.81 [-4.82 - -1.37]	0.321
p-value ^b	<0.001	<0.001	
% decrease T1-day 7 median [IQR]	-100% [-100% - -71.13%]	-100% [-100% - -68.55%]	
mean difference	-0.21 [90%CI -0.43 - 0.02]		
MLM p-value ^c	0.074		
>Q2			
Day 1 T1	N=45	N=40	
median [IQR]	4.95 [4.31 - 5.80]	5.11 [4.55 - 5.97]	
Day 1 T2	N=45	N=40	
median [IQR]	4.15 [3.57 - 4.60]	4.88 [3.83 - 5.52]	
median difference T1-T2 [IQR]	-0.76 [-1.55 - -0.14]	-0.64 [-1.24 - 0.27]	0.122
p-value ^b	<0.001	0.005	
% decrease T1-T2 median [IQR]	-14.07% [-30.72% - -3.25%]	-13.18% [-23.74% - 4.7%]	
Day 1 T3	N=45	N=40	
median [IQR]	4.19 [3.13 - 4.83]	4.35 [3.69 - 5.66]	
median difference T1-T3 [IQR]	-0.81 [-1.81 - -0.21]	-0.49 [-1.47 - 0.18]	0.121
p-value ^b	<0.001	0.002	
% decrease T1-T3 median [IQR]	-16.02% [-35.85% - -3.52%]	-12.34% [-27.76% - 3.11%]	
Day 7	N=42	N=35	
median [IQR]	0.00 [0.00 - 1.32]	0.00 [0.00 - 1.19]	
median difference T1-day 7 [IQR]	-4.14 [-5.05 - -3.84]	-4.47 [-5.17 - -3.49]	0.489
p-value ^b	<0.001	<0.001	
% decrease T1-day 7 median [IQR]	-100% [-100% - -71.44%]	-100% [-100% - -63.42%]	
mean difference	-0.28 [90%CI -0.52 - -0.04]		
MLM p-value ^c	0.03		
>Q3			
Day 1 T1	N=22	N=22	
median [IQR]	5.81 [5.51 - 6.07]	5.87 [5.52 - 6.78]	
Day 1 T2	N=22	N=22	
median [IQR]	4.36 [3.80 - 5.28]	5.16 [4.70 - 6.06]	
median difference T1-T2 [IQR]	-1.37 [-1.90 - -0.72]	-0.81 [-1.83 - 0.05]	0.091
p-value ^b	<0.001	0.017	
% decrease T1-T2 median [IQR]	-23.55% [-33.2% - -11.47%]	-13.28% [-24.23% - 1.05%]	
Day 1 T3	N=22	N=22	
median [IQR]	4.50 [3.16 - 5.43]	4.94 [3.96 - 5.92]	
median difference T1-T3 [IQR]	-1.39 [-2.62 - -0.28]	-1.08 [-1.79 - -0.22]	0.132
p-value ^b	<0.001	0.002	
% decrease T1-T3 median [IQR]	-24.14% [-41.05% - -4.49%]	-17.17% [-29.26% - -3.54%]	
Day 7	N=22	N=22	
median [IQR]	0.00 [0.00 - 2.09]	1.78 [0.00 - 2.55]	

median difference T1-day 7 [IQR]	-5.14 [-5.75 - -3.84]	-4.92 [-5.72 - -3.25]	0.327
p-value ^b	<0.001	<0.001	
% decrease T1-day 7 median [IQR]	-100% [-100% - -62.26%]	-73.97% [-100% - -55.68%]	
mean difference		-0.44 [-0.80 - 0.07]	
MLM p-value ^c		0.029	

Data are expressed in log₁₀ copies per milliliter of saliva or in % for the % of variation. ^aMann-Whitney test of the differences with a unilateral alternative hypothesis (H1 CDCM < Placebo). ^bWilcoxon rank signed test of the differences. ^cMixed linear model of the concentrations over time with a unilateral alternative hypothesis (H1 CDCM < Placebo). CDCM: β-cyclodextrin-citrox mouthwash

Table 2: Salivary SARS-CoV-2 load on the first day at 9 a.m., 1 p.m. and 6 p.m. for the CDCM and placebo groups

