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Reply to Montclair, NJ

August 25, 2020

Via Overnight Courier

United States Securities & Exchange Commission
Office of the Secretary
100 F Street NE
Washington DC 20549

Mailstop 1090
Attn: Secr of the Commission, Vanessa A. Countryman

Re: Lord Global Corporation (LRDG) (CIK No. 0001569568)
Release No. 89627 / August 20, 2020 – File No. 500-1

Hon. Madam Secretary:

This office is counsel for Lord Global Corporation (OTC: LRDG) (the "Company" or "Lord Global"). Enclosed herewith, please find the following documents related to the Commission's Release No. 89627 dated August 20, 2020 and related Order of Suspension of Trading:

- Sworn Petition to Terminate Suspension, with Exhibits A and B
- A copy of the Secretary's Order for reference

Kindly confirm receipt and please advise as to the next steps in the review procedure. Thank you.

Respectfully submitted,

Lawrence R. Lonergan, Esq.

c: Lord Global Corporation

BEFORE THE UNITED STATES
SECURITIES AND EXCHANGE COMMISSION
WASHINGTON, D.C.

LORD GLOBAL CORPORATION,

Petitioner,

vs.

UNITED STATES SECURITIES AND EXCHANGE COMMISSION

Respondent.

SWORN PETITION OF LORD GLOBAL CORPORATION

UNDER RULE 550 OF THE SECURITIES AND EXCHANGE COMMISSSION RULES OF PRACTICE

Securities and Exchange Act of 1934,
Release No. 34-89627 / August 20, 2020

File No. 500-1

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INTRODUCTION

Petitioner Lord Global Corporation ("LRDG" or the "Company") hereby files this timely[1] petition, duly sworn to by the Company's Chief Executive Officer, Mr. Joseph Frontiere[2] (this "Verified Petition" or "Petition") with the United States Securities and Exchange Commission (the "Commission") pursuant to Rule 550 of the Rules of Practice of the Commission (the "Commission Rules"). Petitioner is requesting that the Commission (1) rescind and void its August 20, 2020 order (the "Order") that suspended trading in LRDG's stock for ten days (the "Suspension") and, to the extent necessary, (2) not require the process outlined in Rule 15c2-11 under the Exchange Act ("Rule l5c2-11 "), codified at 17 C.F.R., Section 240.15c2-l l, be followed to recommence trading in LRDG stock. Because LRDG has been adversely affected by the Suspension, the Petitioner is entitled to petition for the above relief and, as more fully explained below, seek to show that such relief is warranted because the Suspension was not necessary in the public interest or for the protection of investors.

A. Background

We respectfully submit this Petition pursuant to 17 CFR 202.5(c) and §12(k)(1)(A) of the Exchange Act, 15 U.S.C. 78l(k)(1)(A), which allows interested parties to show that such suspension is not necessary in the public interest or for the protection of investors. As set forth herein, we believe that the suspension of trading should not continue and, in fact, that in the interest of the investing public that the trading suspension be immediately lifted. We offer the following facts and reasons supporting Petitioner's position.

We understand that the usual purpose of a suspension is to alert the investing public that there is insufficient public information about the issuer upon which an informed investment

[1] The Commission issued the Order suspending trading, effective at 9:30a.m. on August 21, 2020 and, as a result, this petition is timely filed.

[2] The facts and statements contained in this Petition have been sworn to by Mr. Frontiere, CEO of the Petitioner.

judgment can be made or that the market for the securities may be reacting to manipulative forces or deceptive practices. Consequently, the primary issues normally to be considered by the Commission in determining whether or not a 10-day suspension should be instituted are whether or not there is sufficient public information upon which to base an informed investment decision or whether the market for the security appears to reflect manipulative or deceptive activities.

As detailed in this Petition, the Company has issued several press releases designed to provide explicit information regarding CoviGuard™, as follows:

(a) commencing with its release on June 2, 2020, the Company announced the signing of a binding letter of intent between the Company's subsidiary, 27Health Inc. and Coviguard, Inc., a privately owned entity ("Coviguard") for the marketing rights to CoviGuard™;

(b) on June 5, 2020, the Company issued a press release reporting its CEO's "letter to shareholders" and the Company's "Significant expectations for the CoviGuard™ line of products" and reporting its intention of focusing its business efforts on the development of its 27Health Inc. subsidiary. 27Health Inc. is dedicated to financing and marketing innovative, healthcare related products that are and will benefit from the permanent changes caused by the COVID-19 pandemic;

(c) on June 16, 2020, LRDG issued a press release announcing the execution of a Definitive Joint Venture Investment and Distribution Agreement with Coviguard pursuant to which 27 Health, the Company's subsidiary, was granted an exclusive license to distribute CoviGuard™ by Coviguard, as reported in the Company's Form 8-K filed with the Commission on June 19, 2020;

(d) on July 21, 2020, the Company issued a release quoting an article published in the July 18, 2020 issue of Knowridge Science Report, an independent online magazine that reports on matters related to science, medicine and technology, among other technical issues, which article cited a recent review study at Cardiff University and elsewhere reporting that researchers found

that certain mouthwashes used in the dental community may help destroy the lipid envelope of Coronaviruses; and

(e) on August 17, 2020, the Company issued a press release announcing that it had received an order for one million units of the 4oz spray from Global Sanitizers Technologies Inc., further stating that the Company's Oral Sanitizer product is designed to be the first product on the market to significantly reduce the viral and bacterial loads in the oral and mucosa membrane (mouth and throat). Reference is made to the Company's Form 8-K filed with the Commission on August 18, 2020 and the revised press release attached as Exhibit 99.1 to the subject Form 8-K, in which it clarified that the estimate of having "products in the marketplace within 60 days" by stating that it "expects to be able to deliver in or before the end of 2020."

As discussed more fully under Statement of Facts below, the Company's disclosures made in these press releases were based on: (i) scientific case studies and peer-reviewed research that has been published by unrelated third-party professionals; and (ii) the best business judgment of the Company's management supported by representations made to the Company by Coviguard. Claims relating to the Company's belief in the efficacy of CoviGuard™ in preventing the contraction and spread of contagion has been documented and is based upon the active ingredients in CoviGuard™'s formula, as discussed below. There was no manipulation of facts or deception by the Company in any of the press releases nor was there any intention on the part of the Company to financially benefit from the releases and filing with the Commission.

We would also like to address the Commission's concerns about the formation and development of the Company's business, which underwent a change in control transaction earlier this year, at which time a new board of directors and new officers were appointed, as reported in its Forms 8-K filed with the Commission under the Exchange Act. In connection with the change in control and the change in management supported by the infusion of new equity and debt

financing, LRDG has changed its business focus to that of its subsidiary, 27Health Inc., concentrating on products that can potentially address the COVID-19 pandemic by investing in the manufacture, testing and marketing of unique products that could help the public in the battle against the coronavirus. Of course, there can be no claim that CoviGuard™ will cure COVID-19, but the CoviGuard™ product line has the potential to mitigate the spread of infection from its source, the human mouth.

B. The Commission Suspended Trading in LRDG.

Effective at 9:30 a.m. on August 21, 2020, the Commission suspended trading in the securities of LRDG because of questions concerning the accuracy and adequacy of information publicly disseminated about LRDG between June 2 to August 18, 2020, ostensibly due to informational statements disseminated by LRDG.

In a public release dated June 2, 2020, the Company disclosed its binding letter of intent between its subsidiary, 27Health Inc. and Coviguard Inc., a privately owned company ("Coviguard"), to market the CoviGuard™ mouth wash and oral sanitizer spray, a patent pending product line that uses FDA approved ingredients. All of the ingredients that comprise the CoviGuard™ proprietary formula are FDA approved (for human uses other than treating COVID-19), and are primarily used in dental offices or are sold as over-the-counter products. In fact, the Company specifically stated its intent to market CoviGuard™ as a prewash to the dental community. In the Company's June 2, 2020 release, it further stated that: "Studies with the proprietary ingredients have shown a unique ability to dramatically reduce viral loads in the oral cavity and help prevent transmission" and further disclosed the Company's belief that "it will be among the first commercial products to be used by dentists as a 'pre procedural rinse' for patients as well as individuals to protect themselves and others from virus transmission as well as bacterial infections. Covi-Guard™ also contains immune supporting ingredients. There have been several

papers showing that the Covi-Guard™ combination of ingredients have been able to reduce the viral load of the Corona family of viruses including COVID-19 and other viruses" (Exhibit A). In support of the June 2, 2020 release, the Company is aware of an ongoing study in France that has been underway since April 2020 (Exhibit B), which study is examining a formula very similar to that contained in CoviGuard™, which formula purports to be able to reduce the viral load in the mucosal membrane (in the mouth and throat) using cyclodextrin and bioflavinoid (citrox). These are the active ingredients that are virucidal in nature and are the reason why CoviGuard™ could be a very successful product. The French study is seeking to show why that this combination, which contain many of the same active ingredients contained in CoviGuard™, eliminates the viral load in the oral cavity.

In the June 5, 2020 letter to shareholders and contemporaneous press release, the Company announced that studies of the individual components of this [Covi-Guard™] product line has been proven to nearly eliminate the CO-SAR2 (COVID-19) viral load in the mouth. We believe that the market size for this product line could well be in excess of hundreds of millions of dollars. We have begun discussions with several different marketing channels for this product line and have received enthusiastic response. It is possible that this product line could represent sales in excess of $15 million in the first 12 months of launch. We have identified our outsource production supplier and feel that we can have products in the marketplace within 60 days."

