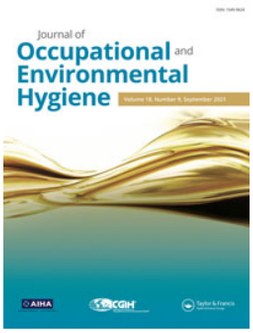

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Journal of Occupational and Environmental Hygiene

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/uoeh20

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To cite this article: Isabella Marchesi, Arianna Sala, Giuseppina Frezza, Stefania Paduano, Sara Turchi, Annalisa Bargellini, Paola Borella & Claudio Cermelli (2021): *In vitro* virucidal efficacy of a dry steam disinfection system against Human Coronavirus, Human Influenza Virus, and Echovirus, Journal of Occupational and Environmental Hygiene, DOI: 10.1080/15459624.2021.1989442

To link to this article: https://doi.org/10.1080/15459624.2021.1989442

Published online: 20 Oct 2021.

JOURNAL OF OCCUPATIONAL AND ENVIRONMENTAL HYGIENE
https://doi.org/10.1080/15459624.2021.1989442

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SHORT REPORT

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In vitro virucidal efficacy of a dry steam disinfection system against Human Coronavirus, Human Influenza Virus, and Echovirus

Isabella Marchesi[a], Arianna Sala[a], Giuseppina Frezza[a], Stefania Paduano[a], Sara Turchi[a], Annalisa Bargellini[a], Paola Borella[a], and Claudio Cermelli[b]

[a]Department of Biomedical Metabolic and Neuro Sciences, University of Modena and Reggio Emilia, Modena, Italy; [b]Department of Surgery Medicine Dentistry and Morphological Sciences with an Interest in Transplant Oncology, University of Modena and Reggio Emilia, Modena, Italy

ABSTRACT

This *in vitro* study was aimed to assess the efficacy of dry steam in inactivating Human Coronavirus OC43 (HCoV-OC43) as surrogate of SARS-CoV-2, Human Influenza Virus A/H1N1/WSN/33 and Echovirus 7 on stainless steel, polypropylene, and cotton. The virus models were chosen on the basis of their transmission route and environmental resistance. Tests were carried out under a laminar flow cabinet, where two panels of each material were contaminated with a viral suspension. The inocula were left to dry and then the virus on untreated panel (control) was collected by swabbing in order to determine the initial titer. The other panel was treated using a professional vacuum cleaner equipped with a dry steam generator. Dry steam is generated in a boiler where tap water is heated up to 155 °C at 5.5 bar pressure and then during the passage along the flexible hose the temperature decreases to a value between 100 °C and 110 °C at the output. The dry steam was applied for four sec with a window wiper on metal and plastic panels or a brush covered by a microfiber cap on cotton, simulating the steam application during routine cleaning. After the treatment, infectious virus possibly remained on the surface was collected following the same swabbing procedure applied for controls. HCoV-OC43 and Echovirus 7 were titrated by end-point method on HCT-8 line cells and Vero cells, respectively, while Human Influenza Virus was quantified by plaque reduction assay on MDCK cells. Dry steam resulted effective against the three viruses on all tested materials, achieving a mean Log_{10} reduction factor $\geq$4 in viral titer of treated samples compared with controls according to UNI EN 14476:2019. Thus, dry steam may be proposed as an ease to use, effective, fast, and non-toxic alternative to chemicals for surface disinfection without damaging materials. Therefore, this device could be employed not only in healthcare facilities but also in occupational, domestic, and community settings, with advantages for environment and human health.

