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Short Report

Methods for SARS-CoV-2 hospital disinfection, *in vitro* observations

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Introduction: Escalation of chemical disinfection during the COVID-19 pandemic has raised occupational hazard concerns. Alternative and potentially safer methods such as ultraviolet-C (UVC) irradiation and ozone have been proposed, notwithstanding the lack of standardized criteria for their use in the healthcare environment.
Aim: Compare the virucidal activity of 70% ethanol, sodium dichloroisocyanurate (NaDCC), chlorhexidine, ozonated water, UVC-222 nm, UVC-254 nm against three SARS-CoV-2 variants of concern cultured *in vitro*.
Methods: Inactivation of three SARS-CoV-2 variants (alpha, beta, gamma) by the following chemical methods was tested: ethanol 70%, NaDCC (100 ppm, 500 ppm, 1000 ppm), chlorhexidine (2%, 1% and 0.5%), ozonated water 7 ppm. For irradiation, a je2Care 222nm UVC Lamp was compared to a Sylvania G15 UV254 nm lamp.
Results: Viral inactivation by >3 log was achieved with ethanol, NaDCC and chlorhexidine. The minor virucidal effect of ozonated water was <1 log. Virus treatment with UVC-254 nm reduced viral activity by 1–5 logs with higher inactivation after exposure for 3 minutes compared to 6 seconds. For all three variants, under equivalent conditions, exposure to UVC-222 nm did not achieve time-dependent inactivation as was observed with treatment with UVC-254 nm.
Conclusion: The virucidal activity on replication-competent SARS-CoV-2 by conventional chemical methods, including chlorhexidine at concentrations as low as 0.5%, was not matched by UVC irradiation, and to an even lesser extent by ozonated water treatment.

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Introduction

During the COVID-19 pandemic a range of disinfection methods, particularly ethanol, hypochlorite and quaternary ammonium compounds were evaluated for decontamination of hospital surfaces at risk of contamination with SARS-CoV-2. Ethanol and hypochlorite consistently demonstrated optimal virucidal activity at concentrations of ≥60% and 1000 ppm, respectively [1]. On the other hand, quaternary ammonium compounds such as chlorhexidine had variable anti-SARS-Co-V2 activity *in vitro* and *in vivo* [1].

Escalation of chemical disinfection during the pandemic raised occupational hazard concerns particularly in relation to respiratory toxicity, skin irritation and sensitisation, and cytotoxicity associated with chlorine by-products [2]. On this basis, alternative and potentially safer methods are desirable, notwithstanding the lack of standardized criteria for their use in the healthcare environment [3]. Ultraviolet-C (UVC) and ozone treatment are especially advocated in the context of SARS-CoV-2 droplet and PPE decontamination, respectively, but have been proposed for a range of materials and applications [1]. However, to the best of our knowledge the efficacy of ozone and UVC has not been directly compared to conventional chemical methods. The aim of this study was to compare the virucidal activity of ethanol, sodium dichloroisocyanurate (NaDCC), chlorhexidine, UVC-222 nm, UVC-254 nm and ozonated water against three SARS-CoV-2 variants of concern cultured *in vitro*.

Methods

All experiments were carried out in a Containment Level 3 laboratory at the Institute of Medical Sciences (University of Aberdeen). Three SARS-CoV-2 variants of concern (WHO label alpha, beta, gamma; Pango lineage B.1.1.7, B.1.351, P.1) were obtained from BEI Resources Repository (NIAID, Maryland USA): hCoV-19/England/204820464 (ATCC NR-54000), hCoV-19/South Africa/KRISP-K005325/2020 (ATCC-54009), hCoV-19/Japan/TY7-503/2021 Brasil P.1 (ATCC NR-54982). Cell line and viral cultures were maintained as described previously by our group [4]. Vero E6 cells (ATCC® CRL-1586™) were used for viral propagation and microtitration assays. First, viral variants were propagated in Vero E6 cells for 72 h at 37°C and 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 2% foetal calf serum (FCS). Virus from second and third cell passages was used in all experiments. The viral inoculum was prepared in 2% FCS-supplemented DMEM at a titre of 7.0×10^7–3.1×10^9 PFU/ml. Inactivation of SARS-CoV-2 variants was tested by two different methods, UV irradiation and chemical. For UVC-222 nm irradiation, a je2Care J8080 lamp was tested: je2Care 222nm UVC Lamp (Model number J8080) and je2Care Safety Controller Simulator Box (Model number J8082) manufactured by John Ellison Electronics Ltd., supporting a Ushio Corporation B1 222 nm KrCl excimer lamp, in turn composed of the Ushio Care222nm Lamp Module UXFL70-222B4-UIA with Inverter PXZ120I2-A; average output was 974 $\mu W/cm^2$ at 100 mm. The UVC-222 nm lamp was compared to a Sylvania G15 UV254 nm lamp after 6 s, 3 min and 10 min of exposure with samples at 100 mm from the UV source.