The Company acknowledges that notwithstanding its belief that it could "have products in the marketplace within 60 days," in fact this belief reported in the June 5, 2020 letter to shareholders was not achieved. As a result, on August 18, 2020, the Company filed a Form 8-K announcing a release that was attached as Exhibit 99.1 thereto, correcting the Company's estimate that it will be able to deliver the products pursuant to the Global Sanitizers' purchase order for 1 million units of CoviGuard™, representing a $5 million order, before the end of the year.

The Company has endeavored in issuing each of its press releases to report that the information released is based upon the Company's best business judgment and that its best estimates about such facts as potential market size, estimated dates for delivery, and other material information are, in fact, based upon the Company's reasonable beliefs. In addition, and in support of this Petition, the Company has relied upon published scientific studies including ongoing studied being conducted by reputable third parties unrelated to the Company.

As indicated in several of the Company's press releases, the Company is also actively testing CoviGuard™ and negotiating with unrelated third parties for support in bringing CoviGuard™ to the market. The Company has financed and will continue to finance Coviguard pursuant to the Definitive Joint Venture Investment and Distribution Agreement (first reported in the Company's release dated June 16, 2020) for the express purpose of facilitating Coviguard's testing and production of sample process and, in connection therewith, the Company has engaged reputable third-party laboratories to design testing protocols and procedures. The CoviGuard™ product has been formulated pursuant to these ongoing protocols and procedures and, following its past and correct practices, will publish the results of ongoing tests once available to the Company.

ARGUMENT

A. The Commission's *Ex Parte* Actions are Not Supported by Law

The Commission issued its Order on an *ex parte* basis without providing LRDG any notice of the action or opportunity to be heard prior to the Suspension; the Commission did not obtain this extraordinary relief from a neutral judicial officer. As such, the Commission did not comply with "the root requirement" of the due process clause to give notice before acting. Cleveland Bd. of Educ. v. Loudermill, 470 U.S. 532, 542 (1985).

With respect to actions taken by administrative agencies like the Commission, federal courts have held that the demands of due process may not require a hearing at the initial stage, or at any particular point in the proceeding, so long as a hearing is held before the final order becomes effective. Opp Cotton Mills v. Administrator, 312 U.S. 126, 152, 153 (1941). For OTC companies, however, a trading suspension is effectively a final order (and the likely demise of the company). Not only is there no further action that the Commission needs to take but also the consequences of the onerous 211 process that the SEC requires have lasting effects for OTC companies.

To the extent that the Commission may rely on a case that held that plaintiffs due process rights were not denied by a prompt post-deprivation review of the trading suspension, Xumanii Int'l Holdings Corp. v. SEC, that case does not control here. 670 Fed. Appx. 508 (9th Cir. Oct. 19, 2016). First, Xumanii, as an unpublished case, not considered precedent. Ninth Circuit Rule 36-3. Second, Xumanii does not establish whether or not the Court considered the onerous burden of the Rule 15c2-11 process that the SEC requires, delaying the re-trading of the stock for two to six months, in its decision that Xumanii's due process rights were adequately protected. See 670 Fed. Appx. at 509. Third, that decision is so bare as to be unusable.

Furthermore, the application of this practice is patently unfair to OTC issuers. A trading suspension for an OTC company equates to an unconstitutional de-listing. It evidently has also become the SEC policy now, as well, to support FINRA against OTC companies. This joint policy of FINRA and the SEC has developed slowly over many years and now imposes an automatic re-filing of a 211 by a market-maker for any OTC company which has any type of trading suspension, whether temporary or permanent. Here, the Commission did not hold a hearing before implementing a "temporary" suspension which, in fact, amounted to a de facto de-listing of LRDG' s stock off of the OTC exchange.

Based upon the steadfast application of FINRA and SEC "policy," the process of temporary suspension is never good for an OTC stock. When the Constitution requires a hearing,

it requires a fair one, held before a tribunal that meets currently prevailing standards of impartiality. Wong Yang Sung v. McGrath, 339 U.S. 33, 50 (1950). A party in LRDG's position must be given an opportunity not only to present evidence, but also to know the claims of the opposing party and to meet them. Those who are brought into contest with the government in a quasi-judicial proceeding aimed at control of their activities are entitled to be fairly advised of what the government proposes and to be heard upon the proposal before the final order is issued. Margan v. United States, 304 U.S. 1, 18-19 (1938).

B. The Commission's Actions Violate the APA

The process here, once the ten-day suspension expires, continues on without disclosure or resolution for LRDG or its shareholders. SEC has an informal rule, which violates the Administrative Procedures Act because this rule was not passed pursuant to the APA that requires the filing of new 15c2-11 filing pursuant to 17 C.F.R Section 240. l 5c2-11 without that regulation actually requiring it after a suspension of an OTC stock. Stewart v. Smith, 673 F. 2d 485, 498 (1982) ("a rule may not be characterized as one of 'management' or 'personnel' if it has a substantial effect on persons outside the agency.").

This illegal process will harm LRDG's shareholders in three ways. (1) LRDG's shareholders now cannot trade for many months and their investment has become illiquid; (2) LRDG's shareholders concurrently will likely see substantial dilution from predatory lenders who may have added shares as the stock now remains dormant or "gray"; and (3) once trading re-opens (after a presumed minimum 6-month delay in 15c2-11 approval), the "old" stock price shall plummet. This whole process hurts investors, and it should not be imposed here.

This is not by law or regulation; rather, 17 CFR § 240. l 5c2-11 amounts to a mere policy requiring that broker-dealers file a "new 211" every time information about the issuer goes stale. See https://www.sec.gov/investor/alerts/tradingsuspensions.pdf. But a suspension does not mean that the information about the OTC filer is stale. SEC policy, especially here, should be waived.

C. The Commission Committed an Illegal Taking.

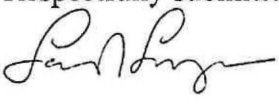
Without a hearing prior to the suspension, the suspension of an OTC stock amounts to an unlawful taking. Suspending trading essentially strips LRGD shareholder's stock of its value, as it makes it illiquid. Suspending trading affects a taking of LRGD shareholder's property by removing its value without compensation until a 211 is filed. See Knick v. Twp. Of Scott, 588 U.S._ slip op. at 8 (2019) ("a property owner has a Fifth Amendment entitlement to compensation as soon as the government takes his property without paying for it. ").

CONCLUSION

The Commission is targeting OTC companies unfairly while relying upon an illegal, informal rule that demands automatic re-filing of a 211 by a market-maker if the issuer's trading is suspended, even temporarily. This is not rooted in law or regulation. To remedy this constitutionally deficient process, LRDG urges the Commission to vacate and rescind the Suspension, provide LRDG with a legitimate, transparent constitutional process to be heard, and have LRDG's arguments considered by a neutral judicial officer. If needed, the Petitioners also request that the Commission permit expedited briefing (including a short reply) and an expedited hearing on this petition and as result of that briefing and hearing, the Commission rescind and void the Suspension and order that no one is required to follow the process outlined in 17 C.F.R Section 240. l 5c2-l l for the shares of LRDG to commence trading again.

Dated August 25, 2020

Respectfully submitted,



The Lonergan Law Firm LLC
Lawrence R. Lonergan, Esq.
Attorneys for Petitioner
Lord Global Corporation

CERTIFICATE OF COMPLIANCE

I certify that the attached PETITION uses a 12-point, Times New Roman font and contains 3181 words.

Dated August 25, 2020

Respectfully submitted,

The Lonergan Law Firm LLC
Lawrence R. Lonergan, Esq.
Attorneys for Petitioner

VERIFICATION

STATE OF New Jersey
SS:
COUNTY OF Monmouth

JOSEPH FRONTIERE, being duly sworn, deposes and says:

I am the president and chief executive officer of petitioner Lord Global Corporation and am personally familiar with the facts and circumstances of this matter. The statements contained in the foregoing Petition are true and accurate to the best of my knowledge and, as to statements made on information and belief, I believe such statements to be true.

Joseph Frontiere

Sworn to before me this 25th
Day of August, 2020

Notary Public

LORI CASTRO
Commission # 2379468
Notary Public, State of New Jersey
My Commission Expires
October 29, 2023

COVID-19: Nasal and Salivary Detection of the SARS-CoV-2 Virus After Antiviral Mouthrinses (BBCovid)

ClinicalTrials.gov Identifier: NCT04352959

Recruitment Status : Recruiting
First Posted : April 20, 2020
Last Update Posted : July 15, 2020

See **Contacts and Locations**

Sponsor:
Claude Bernard University

Collaborators:
University Hospital, Tours
University Hospital, Montpellier
Hospices Civils de Lyon

Information provided by (Responsible Party):
Carrouel Florence, Claude Bernard University

Study Details | **Tabular View** | **No Results Posted** | Disclaimer | How to Read a Study Record

Study Description

Go to

Brief Summary:

Given the current lack of effective COVID-19 treatment, it is necessary to explore alternative methods to contain the spread of the infection, focusing in particular on its mode of transmission. The modes of person-to-person transmission of SARS-CoV-2 are direct transmission, such as sneezing, coughing, transmission through inhalation of small droplets, and transmission through contact, such as contact with nasal, oral and eye mucous membranes. SARS-CoV-2 can also be transmitted directly or indirectly through saliva. The use of antiviral mouthrinses may be used as adjunctive therapy.