KEYWORDS

Fomites; HCoV-OC43; SARS-CoV-2 surrogate; steam generator; surfaces; viruses

Introduction

Human SARS-CoV-2 is a highly contagious virus that is transmitted from human to human through aerosols generated during sneezing, coughing, speaking, as well as through the virus-contaminated surfaces (Krishan and Kanchan 2020; Weber and Stilianakis 2021). It can persist on abiotic surfaces for a few hours to several days, thus a prompt inactivation to block virus transmission is required (van Doremalen et al. 2020). Common household chemicals like ethanol, sodium hypochlorite, and hydrogen peroxide, applied after cleaning, are demonstrated to be effective in killing SARS-CoV-2, but their excessive use can be associated with adverse effects on human health and environment, and materials damage as well (Gharpure et al. 2020; Noorimotlagh et al. 2020; Sharafi et al. 2021). One possible alternative is steam, generated with different technologies, which has demonstrated a high capability to inactivate microorganisms in several *in vitro* studies. In fact, a thermal-accelerated nanocrystal sanitation—equipped steam vapor technology resulted effective in killing numerous nosocomial pathogens, endospores of *Clostridium difficile*, bacteria embedded in biofilms, and Human Norovirus surrogates (Tanner 2009; Song et al. 2012; Buckley et al. 2018). Another study reported that numerous drug-

CONTACT Isabella Marchesi isabella.marchesi@unimore.it Department of Biomedical Metabolic and Neuro Sciences, University of Modena and Reggio Emilia, Via Campi 287, Modena, 41125, Italy.

resistant bacterial pathogens and fungi were inactivated by an overheated dry-saturated steam system (Bagattini et al. 2015). Dry steam was reported effective also in reducing the number of viable mold *Cladosporium sphaerospermum* spores on moist carpet (Ong et al. 2015). Several field studies reported that application of dry steam in hospital ward room surfaces and in residential aged-care settings resulted in good decontamination against heterotrophic bacteria at 36 °C and many pathogens like *Clostridium difficile*, methicillin intermediate and resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus faecalis*, carbapenem-resistant *Pseudomonas aeruginosa*, multidrug-resistant *Acinetobacter baumannii*, etc. (Sexton et al. 2011; De Lorenzi et al. 2012; Gillespie et al. 2013; Oztoprak et al. 2019). Microwave-generated steam is one of the four methods recommended for virus decontamination before reuse of surgical masks and N95 respirators during the COVID-19 pandemic (Seresirikachorn et al. 2021). Similarly, the Italian National Institute of Health during COVID-19 health emergency recommended the dry steam for disinfection in indoor environments of clothing used in fitting or dressing rooms, upholstered furniture, curtains, etc. (ISS COVID-19 Working Group on Biocides 2020). Nevertheless, published literature on the use of steam against SARS-CoV-2 is lacking. The reduction of SARS-CoV-2 contamination in non-healthcare settings is essential, due to the intra-familial and workplace infections observed in this third pandemic phase, and to the numerous contaminable high-touch surfaces like public railings, transport seats, door and window handles, food preparation areas, toilets and taps, touchscreen devices, computer mouse and keyboard, etc.

The aim of our study was to assess the efficacy of dry steam technology in inactivating three pathogenic human viruses experimentally inoculated on different materials (metal, plastic, tissue) selected among the most widespread ones in work, domestic, and community settings.

Methods

The steam system

In this study, a professional portable vacuum cleaner equipped with a dry steam generator (Gioel G400, Gioel S.p.A, Trento, Italy) was used. It combines the vacuuming with the release of high-pressure steam to clean and sanitize floors and high-touch surfaces. Figure 1 shows the main components of the cleaning system, whose operation requires some simple steps. First, cold tap water is added in the collection tank where the aspirated dirt flows. Second, the refill tank of the steam generator is filled with water which is pumped inside the boiler where it is heated through an electrical resistance up to 155 °C at 5.5 bar pressure, conditions generating dry steam. Third, the flexible hose is connected to the specific inlet and the vacuuming function and/or the steam may be selected pushing the buttons on the handle. Last, the dry steam release can be activated pressing the trigger on the handle.