Figures

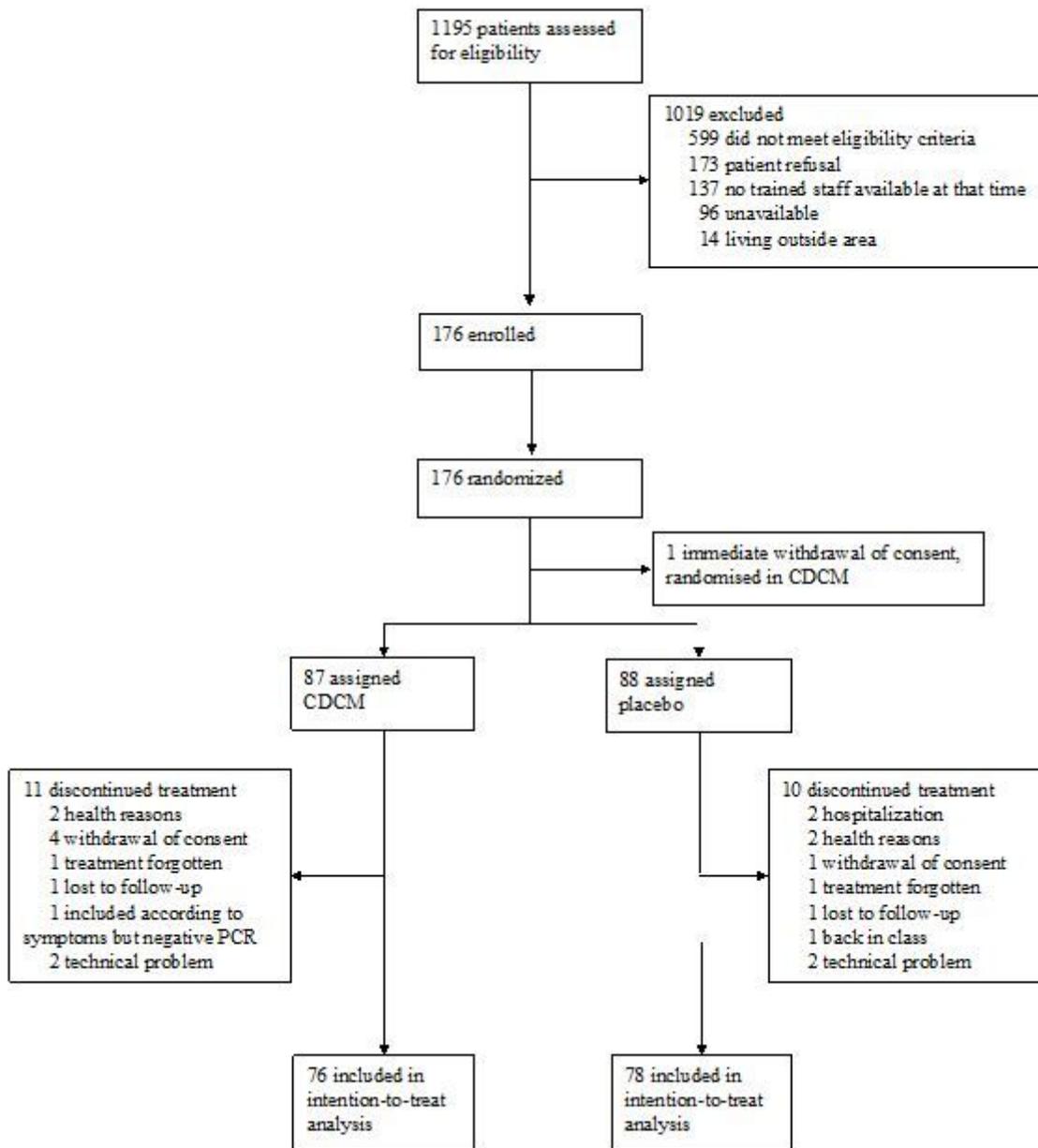


Figure 1

Trial profile

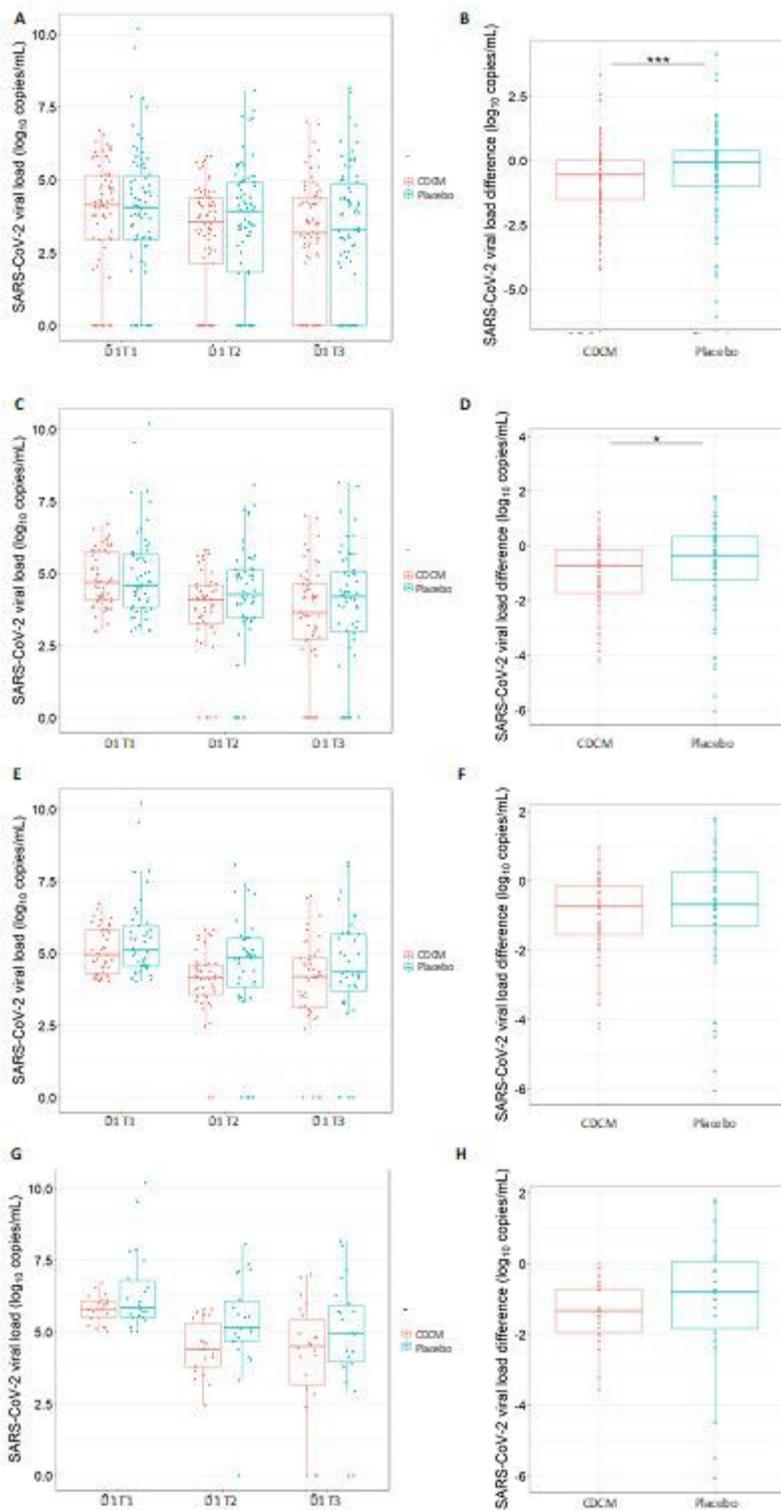


Figure 2

: Evolution of SARS-CoV-2 salivary load within the mouthwash cohorts at day 1. (A) Evolution for all patients. (B) SARS-CoV-2 viral load difference between T1 and T2 for all patients. (C) Evolution for patients with a SARS-CoV-2 viral load greater than 2.95 log₁₀ copies/mL at day 1 T1. (D) SARS-CoV-2 viral load difference between T1 and T2 for patients with a SARS-CoV-2 viral load greater than 2.95 log₁₀

copies/mL at day 1 T1. (E) Evolution for patients with a SARS-CoV-2 viral load greater than 4.12 log₁₀ copies/mL at day 1 T1. (F) SARS-CoV-2 viral load difference between T1 and T2 for patients with a SARS-CoV-2 viral load greater than 4.12 log₁₀ copies/mL at day 1 T1. (G) Evolution for patients with a SARS-CoV-2 viral load greater than 5.16 log₁₀ copies/mL at day 1 T1. (H) SARS-CoV-2 viral load difference between T1 and T2 for patients with a SARS-CoV-2 viral load greater than 5.16 log₁₀ copies/mL at day 1 T1.