Condition or disease	Intervention/treatment	Phase
COVID19 Mouthwash Saliva	Device: mouthrinse with bêta-cyclodextrin and citrox Device: mouthrinse without bêta-cyclodextrin and citrox	Not Applicable

Study Design

Go to

Study Type :

Interventional (Clinical Trial)

Estimated Enrollment :

178 participants

Allocation:

Randomized

Intervention Model:

Parallel Assignment

Masking:

Triple (Participant, Care Provider, Investigator)

Primary Purpose:

Prevention

Official Title:

COVID-19: Nasal and Salivary Detection of the SARS-CoV-2 Virus After Antiviral Mouthrinses: Double-blind, Randomized, Placebo-controlled Clinical Study

Actual Study Start Date :

April 27, 2020

Estimated Primary Completion Date :

November 20, 2020

Estimated Study Completion Date :

December 20, 2020

Arms and Interventions

Go to

Arm	Intervention/treatment
Active Comparator: mouth rinse with antiviral	Device: mouthrinse with bêta-cyclodextrin and citrox 3 daily mouthrinses for 7 days
Placebo Comparator: mouth rinse without antiviral	Device: mouthrinse without bêta-cyclodextrin and citrox 3 daily mouthrinses for 7 days

Outcome Measures

Go to

Primary Outcome Measures :

1. Change from Baseline amount of SARS-CoV-2 in salivary samples at 7 days [Time Frame: 7 days]

   Quantitative PCR experiments will be performed and a quantitative analysis of the salivary samples will be made

Secondary Outcome Measures :

1. Change from Baseline amount of SARS-CoV-2 virus in nasal samples at 7 days [Time Frame: 7 days]

   Quantitative PCR experiments will be performed and a quantitative analysis of the nasal samples will be made

Eligibility Criteria

Go to

Information from the National Library of Medicine

NIH NLM

Choosing to participate in a study is an important personal decision. Talk with your doctor and family members or friends about deciding to join a study. To learn more about this study, you or your doctor may contact the study research staff using the contacts provided below. For general information, Learn About Clinical Studies.

Ages Eligible for Study:

18 Years to 70 Years (Adult, Older Adult)

Sexes Eligible for Study:

All

Accepts Healthy Volunteers:

No

Criteria

Inclusion Criteria:

- Clinical diagnosis of Covid-19 infection by the patient's general practitioner and hospital doctor
- Clinical signs started less than 48 hours ago.
- Virological confirmation: not necessary but possible.
- Understanding and acceptance of the trial.
- Written agreement to participate in the trial

Exclusion Criteria:

- Pregnancy
- Breastfeeding
- Inability to comply with protocol
- Lack of written agreement

Contacts and Locations

Go to

Information from the National Library of Medicine

To learn more about this study, you or your doctor may contact the study research staff using the contact information provided by the sponsor.

Please refer to this study by its ClinicalTrials.gov identifier (NCT number): ***NCT04352959***

Contacts

Contact: florence Carrouel, PhD 0478785745 florence.carrouel@univ-lyon1.fr

Locations

France

Chu Tours **Recruiting**

Chambray-lès-Tours, France, 37170

Contact: Frédérique Denis, PhD frederic.denis@chu-tours.fr

CH Emile Roux **Recruiting**

Le Puy en Velay, France, 43000

Contact: Sophie Lengagne 0471043704 sophlengagne@gmail.com

Sponsors and Collaborators

Claude Bernard University

University Hospital, Tours

University Hospital, Montpellier

Hospices Civils de Lyon

More Information

Go to

Publications:

Carrouel F, Conte MP, Fisher J, Gonçalves LS, Dussart C, Llodra JC, Bourgeois D. COVID-19: A Recommendation to Examine the Effect of Mouthrinses with β-Cyclodextrin Combined with Citrox in Preventing Infection and Progression. J Clin Med. 2020 Apr 15;9(4). pii: E1126. doi: 10.3390/jcm9041126.

Responsible Party:

Carrouel Florence, Associate professor, Claude Bernard University

ClinicalTrials.gov Identifier:

NCT04352959 History of ChangesHistory of Changes

Other Study ID Numbers:

BBCovid

First Posted:

April 20, 2020 Key Record Dates

Last Update Posted:

July 15, 2020

Last Verified:

July 2020

Studies a U.S. FDA-regulated Drug Product:

No

Studies a U.S. FDA-regulated Device Product:

No

Keywords provided by Carrouel Florence, Claude Bernard University:

covid19
SRAS-CoV-2
mouthrinses
antiviral
bêta-cyclodextrin
citrox

Additional relevant MeSH terms:

Betadex
Sequestering Agents
Molecular Mechanisms of Pharmacological Action

Antimicrobial activity of Citrox® bioflavonoid preparations against oral microorganisms

S. J. Hooper,[1] M. A. O. Lewis,[2] M. J. Wilson[3] and D. W. Williams[4]

RESEARCH

IN BRIEF

- Citrox® is a bioflavonoid-containing product derived from citrus fruit, available in two formulations that have been used in a range of cleansers and disinfectants.
- Citrox® showed substantial antimicrobial activity against a range of oral bacteria and *Candida* species.
- Citrox® bioflavonoid preparations may be useful antimicrobial agents in future mouthwash and oral care products.

Background Citrox® is a formulation of soluble bioflavonoids obtained from citrus fruits. The non-toxic and antimicrobial properties of natural bioflavonoids are well documented, and consequently there has been interest in the therapeutic application of these substances. **Objective** To determine the antimicrobial activity of two Citrox® formulations (BC30 and MDC30) with different bioflavonoid combinations against a range of oral microorganisms. **Methods** The antimicrobial activity of both formulations was tested against 14 bacterial species and six *Candida* species. The two Citrox® formulations (dilution range 0.007–8% v/v) were firstly evaluated by determining the *in vitro* Minimal Inhibitory Concentration (MIC) against planktonic microorganisms in a broth microdilution assay. Secondly, the ability of the same serial dilutions to inhibit microbial growth was assessed in a modified microtitre biofilm assay. **Results** Both Citrox® formulations exhibited antimicrobial activity. The BC30 formulation demonstrated greater activity than MDC30 and significantly inhibited growth of all bacterial species and most candidal species tested at a concentration of 1% (v/v) in both the broth and the biofilm assay. **Conclusion** Bioflavonoid preparations of Citrox® have a broad-spectrum of antimicrobial activity against oral microorganisms, and as such have the potential to be used within therapeutic preparations for the control of the oral microflora.

INTRODUCTION

A variety of antimicrobial mouthwashes are available commercially and these have been shown to be clinically beneficial in the management of oral disease.[1–4] However, for a variety of reasons the search for novel and more effective antimicrobial agents continues. In addition, adverse effects associated with some available preparations, in particular unpleasant taste and staining of the teeth, have reduced patient acceptability and compliance.[5] Furthermore, the presence of alcohol in some mouthwashes has been shown to cause mucosal irritation in certain patients, particularly those with mucositis, and is felt to be inappropriate by some because of the association of denatured alcohol with the development of oral cancer.[4,6–8] Finally, the long term use of antimicrobial agents has raised concern regarding the potential for an undesirable shift in the composition, site colonisation and emergence of resistance within the complex oral microflora.[9,10]

In recent years an association between members of the oral microflora and the development of some forms of systemic diseases has been reported. There is increasing evidence that poor control of the oral flora and severe periodontal disease may be important factors in the onset and progress of coronary heart disease and diabetes.[11] In addition, it is well documented that the composition of the oral flora in hospitalised and debilitated patients undergoes an early microbial shift to one predominated by Gram-negative bacteria. It is now recognised that plaque can therefore act as a reservoir for potential pathogens, including highly resistant microorganisms, for infection at other body sites.[12–15] Specifically, it has been demonstrated that the oropharyngeal microflora has a role in ventilator-associated pneumonia, and the use of therapeutic preparations containing either chlorhexidine or essential oils can reduce the incidence of this significant infection.[16] As the number of immunocompromised individuals in the population continues to increase, so too does the incidence of mucosal infections in the mouth, in particular oral candidosis. Given the concern over the emergence of resistance of yeasts to systemic antifungal agents, there is a clinical requirement for new and effective topical anticandidal strategies.[17] It is against this background that the need for alternative antimicrobial agents with improved antimicrobial profiles and fewer adverse effects becomes greater.

There has been considerable interest in the use of 'natural' antimicrobial agents. Natural antimicrobial agents can be defined as bioactive compounds derived from biological sources. Although traditional antibiotics strictly fall into this definition, there is concern regarding the prophylactic use of antibiotics due to the high potential for promotion of microbial resistance.[18] However, there is a range of alternative non-microbial 'natural agents' that have

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Online article number E22
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DOI: 10.1038/sj.bdj.2010.1224

antimicrobial activity and the effectiveness of plant extracts, such as bacteriocins, defence peptides and phenolics has been demonstrated.[19-21] Polyphenolic plant derivatives, which are part of a plant's natural defence mechanisms against viral and bacterial pathogens, have been the main focus of investigation.

In addition to greater antimicrobial activity and improved safety profiles, there is also a perception that natural agents may be more acceptable to patients. Although the scientific evidence is unclear, there is a view that natural agents may be less likely to promote the development of resistance.[18] Many of the 'natural' antimicrobials have the added advantage that they may be used in aqueous solution, removing the need for inclusion of alcohol in therapeutic preparation.

Citrox® is an antimicrobial whose components are based on soluble bioflavonoids derived from citrus fruits. Bioflavonoids are hydroylated phenolic structures synthesised by plants and have previously been shown to have activity against bacteria, fungi and viruses.[22-25] Citrox® BC and Citrox® MDC formulations both contain bioflavonoids with the former comprising of a blend of bioflavonoids with small amounts of malic and citric acids, designed to be primarily anti-bacterial. Citrox BC is present in OralClens® mouthrinse and toothpaste, while MDC is currently used in a range of sanitising products for surface disinfection. The aim of the present study was to determine the antimicrobial activity of these two Citrox® formulations against a variety of bacterial species encountered in the mouth, including those implicated in periodontal disease, and a range of candidal species. The formulations would be assessed against the test strains in planktonic state and within *in vitro*-generated biofilms.