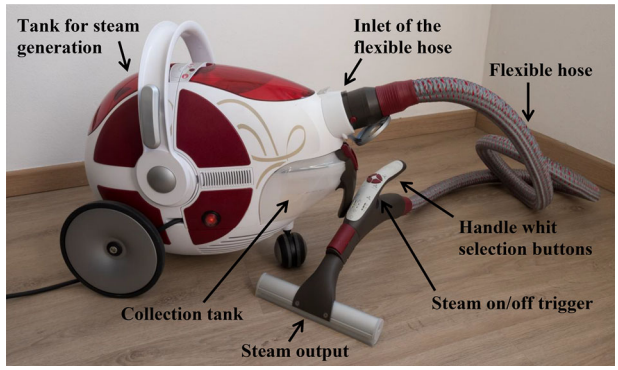

Figure 1. The vacuum cleaner equipped with a dry steam generator used in this study.

Viruses and cells

The tests were conducted on three viruses: Human Coronavirus (HCoV) OC43 (ATCC VR1558), a surrogate of novel SARS-CoV-2 responsible for COVID 19 pandemic; Human Influenza Virus subtype A/H1N1/WSN/33, kindly provided by Prof. Arcangeletti (University of Parma, Italy); and the Echovirus 7 (ATCC VR37), chosen for its highest persistence in the environment and resistance to physical and chemical treatments (Sofer et al. 2003; Sauerbrei and Wutzler 2009). HCoV-OC43 was grown in HCT-8 line cells, Human Influenza Virus in MDCK cells, and Echovirus 7 in Vero cells. All the cell lines were cultivated in Dulbecco's Minimum Essential Medium containing 10% (growth medium) or 5% (maintenance medium) fetal bovine serum, penicillin (100 U/mL), streptomycin (100 µg/mL), ciprofloxacin (100 µg/mL) and L-glutamine (2 mM). All reagents were purchased from Merck Life Science, Milano, Italy.

In vitro tests

For each tested virus and material, an area of 2.5 × 2.5 cm was delimited with a permanent marker on two panels (40 × 40 cm) put under a laminar flow cabinet. Both areas were contaminated with 100 µL of viral suspension left to dry for 20 min (see Figure 2A showing an experiment on stainless steel panels as an