For chemical assays, ethanol (National Health Service Grampian Supply Chain) at 70% in water, ozonated water (7ppm generated by EORG mini submerged unit, EOD Europe, Finland), sodium dichloroisocyanurate (2.5g NaDCC, Actichlor™, Ecolab, UK) and chlorhexidine (Sigma-Aldrich, Merk C9394, 20% in H_2O) were used. For ozonated water, a 7ppm O_3 solution was mixed with the virus (9:1 volume ratio) and used immediately. For NaDCC, three different concentrations were tested: 100 ppm, 500 ppm, 1000 ppm. For chlorhexidine 2%, 1% and 0.5% concentrations were used. As for the ozonated water assay, virus and test solution were mixed at a volume ratio of 1:9 and pre-incubated for 3 min and 10 min. A cytotoxicity control condition with test solution alone (without virus) was tested alongside all assays for each one of the disinfectants. A virus alone condition (without test solution) was used as positive control and reference. For microtitration assays and infectivity measurements, the treated virus was added to Vero E6 cells (3×10^4/well) and incubated for 1 h at 37°C and 5% CO_2. Infected cells were covered with 1.2% Avicel® PH-101 and incubated again for 72 h at 37°C and 5% CO_2. After 72 h cells were fixed in 10% neutral buffered formalin and stained with 1% crystal violet in 20% ethanol solution. Each condition was tested in four replicates based on which viral titres were determined as TCID50/ml by the Reed-Muench Method [5]. Two independent experiments were carried out.

Results

Viral inactivation of >3 log, reflecting a reduction of viral inoculum below the detection limit (>99.9%), was achieved after 3 min exposure to chlorhexidine, NaDCC and ethanol 70% (Figure 1A–C). Chlorhexidine and NaDCC at the lowest concentration of 0.5% and 100 ppm respectively, inactivated SARS-CoV-2 variants by >5 log (>99.999% inactivation). Ozonated water had the poorest activity on SARS-CoV-2 variants with a reduction of viral activity of <1 log (Figure 1D). Virus treatment with UVC-254 nm reduced viral activity by 1–5 logs, with higher virucidal effect after exposure for 3 minutes compared to 6 seconds (Figure 1E). Under equivalent conditions, exposure to UVC-222 nm did not achieve time-dependent inactivation (Figure 1E–F). Increased time of virus exposure at 10 min to test solutions or UVC irradiation had no further effect on virucidal activity (data not shown). Differences in susceptibility to disinfection methods under any condition amongst the three SARS-CoV-2 variants were within 1 log when compared to the respective controls (Figure 1).

Discussion

The virucidal activity of ethanol at >60% and hypochlorite at 1000 ppm are undisputed. However, health and environmental risks associated with these agents have accelerated efforts to develop safer alternatives particularly in the context of management of epidemics requiring escalation of general disinfection of hospital surfaces.

Chlorhexidine gluconate is one of the less toxic agents employed for general disinfection but its suitability for inactivation of SARS-CoV-2 has been called into question in view of studies suggesting lower virucidal activity compared