METHODS

Preparation of microorganisms

A total of eight bacterial species and six fungal species (Table 1) were used to evaluate the Citrox® formulations. All bacteria were initially cultured on Fastidious Anaerobic Agar (FAA) supplemented with 5% defibrinated sheep blood (TCS Biosciences Ltd., Buckingham, UK) and then, before use in experiments, in Brain-Heart Infusion (BHI) broth. *Candida* species were cultured on Sabouraud's dextrose agar (SDA) and subsequently in liquid Sabouraud's medium. All media, unless otherwise stated, was obtained from Lab M™ (International Diagnostics Group plc, Bury, UK). *Candida* isolates and the two streptococcal species were maintained at 37°C under aerobic conditions, whereas the remaining six species of bacteria

Table 1 Identity of the 14 test strains used to assess the antimicrobial activity of two formulations of Citrox®

Microorganism	Strain designation
Bacteria	
Actinomyces odontolyticus	Clinical isolate N19-25
Actinomyces viscosus	Clinical isolate N19-21
Clostridium difficile	Clinical isolate R8651
Porphyromonas gingivalis	NCTC 11834^T
Prevotella buccae	NCTC 13063^T
Prevotella intermedia	Clinical isolate S13-12
Streptococcus gordonii	Clinical isolate T06-01
Streptococcus sanguinis	NCTC 7863^T
Yeasts	
Candida albicans	ATCC 90028
Candida dubliniensis	CD36^T
Candida glabrata	Clinical isolate 501/02
Candida krusei	Clinical isolate 141/03
Candida parapsilosis	ATCC 22019T
Candida tropicalis	Clinical isolate 243/01

^{T}indicates the type strain

Table 2 MIC of Citrox® BC30 and Citrox® MDC30 against 14 test strains in broth suspension

Microorganism	MIC (% Citrox®, v/v)	
	BC30	MDC30
Actinomyces odontolyticus	0.015625	>8*
Actinomyces viscosus	2	4
Clostridium difficile	2	>8*
Porphyromonas gingivalis	2	2
Prevotella buccae	2	>8*
Prevotella intermedia	2	>8*
Streptococcus gordonii	0.0625	2
Streptococcus sanguinis	0.0625	4
Candida albicans	0.125	>8*
Candida dubliniensis	0.125	>8*
Candida glabrata	0.125	>8*
Candida krusei	0.03125	4
Candida parapsilosis	0.5	>8*
Candida tropicalis	0.125	8

*No inhibition of growth at the highest concentration used

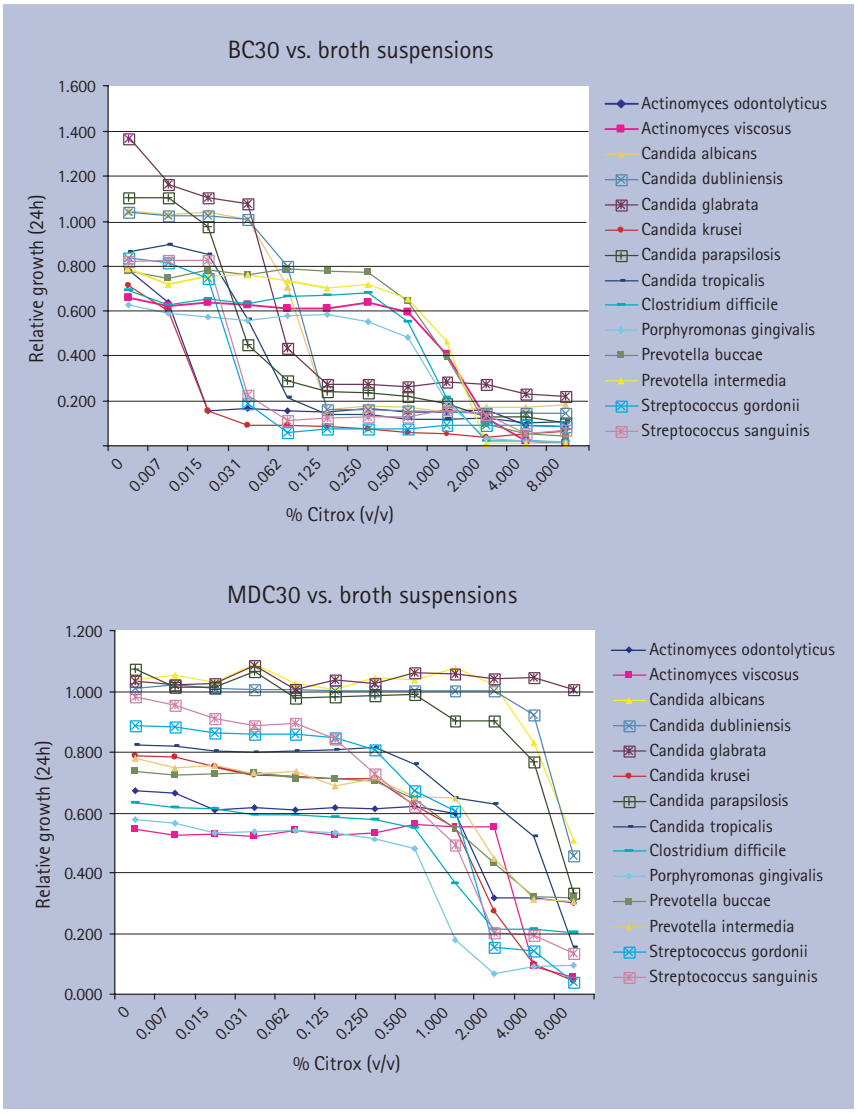

Fig. 1 Relative growth in broth of planktonic forms of the 14 test strains in the presence of varying concentrations of Citrox® BC30 and MDC30

were grown in an anaerobic environment (10% v/v CO_2, 20% v/v H_2, 70% v/v N_2) at 37°C.

Planktonic assay

The two formulations of Citrox® (BC30 and MDC30) were first assessed with regard to their antimicrobial activity against planktonic suspensions of the test species. In these experiments an overnight culture of each strain was prepared in the appropriate liquid medium to a turbidity level equal to a MacFarland standard 3.0. Serial dilutions were then made of the two Citrox® formulations using either BHI or liquid Sabouraud's medium as the diluent. A 100 µl volume of each Citrox® dilution was added to an equal volume of the microbial suspension, giving a range of Citrox® concentration between 0.007% and 8% (v/v). Controls included bacterial suspensions containing no Citrox® and uninoculated broth.

A 200 µl volume of the mixed preparations was incubated in 96-well microtitre plates for 24 hours at 37°C, under the appropriate atmospheric conditions. After incubation, the relative amount of each microbial species was estimated by measuring the turbidity of the well by spectrophotometric absorbance at 544 nm. Absorbance readings were standardised using 'microbial-free' Citrox® dilutions. As recommended by Espinel-Ingroff and Cantón,[26] the minimal inhibitory concentration (MIC) value was recorded as the lowest concentration of Citrox® that showed ≥80% reduction in absorbance compared to the control.

Biofilm assay

The concentrations of both Citrox® formulations required to inhibit the growth of microbial biofilms were determined. Suspensions of each organism (MacFarland standard 3.0) were incubated for 24 hours at 37°C in the appropriate broth and atmospheric conditions, without agitation so as to allow the formation of biofilms. The medium was then removed by gentle aspiration and the biofilm washed with 200 µL of phosphate buffered saline (PBS) to remove planktonic cells. Fresh medium containing Citrox® at concentrations ranging between 0.007–8% v/v was then added to the biofilm. Each antimicrobial concentration was prepared in triplicate and a control broth containing no Citrox® was also used. Biofilms were then incubated for a further 24 hours without movement under the same conditions as before. The medium was subsequently removed by gentle aspiration and the biofilm again washed with PBS. Fresh broth was added and the biofilms disrupted by pipetting and agitation. The turbidity of the resuspended biofilm was then observed by measuring the absorbance at a wavelength of 620 nm. A second absorbance reading (at 620 nm) was taken after a further incubation over 6 hours. The relative growth of the microorganisms was then determined by the change in absorbance over this 6 hours period. The mean value was calculated from triplicate results and the MIC recorded as the lowest concentration of Citrox® that demonstrated a ≥80% reduction in absorbance compared to the control.

RESULTS

Planktonic assay

The MICs of each of the two Citrox® formulations against the 14 microorganisms are shown in Table 2 and Figure 1. The planktonic growth of all of the microorganisms studied was inhibited by Citrox® BC30. The growth of most of the species did not appear to be significantly inhibited by Citrox® MDC30, even when it was present at the highest concentration 8% (v/v) used in this study. The MIC for each microorganism was lower with BC30 than MDC30, with the exception of *Porphyromonas gingivalis* for which the MIC of both formulations was the same at 2% (v/v). Overall, this suggested that BC30 was more effective than MDC30 at

inhibiting microbial growth. Furthermore, as can be seen in Figure 1, BC30 inhibited the growth of each microorganism at concentrations of between 1-2% (v/v).

Biofilm assay

The MIC values recorded in biofilm assay for both formulations are shown in Table 3 and Figure 2. In general the BC30 formulation was more effective at inhibiting the growth of microorganisms. One notable exception was that MDC30 appeared to be more effective against *C. albicans* and *C. dubliniensis* biofilms compared to BC30, for which there was no apparent growth inhibition even with the highest concentration of 8% (v/v).