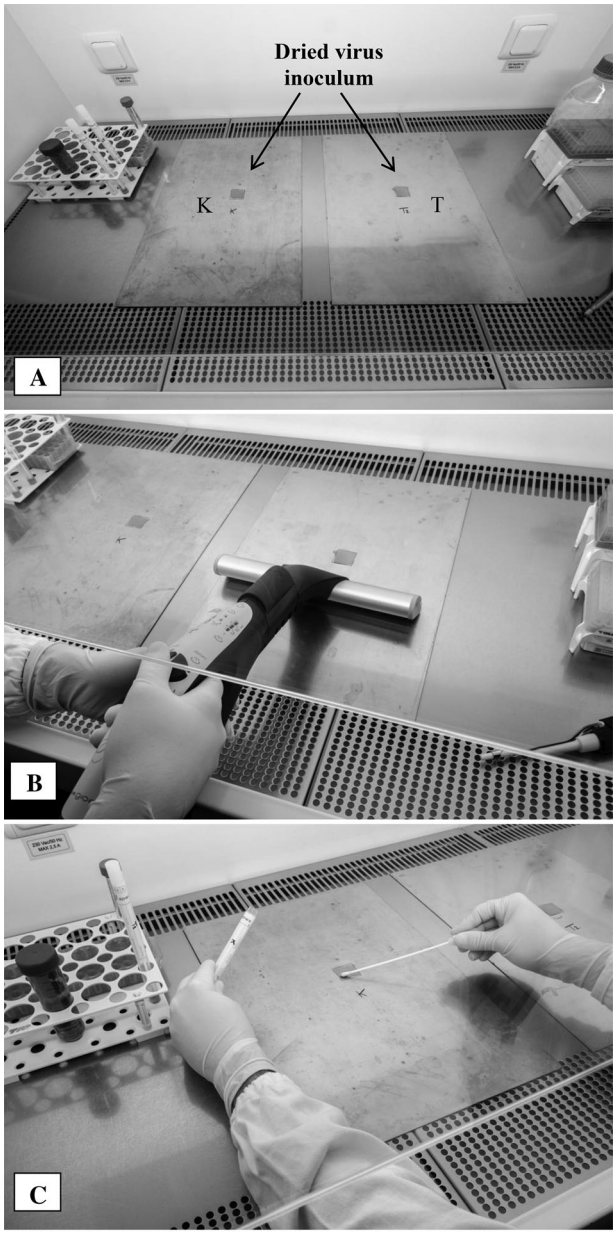

Figure 2. The pictures show the main phases of an experiment performed on stainless steel panels. (A) Virus suspension dried on squared areas in the middle of control (K, left panel) and test (T, right panel) panels. (B) Treatment with window wiper on the test panel. (C) Virus harvesting with swab from control area K.

example). One of the two panels (the test panel) was treated with the steam, while the other was not exposed to steam and served as a control. Fifteen minutes before use, the device was activated and the steam delivery was set to level 2. During the passage along the flexible hose the steam temperature decreases from 155 °C to a value between 100 °C and 110 °C at the output, according to the manufacturer's instructions. For experiments on metal and plastic panels, a window wiper was connected to the handle of the dry steam device (Figure 2B), while a triangular brush with a microfiber cap was used on cotton. Each treatment was conducted simulating the routine cleaning and consisted of three back and forth passages of vaporization, followed by an only one final step of vacuuming to remove condensation, for a steam application total time of 4 sec. The virus suspension dried on the control panel was collected with a cotton swab (Biolife Italiana, Milan, Italy) by scraping the area for 1 min (Figure 2C). After the steam treatment, the same procedure was applied for harvest the virus on the test panel. Swabs were resuspended in 1 mL of phosphate buffer saline by vortexing for 1 min. Serial 10-fold dilutions of these suspensions were seeded in duplicate on the suitable cell cultures in 96-well-plates (Euroclone, Milano, Italy). After incubation, the cell cultures were observed daily by inverted light microscope (Carl Zeiss, Milano, Italy) to check the cytopathic effect of tested virus. The assessment of the viral cytopathic effect was possible even at the lowest dilutions because no confounding cytotoxicity was observed, as expected in the absence of any chemicals potentially damaging the cell cultures. HCoV-OC43 and Echovirus 7 were quantified by end-point titration, as previously described (Ascione et al. 2017). The titer of both viruses was established as the highest dilution showing the typical viral cytopathic effect (rounding and detachment of cells) and the results expressed as 50% Tissue Culture Infectious Dose ($TCID_{50}$). A/H1N1 virus was titrated by plaque reduction assay, as reported by Ascione and colleagues (2017). In this case, viral titer was defined by counting the cytolysis plaques and the results were expressed as plaque forming units (PFU/mL). The limit of detection (LoD) was 1 $TCID_{50}$ for end point titration and 5 PFU/ml for plaque reduction assay.

For each material, we performed three independent experiments with each virus. Samples were assayed in duplicate in all the experiments. The virucidal efficacy of steam was defined as a ≥ 4 Log_{10} reduction in virus titer of treated samples compared with controls, according to UNI EN 14476:2019.

Moreover, limited to tests with Echovirus 7, 1 L of dirty water remaining in the collection tank of the vacuum cleaner after use was concentrated by ultrafiltration (Pellicon XL system, Merck Life Science, Milano, Italy) to assess the possible presence of infectious virus. The potential viral load of these samples was end-point titrated as described above.

Results

The mean HCoV-OC43 titer in controls was 2.1×10^4 $TCID_{50}$ on stainless steel and 1.0×10^4 $TCID_{50}$ on