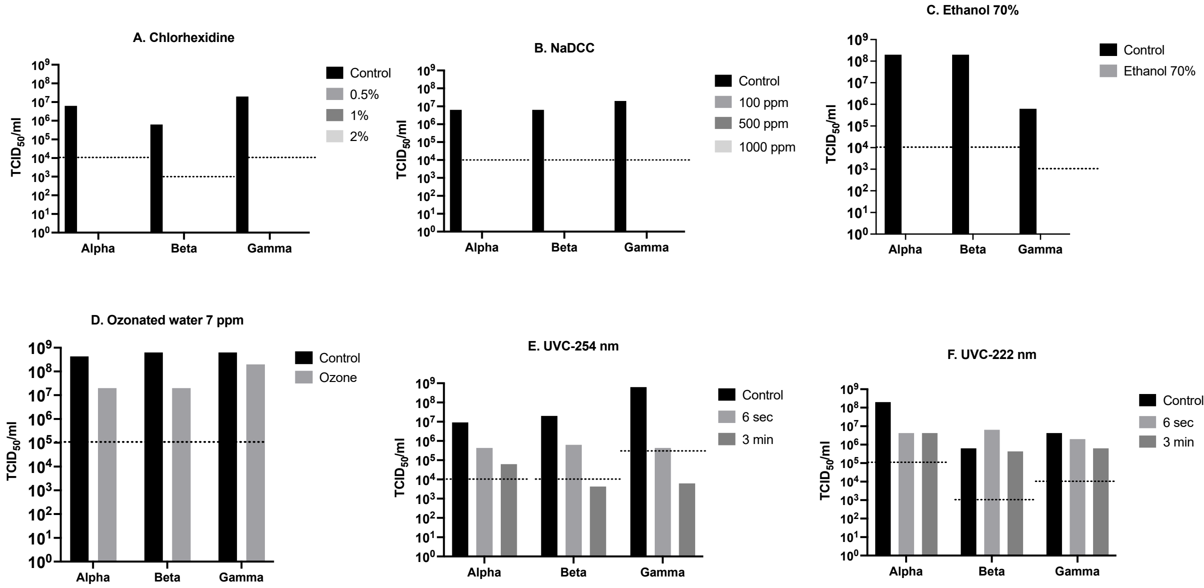

Figure 1. Activity of SARS-CoV-2 exposed to conventional chemical methods, ozonated water and ultraviolet irradiation. Viral activity of three variants of concern exposed to chlorhexidine at 0.5—2% (A), sodium dichloroisocyanurate (NaDCC) at 100—1000 ppm (B), ethanol 70% (C), ozonated water at 7 ppm (D), UVC-254 nm (E) and UVC-222 nm (F) in relation to non-treated virus (control) represented as $TCID_{50}$/ml. Reduction of viral activity below the detection limit (>3 log relative to the control) is indicated by the horizontal dashed line. Time of exposure was 3 minutes unless otherwise specified. One representative experiment of two replicates is shown.

to other disinfectants [1]. It has been suggested that the antiviral activity of disinfectants, including chlorhexidine, can be affected by protein content in the test environment [6]. In this study, we show that chlorhexidine at the lowest concentration of 0.5% displayed equal virucidal activity as NaDCC at 100ppm and 70% ethanol against viral cultures grown in relatively low-protein medium (2% FCS). This being said, the intensive use of chlorhexidine is less than optimal due to a range of concerns including selection of multidrug resistant lineages of hospital-acquired pathogens [7] as well as possible sensitisation in health care workers, although likely resulting from the direct contact of chlorhexidine with the skin and mucous membranes [8].

The virucidal activity of UVC, including the presumed safer low wavelength (200—300) UVC, on SARS-CoV-2 has been demonstrated previously to varying degrees depending on UV spectrum, type of device, UV intensity, exposure time and distance from UV source. UVC was most effective at 254 nm for which complete inactivation of SARS-CoV-2 was observed at exposure time of up to 15 minutes and irradiation distance of up to 1 meter [9] (Supplementary Table A1). *In vitro* culture conditions such as the composition and volume of culture medium or surface substrate may also affect the absorption dose of the UV light [10]. Irradiation dose requirement was lower for inactivation of aerosolised viral particles, and inversely correlated with the volume of liquid culture medium [10]. In view of the wide variation of experimental conditions, it is difficult to draw conclusions on the highest performing UVC device and treatment conditions. Our study confirmed the higher efficacy of the conventional UVC-254 nm compared to UV-222 nm in agreement with a previous study showing a median lethal dose of 8.8mJ/cm^2 for UVC-245nm and 106.3 mJ/cm^2 for UV-222 nm [11]. In our study, reduction of viral activity below the detectable limit was not observed even at a UVC-222 nm dose as high as 584.4mJ/cm^2. Further, neither UVC irradiation method matched the antiviral activity of NaDCC, ethanol and chlorhexidine at equal experimental conditions.

Ozonated water has been proposed as a ‘green’ disinfection method with a study suggesting that anti-SARS-CoV-2 activity occurs via direct disruption of the SARS-CoV-2 viral RNA and spike surface protein [6]. Our study did not show clinically significant antiviral activity of ozonated water at 7 ppm and at a virus:test solution ratio of 1:9. A previous study showed that virus:test solution ratio is a key factor affecting antiviral activity of ozonated water as viral inactivation to undetectable limits was demonstrated at a virus:test solution ratio of 1:99 but not 1:19 or 1:9 [6]. It has been suggested that the effective concentration of ozone is reduced by increased time of exposure, although the very short half-life of ozone in water must be considered (2—4 min at 25C in aqueous solution, pH 7) [6]. On the other hand, it is not clear to what extent the presence of organic substances may affect the activity of ozonated water as reduction of FCS concentration *in vitro* did not result in higher antiviral activity [6]. Naturally, the experimental conditions in this study do not recapitulate real-life conditions in the hospital environment as both temperature and humidity are known to affect the efficacy of disinfectants as well as viability of viruses [1]. Nonetheless, the virucidal activity of UVC in liquid suspension as measured in this study, is likely to occur more readily than with the virus dried onto surfaces which is a key challenge in the healthcare environment.

Conclusions

To the best of our knowledge, this is the first study to compare the activity of different disinfection methods (conventional chemical methods, ozonated water and ultraviolet irradiation) proposed for SARS-CoV-2 inactivation in hospital settings. Within the limitations of the *in vitro* experimental conditions described, we showed that the virucidal activity on three variants of replication-competent SARS-CoV-2 by UVC irradiation and ozonated water, did not match the virucidal activity of conventional chemical methods, including chlorhexidine generally deemed inferior to ethanol and hypochlorite.

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Competing interest

The authors declare that there is no competing interest.

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Ethics

Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.infpip.2024.100339.

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