DISCUSSION

There is a need for novel antimicrobial agents with improved activity and safety profiles. A range of substances extracted from plants have shown promise in this respect. Peel extracts from *Citrus sudachi* have been found to have antimicrobial activity against bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) and *Helicobacter pylori*.[23] Other bioactive plant extracts, containing flavonoids and phenols as major components, have proven effective against MRSA and a range of Gram-negative bacteria.[27,28] From the oral perspective, naringin, a flavonoid extract from grapefruit, has shown promise in the growth inhibition of *A. actinomycetemcomitans* and *P. gingivalis* in planktonic phase.[29] Polyphenols extracted from hop extracts and cranberry have shown activity against streptococcal species associated with dental caries[30-32] and a citrus extract combined with lemon juice and lemon grass has been used successfully to treat oral candidosis.[33]

The results of the present study show for the first time the impact of a citrus fruit bioflavonoid based product (Citrox®) on the growth of a range of oral microorganisms. Both formulations of Citrox® tested showed substantial antimicrobial activity, with BC30 exhibiting biofilm MIC values below 0.5% for all bacterial strains tested. Although the MIC values are not directly comparable, the antimicrobial activity and range compare favourably with those reported for chlorhexidine, an essential oil mouthwash and a herbal mouthwash containing grapefruit seed extract when tested against

Table 3 MIC of Citrox® BC30 and Citrox® MDC30 against 14 test strains in biofilm

Microorganism	MIC (% Citrox, v/v)	
	BC30	MDC30
Actinomyces odontolyticus	0.0625	>8*
Actinomyces viscosus	0.25	>8*
Clostridium difficile	0.0625	>8*
Porphyromonas gingivalis	0.5	>8*
Prevotella buccae	0.25	>8*
Prevotella intermedia	0.25	>8*
Streptococcus gordonii	0.125	>8*
Streptococcus sanguinis	0.125	>8*
Candida albicans	>8*	1
Candida dubliniensis	>8*	1
Candida glabrata	1	>8*
Candida krusei	1	>8*
Candida parapsilosis	0.25	>8*
Candida tropicalis	0.25	>8*

*No inhibition of growth at the highest concentration used

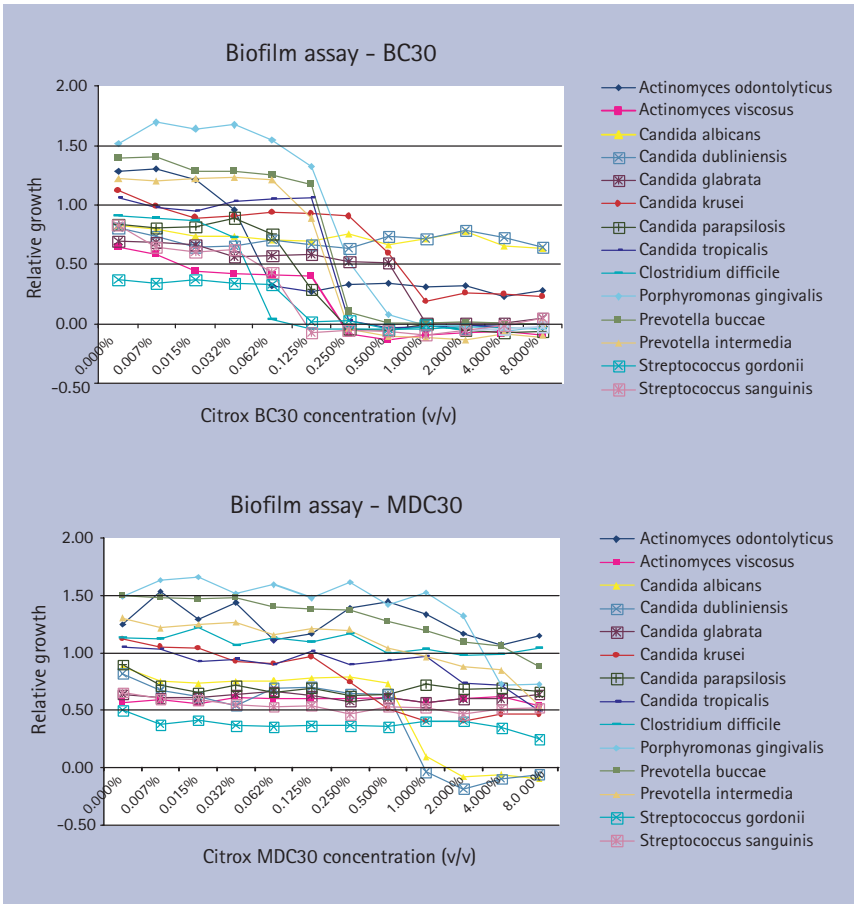

Fig. 2 Relative growth in biofilm of the 14 test strains in the presence of varying concentrations of Citrox® BC30 and MDC30

a similar range of oral bacteria.[34] In general the BC30 formulation demonstrated higher activity against both bacteria and yeasts. Interestingly, the two species of *Candida* demonstrating a high MIC with the BC30 preparation (*C. albicans* and *C. dubliniensis*) were significantly more susceptible to the MDC30 formulation. For these two species MDC30 demonstrated greater activity than that previously reported for chlorhexidine.[35] Citrox® MDC contains the same bioflavonoids as BC30 but, in addition, incorporates citric acid and choline ascorbate.

The antimicrobial activity of the bioflavonoid preparations when tested against bacterial and fungal biofilms is significant and of direct clinical relevance. Biofilms can be defined as microbial cells attached to a surface and organised into structured communities embedded within a matrix of extracellular material that has been produced by the biofilm cells.[36] Bacterial dental plaque is perhaps the most widely studied biofilm due to its association with caries and periodontal disease and there is increasing interest in the nature of biofilms formed by *Candida spp*.[37] It is widely recognised that both the biofilm structure and the phenotype of cells within a biofilm can afford protection against both host defence processes and administered antimicrobial agents.[36,38] Enhanced resistance of a biofilm to an antimicrobial has been related to failure of the agent to diffuse the biofilm, sequestering of the agent within the biofilm matrix, and the presence of persister cells within the biofilm that have a low activity status that promotes their survival in the presence of an antimicrobial. Studies have shown that antimicrobial activity is elevated up to 500-fold in biofilms when compared with equivalent planktonic studies.[39] Potentially useful antimicrobial agents must therefore demonstrate activity against bacteria in both planktonic and biofilm states.[35] In this context, it is of interest that chlorhexidine at its working concentration of 0.2% does not appear to effect a total kill in a biofilm and that the essential oil-containing preparations would appear to have greater activity against microorganisms in biofilm state than planktonic phase.[35,40]

Bacterial biofilm susceptibility to Citrox BC30 was exhibited at concentrations below 1%. However, elevated MICs were recorded against biofilms generated by the two closely related yeast species of *C. albicans* and *C. dubliniensis*. As in the planktonic assay, the MICs of the MDC30 preparations were correspondingly low for these species. Further investigation is required to explain the mechanisms behind this phenomenon.

In summary, the present study has demonstrated that working concentrations (1-2% v/v) of Citrox® BC30 are effective at inhibiting the growth of a range of bacteria and *Candida* when cultured in either broth suspension or as a biofilm. In addition, Citrox™ MDC demonstrated activity against microorganisms exhibiting high MICs with BC30. The findings support the further investigation of both formulations of Citrox® as potentially significant antimicrobial agents in future mouthwash preparations and other oral care products.

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Editorial

COVID-19: A Recommendation to Examine the Effect of Mouthrinses with β-Cyclodextrin Combined with Citrox in Preventing Infection and Progression

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Abstract: Considered to be a major portal of entry for infectious agents, the oral cavity is directly associated with the evolutionary process of SARS-CoV-2 in its inhalation of ambient particles in the air and in expectorations. Some new generations of mouth rinses currently on the market have ingredients that could contribute to lower the SARS-CoV-2 viral load, and thus facilitate the fight against oral transmission. If chlorhexidine, a usual component of mouth rinse, is not efficient to kill SARS-CoV-2, the use of a mouth rinses and/or with local nasal applications that contain β-cyclodextrins combined with flavonoids agents, such as Citrox, could provide valuable adjunctive treatment to reduce the viral load of saliva and nasopharyngeal microbiota, including potential SARS-CoV-2 carriage. We urge national agencies and authorities to start clinical trials to evaluate the preventive effects of βCD-Citrox therapeutic oral biofilm rinses in reducing the viral load of the infection and possibly disease progression.

Keywords: COVID-19; 2019-nCoV; SARS-CoV-2; oral cavity; mouthrinse; β-cyclodextrins; Citrox; viral load; microbiome

1. Introduction

The epidemic of infection COVID-19 (or 2019-CoV) by an emerging coronavirus SARS-CoV-2 in December 2019 has generated severe threats to international health security, global health, and the economy [1]. Given the current lack of effective treatment there is a need to explore alternative methods to contain the propagation of the infection, focusing in particular on its mode of transmission. The person-to-person modes of transmission of SARS-CoV-2 are direct transmissions, such as sneezing, coughing, transmission through inhalation of small droplets, and transmission by contact such as contact with nasal, oral, and ocular mucous membranes [2]. SARS-CoV-2 may also be transmitted directly or indirectly by the saliva, and the fetal–oral routes can be a possible route of person-to-person transmission as well [3,4]. Moreover, high viral loads have been found in the oropharynx of infected patients, as well as in the asymptomatic subjects [5]. This could suggest that the potential of SARS-CoV-2

transmission is wider than originally thought. The oral cavity is therefore directly associated with the evolutionary process of SARS-CoV-2 in its inhalation of ambient particles in the air and in expectorations.