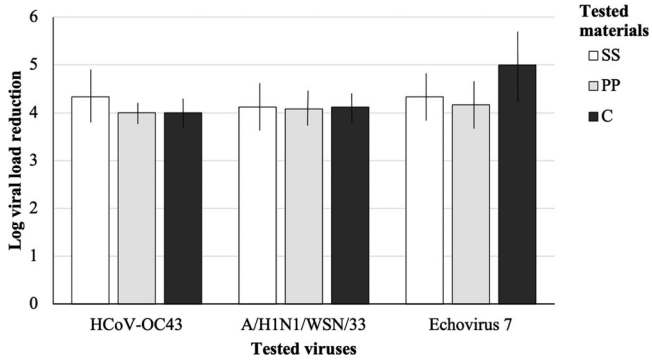

Figure 3. Logarithmic viral load reduction by the dry steam system on the different surfaces. Data reported in figure are mean values (± standard deviation) from three different experiments performed. SS = stainless steel, PP = polypropylene, C = cotton fabric.

both polypropylene and cotton. Similarly, A/H1N1 virus showed a mean initial load of 1.3×10^4 PFU/mL on both stainless steel and cotton, and 1.2×10^4 PFU/mL on plastic. Figure 3 shows the logarithmic viral load reduction of each virus on the three studied surfaces, calculated as the difference between viral titer of controls expressed as Log_{10} and that of steam treated samples. The dry steam treatment led to complete inactivation of both viruses (titer $<$ 1 $TCID_{50}$ and $<$ 5 PFU/mL for HCoV-OC43 and A/H1N1 virus, respectively) on all tested surfaces, achieving a mean Log_{10} reduction factor $\geq$ 4 in virus titer, corresponding to a percent reduction of 99.99% (Figure 3). The initial loads of Echovirus 7 recovered from tested surfaces were 2.1×10^5, 1.5×10^5, and 1.5×10^4 $TCID_{50}$ on stainless steel, cotton and polypropylene, respectively. After dry steam treatment, Echovirus 7 was still detected on stainless steel and cotton, even though at a minimal load (10.0 and 1.5 $TCID_{50}$, respectively), while on plastic it was found below the LoD. Thus, a mean logarithmic reduction factor $\geq$ 4 on all surfaces was reached (Figure 3). Moreover, infectious Echovirus 7 was not detected even in the dirty water inside the collection tank where aspirated impurities are concentrated.

Discussion

To our knowledge, this is the first *in vitro* study demonstrating the virucidal efficacy of the dry steam against Human Coronaviruses (HCoVs) contaminating different surfaces. We tested HCoV-OC43, which has a structural similarity with SARS-CoV-2 and is commonly considered a surrogate for the highly pathogenic HCoVs (Gerchman et al. 2020). Several studies confirm similar persistence on environmental surfaces among human and animal CoVs (Aboubakr et al. 2020; Bueckert et al. 2020; Malik 2020; Ren et al. 2020) supporting a comparable efficacy of dry steam against HCoV-OC43 and SARS-Cov-2. Moreover, this similarity is also suggested by the evidence that T cells that react to SARS-CoV-2 epitopes cross-react with corresponding homologous sequences from HCoV-OC43 and other commonly circulating HCoVs (Mateus et al. 2020; Steiner et al. 2020). We also tested other two viruses, chosen on the basis of their transmission routes (via droplets and/or fecal-oral route) and environmental resistance. Respiratory Human Influenza A Virus is fragile and less stable than Coronaviruses outside the host, while Echovirus 7, mainly transmitted by feces, is among the most resistant viruses (Sofer et al. 2003; Sauerbrei and Wutzler 2009). The remarkable virucidal activity of dry steam against such a resistant virus provides further support to the possibility of an effective action against the less stable SARS-CoV-2. Interestingly, SARS-CoV-2 has been proved present in feces, suggesting a transmission through fecal contaminated fomites (Amirian 2020; Tian et al. 2020). Therefore, cleaning with dry steam, along with proper hand hygiene, could help to prevent the risk of fecal-oral transmission of SARS-CoV-2 in environments where fecal spills may easily occur, such as childcare centers and facilities with incontinent residents.

Our findings, showing a high efficiency in virus inactivation by dry steam, fulfill the European standard for antiviral efficacy (4-Log_{10} reduction) against the tested viruses, without differences among hard (steel and plastic) and porous (cotton) materials (Ente Nazionale Italiano di Unificazione 2019). Nonporous surfaces are known to allow a longer viral persistence than the porous ones, from which, however, it is more difficult to remove viruses with chemical treatments (Noorimotlagh et al. 2020).