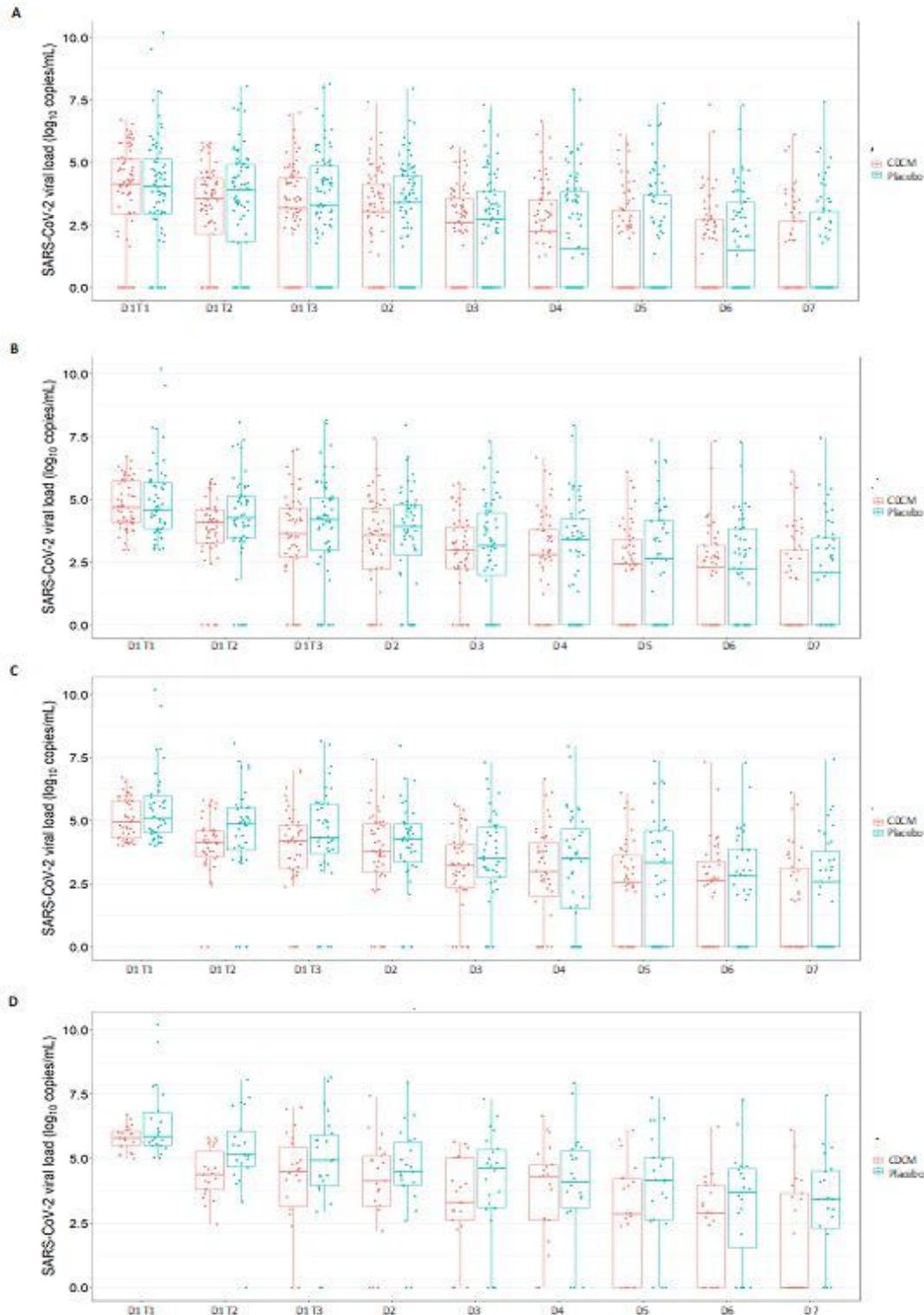


Figure 3

Evolution of SARS-CoV-2 salivary load within the mouthwash cohorts from day 1 to day 7. (A) Evolution for all patients. (B) Evolution for patients with a SARS-CoV-2 viral load greater than 2·95 log₁₀ copies/mL at day 1 T1. (C) Evolution for patients with a SARS-CoV-2 viral load greater than 4·12 log₁₀ copies/mL at day 1 T1. (D) Evolution for patients with a SARS-CoV-2 viral load greater than 5·16 log₁₀ copies/mL at day 1 T1.

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