Considered to be a major portal of entry for infectious agents [6], the oral cavity is colonized by a large number and variety of micro-organisms, including bacteria, fungi, and viruses termed microbiota [7]. These microbial communities and their inevitable multiple synergistic and antagonistic interactions are reflected in an individual's oral and general health. The oral cavity and nasopharyngeal regions can be considered as the anatomical transition between external and internal environments. The standard oral cavity temperature is on average 37 °C with no notable variations, which gives the microorganisms a secure environment to survive [8]. Saliva is also pH stable at 6.5–7, which is the favorable pH to oral microbiota (bacteria species and virus such as coronavirus) [9–11]. Variations in saliva composition are often associated with microbiota dysbiosis and oral diseases [12]. Moreover, salivary composition influences oropharyngeal colonization characteristics and bacterial profile [13]. Oral microbiota comprises of commensal bacterial populations that sustain mutual benefits with the host and keep potentially pathogenic bacteria in balance through a number of negative feedback mechanisms. In the oral biofilm, these bacteria combine to make a barrier which resists to antibiotics, disinfectants, mechanical removal, and other stresses [14]. In addition, bacteria within biofilms have 1000 times more resistance to antibacterial treatments compared to planktonic microorganisms [15]. In the absence of proper oral hygiene, the percentage of potentially health damaging bacteria in biofilm are seen to increase, contributing to the development of chronic infection [16]. Moreover, in biofilms, bacteria can escape the immune system producing so-called superantigens [17]. In addition to host–microbe interactions, the interfaces of periodontal pathogens with other non-host pathogens, such as herpesviruses like Epstein–Barr virus and cytomegalovirus, can contribute to the pathogenesis of the periodontal disease [18], or can affect the outcome of viral infection and dissemination [19]. These risks are often significantly underestimated. In the future, oral care measures that are effective in reducing infection should be given higher priority.

2. Mouth Rinses for Infection Containment

There is currently a large variety of over-the-counter mouth rinses available, which contain a wide range of active ingredients with for each having specific indication [20]. Cosmetic mouth rinses can temporary control halitosis and taste pleasant, but have no biological or chemical application that goes beyond their immediate benefit [21]. On the other hand, therapeutic mouth rinses include active ingredients that are designed to modify the oral microbiota for conditions such as gingivitis, dental caries, plaque, and bad breath. A new generation of therapeutic oral biofilm rinses developed to control virulent bacteria have added metals, metal oxides, and other nanoparticles, which appear to be promising alternatives due to their distinct physio–chemical properties [20].

2.1. Chlorhexidine

Effective ingredients that may be applied in therapeutic mouth rinses includes: chlorhexidine (CHX), fluoride, cetylpyridinium chloride, essential oils, and peroxide [22]. Conceived for short-term usage, CHX 2% is a cationic bisbiguanide widely used in general medical practice as a broad-spectrum antiseptic. CHX increases the permeability of the bacterial cell wall, resulting in bacterial lysis [23]. Its activity includes gram-positive and gram-negative bacteria (gram-positive bacteria being more susceptible), aerobes, facultative anaerobes, fungi, and selected viruses [24]. While chlorhexidine significantly reduces the risk of ventilator-associated pneumonia, no differences were found in terms of mortality, mechanical ventilation, or length of stay in the intensive care unit [25]. However, and of major importance for the care of ventilator-associated COVID-19 patients, several drawbacks have been cited i.e., a reduced susceptibility to CHX of number of ventilator-associated pneumonia pathogens and an increased risk of death in the less severe patients [23].

In oral health, it is commonly accepted that a preoperative antimicrobial mouth rinse decreases the number of oral pathogens. One of the more common CHX indications are gingivitis, periodontitis,

post-surgery periodontal disease, and implantology [26]. While CHX at lower concentrations is bacteriostatic, at higher concentrations it also has a bactericidal effect. CHX has been reported to penetrate the oral biofilms as well, affecting their growth or directly having a bactericidal impact [27]. CHX nanoparticles have the potential to inhibit the development of a multi-species oral biofilm made up of *Porphyromonas gingivalis*, *Streptococcus sobrinus*, and *Fusobacterium nucleatum* [28].

2.2. Flavonoids

Flavonoids constitute an important category of natural products and include various subgroups, such as flavones, chalcones, isoflavones, and flavonols [29]. Bioflavonoids are phenolic hydroxylated structures that have been synthesized from plants and have been shown to be active against fungi, bacteria, and viruses [30,31]. Flavonoids are a group of phytochemicals with a wide range of biological activities, mainly due to their antioxidant properties and their ability to regulate several cell receptors or enzymes [29]. Flavonoids have also been identified as having antiviral activity, antibacterial and anti-inflammatory effect, anti-allergic and antiangiogenic effects, and cytostatic, analgesic, apoptotic, hepatoprotective, antiestrogenic, and estrogenic properties [30,32–34]. Shimizu et al., discovered that flavonoids of Pterogyne nitens were able to inhibit the entry of the hepatitis C virus [35]. Jo et al., also suggested that the anticoronaviral activity of certain flavonoids (Rhoifolin, herbacetin, and pectolinarin) was attributable to the inhibitory effect of the 3C protease type [36]. Some others flavonoids (Isobavachalcone, herbacetin, helichrysetin, quercetin, and 3-β-d-glucoside) were able to inhibit the enzyme activity of MERS-CoV/3CLpro [36]. Ryu et al., in addition, reported that the biflavonoids of Torreya nucifera were also inhibitors of MERS-CoV/3CLpro [37].

To achieve a stronger impact, CHX mouth rinses were combined with plant products, as previous research has reported the efficient and effective utilization of natural antimicrobials inhibiting oral microbiota [38]. Several types of oral rinses are currently available, with additional ingredients containing mainly medical plants, alcohol, and natural substances [39]. More specifically, oropharyngeal microbiota has been identified as being involved in ventilator-associated pneumonia, and the utilization of therapeutic formulations using essential oils can decrease the incidence of this significant infection [40]. In vitro investigations were conducted examining the antimicrobial activities of five various mouth rinses using 14 test strains comprising *Candida* species and bacteria, cultivated both in plankton and as biofilms.

In four of these mouth rinses formulations, Citrox, a combination of natural bioflavonoids, was present. Three of these Citrox mouth rinses were complemented with hyaluronic acid, chlorhexidine or phenoxetol [41]. Citrox is an antimicrobial with ingredients based on natural soluble bioflavonoids extracted of citrus fruits. Citrox bioflavonoid preparations have a broad spectrum of antimicrobial activity on oral microorganisms and as such can be used within therapeutic formulations for the oral microbiota control [40].

2.3. Cyclodextrins

Cyclodextrins (CDs) are natural derivatives of glucose, with a rigid cyclic structure, composed of α(1-4)–linked gluco-pyranoside units [42]. The most usual CDs, called, α, β, and γ, contain 6-, 7-, and 8-glucopyranoside units, respectively. CDs are cyclic oligosaccharides used for improving bioavailability of medicinal products and water-solubility [43]. Also, CDs can be employed to prevent or reduce ocular and gastrointestinal irritation, decrease or eliminate disagreeable tastes or smells, prevent interactions between drugs or drug additives in a formulation [44]. They have been used in a multitude of commercial sectors, such as deodorants, drug delivery, food, and cosmetics. On the European market, examples of use of CDs in drugs are γ-CD in a minoxidil solution, β-CD in cetirizine tablets and cisapride suppositories and examples of use of derivatives of β-CD derivatives are HP-β-CD in itraconazole antifungal, in intravenous and oral solutions, SBE-β-CD in intravenous voriconazole antimycotic, and RM-β-CD in a nasal spray for hormone replacement therapy with 17β-estradiol [45].

Cyclodextrins have many advantages. They are more biocompatible than most oxides contained in oral products (i.e., silver and gold), simpler to use, they do not generate a resistance reaction, and they are not toxic [46]. Cyclodextrins have no harmful effects and are considered "generally regarded as safe" for humans [46]. The fields of pharmaceutical application of CDs are notable due to their low immunogenicity, low toxicity, cost-effectiveness, and accessibility [47]. These applications include: increasing drug stability and solubility, improving drug absorption, masking undesirable tastes and odors, controlling drug release, eliminating local and systemic toxicity, and improving drug permeability via biological barriers [48,49].

Their strucutre of cyclodextrins can be modified and used for containment of infections or as virucidal agents [50]. For example, it has been established that methylated beta-cyclodextrin can reduce influenza A virus and coronavirus infectivity by sequestering cholesterol from viral particles or depleting it from the host cell membranes [51]. Hydroxypropyl-β-cyclodextrin was used as a vaccine adjuvant providing protection against the H1N1 influenza virus in the cynomolgus monkey model [52,53]. One point to emphasize is that for natural CDs, the intramolecular hydrogen bond with the CD molecule decreases their hydrogen bond formation with the surrounding water molecules [54]. This could lead to a potential negative antiviral action against the coronavirus.

Recently, Jones et al. further developed the concept of cyclodextrins modified with mercaptoundecane sulfonic acids to provide the key nontoxic virucidal action and to mimic heparan sulfates. Very promising studies have indicated that the modified sugar molecules attract viruses before irreversibly inactivating them [55]. By disrupting the outer shell of a virus, cyclodextrins, modified with mercaptoundecane sulfonic acids can destroy infectious particles by simple contact, rather than simply blocking viral growth. This mechanism seems to be the same regardless of the virus concerned. These modified cyclodextrins are biocompatible, broad-spectrum, and virucidal at in vitro micromolar concentrations against many viruses, including respiratory syncytial virus (RSV), herpes simplex virus (HSV), Zika virus, and dengue virus. They are effective ex vivo against both laboratory and clinical strains of RSV and HSV-2 in respiratory and vaginal tissue culture models, respectively [55].