In order to assess the dry steam virucidal efficacy, we chose to perform a biological test on tissue cultures, because although it is less sensitive than detection and quantification of RNA, is the only test that provides results on the infectivity of the virus recovered from the surfaces. As reported by Atkinson and Petersen (2020), the presence of nucleic acid alone cannot be used to define viral shedding or infection potential. Moreover, it is well known that RNA of many other viruses like SARS-CoV, MERS-CoV, Influenza Virus, etc. can be detected long after the disappearance of infectious virus (Bueckert et al. 2020).

Although it cannot be excluded a virus removal by device brush, the lack of infectious Echovirus in the

dirty water supports the hypothesis that the highly remarkable viral load reduction detected after treatment is due to virus inactivation by the dry steam. Moreover, neither swab rubbing to rescue residual virus nor physical-chemical reaction between the steam and the material generated toxic compounds, as suggested by the absence of any cytotoxicity observed at virus titration, even at the lowest dilutions.

Importantly, in this study all experiments have been conducted on virus suspensions obtained in complete cell culture media, with fetal bovine serum and other organic material, thus simulating the soiled condition that can be found on environmental real surfaces.

A possible limitation of this study is that we did not measure the actual temperature of the output steam during each experiment, but we merely reported the value declared by the manufacturer.

Dry steam inactivates viruses in seconds without leaving residues, while chemicals require an initial cleaning step to remove the organic matter and a 5–10 min contact time, may need extra-ventilation after disinfection and may release disinfection-by-products (Sexton et al. 2011; De Lorenzi et al. 2012; Gillespie et al. 2013; Oztoprak et al. 2019). These features, together with the ease of use comparable to that of any other domestic vacuum cleaner, make the dry steam particularly useful for surface disinfection both in work and community environments such as offices, public transportation, hotels, restaurants, gyms, schools, home, etc. Furthermore, due to its activity against enteric viruses, dry steam could be effectively used in presence of fecal contamination in kindergartens, facilities for the elderly, pediatric wards, etc.

Other advantages of dry steam are the lack of slippery surfactants and the negligible volume of water left on treated surfaces after dry steam disinfection (Tanner 2011). Thus, potential slippage risks are insignificant. Furthermore, textiles are not damaged by dry steam (ISS COVID-19 Working Group on Biocides 2020). In addition, the scalding risk is minimum since the steam temperature rapidly decreases at few centimeters from the emission nozzle. Of course, care must be taken not to cause damage when disinfecting electrical devices and electronic equipment with dry steam. Last, infectious Echovirus 7 was not found in the dirty water tank of the steam generator, indicating that cleaning the device is not hazardous for users.

Conclusion

In conclusion, our study confirms that dry steam may be proposed as an effective, environmentally friendly, and fast alternative to chemicals for disinfection of environmental surfaces potentially contaminated by viruses, preventing users from being exposed to toxic or irritating by-products.

Competing interest disclosure statement

The authors declare that they have no competing financial interests or personal relationships with the company Gioel S.p.A. that could have appeared to influence the work reported in this paper. The company Gioel S.p.A. provided the dry steam device used in the study and technical support to use it properly on the different materials tested, but was not involved in the study design, in data analysis and presentation, or in writing the manuscript. Preliminary results of this study, limited to the efficacy of dry steam in inactivating the Human Coronavirus OC43 on stainless steel and cotton substrates, were used by Gioel S.p.A. for commercial purposes.

ORCID

Isabella Marchesi http://orcid.org/0000-0002-0905-5783
Stefania Paduano http://orcid.org/0000-0003-3640-9177
Annalisa Bargellini http://orcid.org/0000-0002-7562-1472
Paola Borella http://orcid.org/0000-0001-9474-3566
Claudio Cermelli http://orcid.org/0000-0003-3396-0000

Data availability statement

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

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