Amphiphilic β-cyclodextrin nanoparticles (C42H70O35) with seven glucose units has the mid-size cavity of the parent cyclodextrins, has been added to the composition of commercial mouth rinses to prevent flocking out of hyaluronic acid in the combination of CHX-Polylysine and hyaluronic-acid. Two commercial mouth rinses including β-CD are today available.

Amphiphilic CDs, useful for solubilizing, stabilizing, or releasing intermediate-sized molecules, have been produced by synthesis to solve multiple difficulties of parent cyclodextrins that restrict their pharmaceutical uses [56]. The main justifications for the synthesis of amphiphilic CDs were to: improve the interactions of cyclodextrins with biological membranes, increase the interaction of CDs with hydrophobic drugs, and enhance self-assembly capability in aqueous solutions [57].

3. Components of Mouth rinses and CoVID-19-Specific Treatment

In the absence of vaccines or medicines that will unfortunately arrive too late for many patients, it will be crucial to explore how existing treatments might be used as coronavirus specific interventions. Some mouth rinses currently on the market have ingredients that could contribute to the reduction of the SARS-CoV-2 viral load and thus facilitate the fight against oral transmission. We urge consideration be given to the following statements. While we recognize these statements do not have coronavirus specific evidence, they nonetheless can provide valuable information and insight to health workers and can support system-wide efforts to rapidly identify effective treatment protocols.

3.1. Mouth Rinses with Chlorhexidine for CoVID-19-Specific Treatment

In accordance with the Guidelines for the Diagnosis and Treatment of New Coronavirus Pneumonia (5th edition) of the National Health Commission of the Republic of China, CHX as mouth rinse may not be efficient to kill SARS-CoV-2 [4].

3.2. Mouth Rinses with Citrox for CoVID-19-Specific Treatment

Flavonoids as coronaviral chymotrypsin-like protease inhibitors have an essential function for coronaviral replication and also have additional functions for inhibition of host innate immune responses and should be useful in fighting COVID-19 [1]. Moreover, as SARS-CoV-2 is vulnerable to oxidation, it is recommended to use a mouth rinse containing oxidizing agents such as Citrox to reduce the salivary load viral of oral microbiota, including potential SARS-CoV-2 carriage.

3.3. Mouth Rinses with Amphiphilic β-Cyclodextrin for CoVID-19-Specific Treatment

As for the justification for the use of Citrox delivery, and the fact that SARS-CoV-2 is vulnerable to oxidation, mouth rinses containing oxidative agents such as amphiphilic CDs, appear indicative for the purpose of reducing the salivary load of oral microbes. The modified sugar molecules attract viruses before irreversibly inactivating them. By disrupting the outer shell of a virus, they can destroy infectious particles by simple contact, rather than just blocking viral growth [20]. This property of β-CDs can potentially be exploited for the reduction of viral load in the oral cavity with the use of disinfectant solutions. In addition, the use of therapeutic oral biofilm rinses and/or nasal applications might be considered in preventing viral transmission via the oropharyngeal route.

3.4. Mouth Rinses with Cyclodextrins Combined with Citrox for CoVID-19-Specific Treatment

The use of a mouth rinses and/or nasal applications that contain cyclodextrins combined with Citrox could provide a valuable adjunct treatment. Both are locally administered delivery systems that could lower the SARS-CoV-2 viral load and reduce the nasopharyngeal microbiota, which tends to coat the surface aerosol particles and droplets during coughing or sneezing.

Such products are available in Europe as oral health products and have been well tested in clinical profiles. We urge national agencies and authorities to start clinical trials to evaluate the preventive effects of βCD-Citrox therapeutic oral biofilm rinses in reducing the viral load of the infection and possibly disease progression.

We ask that consideration is given to CDs combined with Citrox in the form of therapeutic oral biofilm rinses and/or nasal applications, which can augment other approaches and treatment modalities.

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Virucidal efficacy of different oral rinses against SARS-CoV-2

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Summary: Several oral rinses show significant SARS-CoV-2 inactivating properties *in vitro*, supporting the idea that oral rinsing might reduce the viral load of saliva and could thus lower the transmission of SARS-CoV-2.

Conflict of interest: The authors do not have a conflict of interest.

Abstract

The ongoing SARS-CoV-2 pandemic creates a significant threat to global health. Recent studies suggested the significance of throat and salivary glands as major sites of virus replication and transmission during early COVID-19 thus advocating application of oral antiseptics. However, the antiviral efficacy of oral rinsing solutions against SARS-CoV-2 has not been examined. Here, we evaluated the virucidal activity of different available oral rinses against SARS-CoV-2 under conditions mimicking nasopharyngeal secretions. Several formulations with significant SARS-CoV-2 inactivating properties *in vitro* support the idea that oral rinsing might reduce the viral load of saliva and could thus lower the transmission of SARS-CoV-2.

Introduction

The current severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has created a significant threat to global health. Since effective treatments and vaccines are currently not available, diligent attention on transmission-based precautions is essential to limit viral spread. According to current evidence, SARS-CoV-2 is mainly transmitted through respiratory droplets exhaled from infected individuals [1]. Importantly, viral loads are high in the nasal cavity, nasopharynx and oropharynx and viral shedding can be detected before, during and after the acute clinical phase of illness [2]. Aerosols produced by asymptomatic individuals during breathing, speaking and singing are therefore considered as critical drivers of the enhanced spread of SARS-CoV-2 [3]. The host cell-derived envelope of SARS-CoV-2 is highly susceptible to chemical agents (i.e. various alcohols) that disrupt lipid bio-membranes [4]. Chemical antisepsis thus provides a critical tool to decontaminate fomites and (body-) surfaces like human hands. In this context, nasal and oral antisepsis have been suggested to lower the number of active aerosolized virus particles from the nasal passages and oral cavity and consequently reduce transmission risk of SARS-CoV-2 [5]. Antiseptic mouth rinses with antimicrobial activity are used in various clinical situations for prophylactic and therapeutic purposes and have further been applied in the context of viral infections [5]. Although various commercially available dental mouthwashes contain membrane-damaging agents (i.e. ethanol, chlorhexidine, cetylpyridinium chloride, hydrogen peroxide and povidone-iodine), their ability to inactivate SARS-CoV-2 under biologically-relevant conditions has not been evaluated systematically [5]. Here, we tested the virucidal activity of eight commercially available oral rinses containing different active compounds against three different SARS-CoV-2 isolates under conditions mimicking nasopharyngeal secretions.

Methods

Virus strains and propagation

To isolate SARS-CoV-2 at the University Ulm Medical Center (Ulm, Germany), 50,000 Vero E6 cells were seeded in 24-well plates in 500 µL medium incubated overnight at 37°C. The next day, medium was replaced by 400 µL of 2.5 µg/mL amphotericin B containing medium. Then, 100 µL of throat swabs that were tested positive for SARS-CoV-2 by qRT-PCR were titrated 5-fold on the cells and incubated for 3 to 5 days. Upon visible CPE, supernatant was taken and virus expanded by inoculation of Vero E6 cell in 75 cm^2 flasks and propagated as above described. Thereby, the viral isolates BetaCoV/Germany/Ulm/01/2020 (strain 2) and BetaCoV/Germany/Ulm/02/2020 (strain 3) were obtained. In Essen, Germany, SARS-CoV-2 was isolated from a nasopharyngeal swab of a patient suffering from COVID-19 disease and named UKEssen strain (strain 1). The swab was taken using a Virocult® vial (Sigma, Germany). The Virocult® medium was then incubated on Vero E6 cells cultured in DMEM containing 10% (v/v) fetal calf serum and supplemented with penicillin (100 IU/mL), streptomycin (100 µg/mL), ciprofloxacin (10 µg/mL) and amphotericin B (2.5 µg/mL). Five days after infection, the supernatant was harvested and cell debris were removed by centrifugation. Afterwards, 100 µL of the clear supernatant was used for subsequent infection of fresh Vero E6 cells. After five days of incubation, the virus suspension was harvested and cleared from cellular debris by centrifugation and stored at -80°C. Viral titers of the three stocks were determined by endpoint dilution assay and the 50% tissue culture infective dose ($TCID_{50}$/mL) was calculated.

Quantitative Suspension Test and Virus Titration

Virucidal activity was determined with a quantitative suspension test with 30 s exposure time. Briefly, one part virus suspension was mixed with one part organic load mimicking respiratory secretions (100 µL mucin-type I-S (Sigma-Aldrich), 25 µL BSA Fraction V (Sigma-Aldrich) and 35 µL yeast extract (Sigma-Aldrich) and eight parts of the oral rinse [6] . Medium served as a control. Following 30 seconds exposure time, activity was immediately stopped by serial dilution. $TCID_{50}$/mL values were determined by crystal violet staining and subsequent scoring the amounts of wells displaying cytopathic effects. $TCID_{50}$ was calculated by the Spearman & Kärber algorithm. The titre reduction including its 95% confidence interval is calculated as the difference between the virus titre after contact with the oral rinse and the control virus titre with medium (reduction factor = RF). Cytotoxic effects of oral rinses were monitored by crystal violet staining using non-infected cells and used to determine the lower limit of quantification (LLOQ). An optical analysis for altered density and morphology of the cellular monolayer in the absence of virus was performed and was quantified analogous to the $TCID_{50}$/mL of the virus infectivity.

Results

We examined the virucidal activity of eight commercially available oral rinses based on different active compounds (Table 1) using a quantitative suspension test with three different SARS-CoV-2 isolates mixed with an interfering substance mimicking a respiratory secretion. A medium control after 30 s exposure time did not reduce viral infectivity, thus implying that the used interfering substance mimicking nasal secretions did not alter virus stability. In contrast, the different SARS-CoV-2 strains (strains 1-3) were highly susceptible to various oral rinses. Three of the eight formulations, including product c, product e and product f, significantly reduced viral infectivity to up to three orders of magnitude to background levels

(Fig. 1, Table 1). Also, for the other products containing different active compounds (Table 1) virucidal activities could be observed with log reduction factors ranging between 0.3 to 1.78 (Fig. 1, Table 1). In case of product h, which is based on polyhexamethylene biguanide, the strain 1 was only moderately reduced, whereas the other two strains were inactivated to the lower limit of quantification, which was determined by monitoring the cytotoxic effects of the products in non-infected cells. (Fig. 1). In summary, we provide evidence that SARS-CoV-2 can be efficiently inactivated by commercially available oral rinses within short exposure times of 30 seconds.

Discussion

The main route of transmission of SARS-CoV-2 is suspected to involve direct contact with respiratory aerosols or droplets of infected individuals, produced during sneezing, coughing or talking, and subsequent contact to nasal, oral or ocular mucosal membranes [1]. SARS-CoV-2 initially colonizes the upper respiratory tract of infected individuals [2]. High viral loads in the oral cavity provide a rich source of potentially infectious virus as well as an entry route for new infections. Hence, if assuming that the throat functions as a major site of viral replication during early stages (even before symptom onset), oral antisepsis could lower the number of infectious aerosolized virus particles and consequently the risk of transmission or infection. Experimental and clinical research studies on SARS-CoV-2-related viruses (e.g. SARS-CoV, MERS-CoV, and influenza virus H5N1) showed that antiseptic solutions, containing chlorhexidine gluconate (CHG), polyvinylpyrrolidone iodine (PVP-I), chlorine dioxide (ClO2), cetylpyridinium chloride (CPC), and hydrogen peroxide (H_2O_2) can indeed reduce viral loads [7]. We found that different SARS-CoV-2 strains can be efficiently inactivated with commercially available oral rinses under biologically relevant conditions mimicking respiratory secretions. In particular, we observed that three formulations (products c, e and f) containing different active compounds

significantly reduced viral infectivity to undetectable levels. In agreement with our observation, different studies using Listerine (product f) observed antiviral activities specifically against enveloped viruses, implying an impact on the viral lipid envelope [8–10]. The *in vivo* effects of the oral solutions require further analysis during clinical studies. First trials with the aim to reduce the viral load in confirmed COVID-19 patients have been registered. One study aims to compare three antiseptic mouthwash/gargling solutions compared to a control (distilled water) to reduce SARS-CoV-2 load in 120 confirmed COVID-19 individuals (https://clinicaltrials.ucsf.edu/trial/NCT04409873). Another blind, randomized controlled pilot trial plans to determine the potential of various gargling agents in reducing intraoral viral load among laboratory-confirmed COVID-19 patients (https://clinicaltrials.gov/ct2/show/NCT04341688). Our findings clearly advocate the evaluation of selected formulations in clinical context to systematically evaluate the decontamination and tissue health of the oral cavity in patients and healthcare workers to potentially prevent virus transmission.

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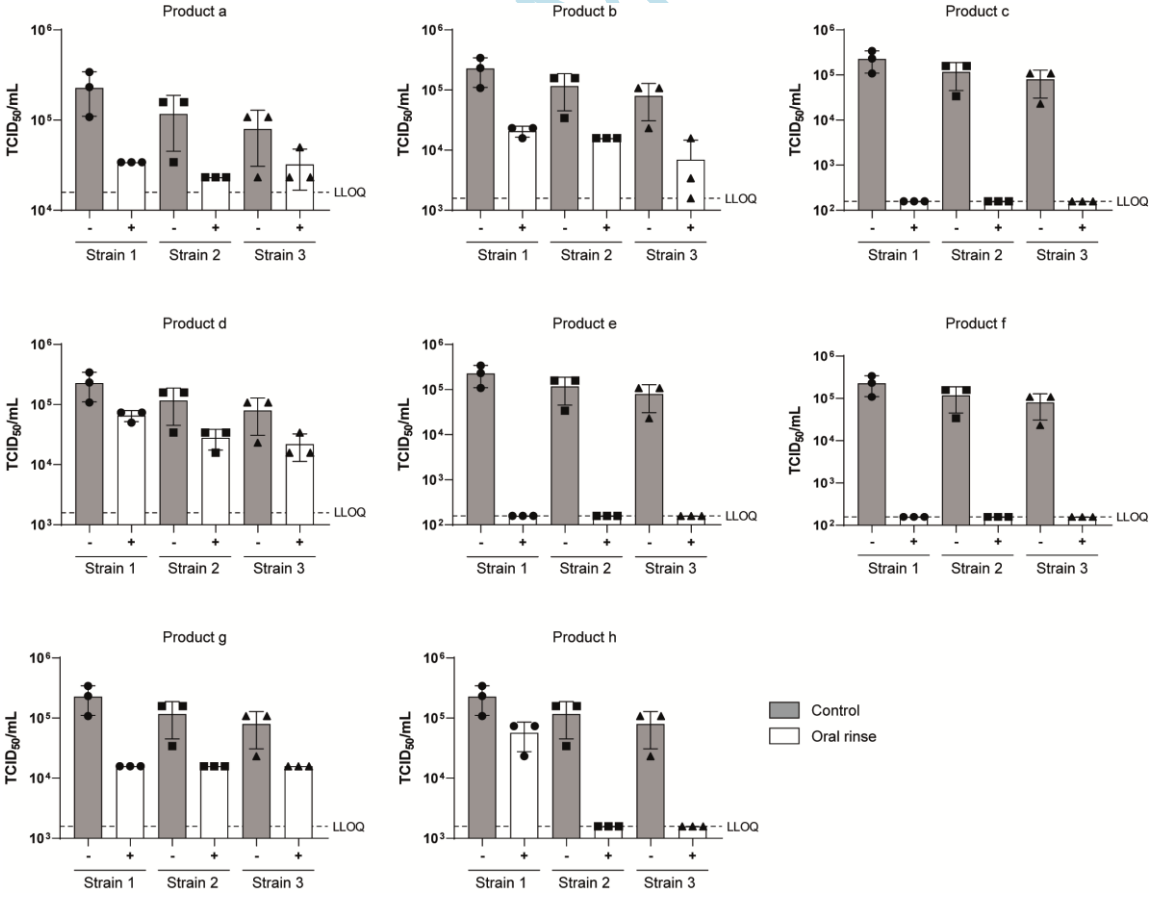
Figure legend

Figure 1. Virucidal activity of oral rinses against SARS-CoV-2. SARS-CoV-2 strain 1 (dot; UKEssen), strain 2 (square; BetaCoV/Germany/Ulm/01/2020), or strain 3 (triangle; BetaCoV/Germany/Ulm/02/2020) were incubated with medium (control) or various oral rinses for 30 s. Both conditions were supplemented with an interfering substance mimicking respiratory secretions. Viral titers were determined upon titration on Vero E6 cells. The cytotoxic effect was monitored using non-infected cells incubated with the different products, defined as lower limit of quantification (LLOQ). Tissue culture infectious dose 50 ($TCID_{50}$/mL was calculated according to Spearman-Kärber. Data indicate averages and standard deviation of three independent experiments.



Tables

Table 1: Overview of oral rinses used in the study with product name, active compounds and calculated reduction factors. The exact formulations for these oral rinses are not publicly available due to patent-related restrictions.

Product	**Trade name**	**Active compound**	**Log reduction factor (mean of n=3)**		
			Strain 1	**Strain 2**	**Strain 3**
a	Cavex Oral Pre Rinse	hydrogen peroxide	0.78	0.61	0.33
b	Chlorhexamed Forte	chlorhexidinebis (D-gluconate)	1.00	0.78	1.17
c	Dequonal	dequalinium chloride, benzalkonium chloride	≥3.11	≥2.78	≥2.61
d	Dynexidine Forte 0.2%	chlorhexidinebis (D-gluconate)	0.50	0.56	0.50
e	Iso-Betadine mouthwash 1.0%	polyvidone-iodine	≥3.11	≥2.78	≥2.61
f	Listerine cool mint	ethanol, essential oils	≥3.11	≥2.78	≥2.61
g	Octenident mouthwash	octenidine dihydrochlorid	1.11	0.78	0.61
h	ProntOral mouthwash	polyaminopropyl biguanide (polihexanide)	0.61	≥1.78	≥1.61

IN THE MATTER OF	:	
	:	
Lord Global Corp.	:	**ORDER OF SUSPENSION OF TRADING**
	:	
File No. 500-1	:	

It appears to the Securities and Exchange Commission that the public interest and the protection of investors require a suspension in the trading of the securities of Lord Global Corp. ("LRDG") (CIK No. 0001569568) because of questions regarding the accuracy and adequacy of information in the marketplace concerning LRDG since at least June 2, 2020. The questions relate to statements LRDG made about oral and nasal sanitizers that the company claims protect against the virus that causes COVID-19, which LRDG made in press releases and Commission filings between June 2 and August 18, 2020.

LRDG is a Nevada corporation whose principal place of business is in Carson City, NV. LRDG's common shares are registered with the Commission under Section 12(g) of the Securities Exchange Act of 1934 and are quoted on OTC Link LLC (previously "Pink Sheets") operated by OTC Markets Group Inc., under the symbol LRDG. As of August 10, 2020, LRDG had ten market makers and was eligible for the "piggyback" exception of Rule 15c2-11(f)(3) under the Securities Exchange Act of 1934. The Commission is of the opinion that the public interest and the protection of investors require a suspension of trading in the securities of the above-listed company.

Vanessa A. Countryman
Secretary

By: Jill M. Peterson
Assistant Secretary