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BRIEF COMMUNICATION

Use of a Hydrogen Peroxide Nebulizer for Viral Disinfection of Emergency Ambulance and Hospital Waiting Room

Marie Estienney[1,2] · Philippe Daval-Frerot[1] · Ludwig-Serge Aho-Glélé[3] · Lionel Piroth[4] · Pascal Stabile[5] · Jean-Yves Gerbet[5] · Romain Rouleau[6] · Alexis de Rougemont[1,2] · Gaël Belliot[1,2]

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Abstract

Disinfection of hospital facilities and ambulances is an important issue for breaking the chain of transmission of viral pathogens. Hydrogen peroxide has provided promising results in laboratory assays. Here, we evaluate the efficacy of a hydrogen peroxide nebulizer for the inactivation of surrogate MS2 bacteriophage and murine norovirus (MNV) in a patient waiting room and the fully equipped cabin of a medical ambulance. We observed an average 3 $\log_{10}$ titer reduction in both settings, which represents the destruction of over 10^6 and 10^9 infectious particles of MNV and MS2 per cm^2, respectively. The potential for viral exposure is high for health workers when disinfecting confined and cluttered spaces, so the use of a hydrogen peroxide mist might offer an affordable and efficient solution to minimize the risk of viral contaminations.

Keywords Virus · Hydrogen peroxide · Ambulance · Hospital room · Sanitation

Introduction

In January 2020, Cohen and Normile published an editorial discussing the potential threat posed by a new severe acute respiratory syndrome (SARS)-like coronavirus which had just emerged in China (Cohen & Normile, 2020). Nowadays, virus containment, the chain of transmission within the population, and prevention are important issues as exemplified for COVID-19 (Coronavirus disease) and its etiological agent, SARS-CoV-2. Infectious patient handling requires reinforced hygiene measures to prevent the accidental infection of health workers with viruses, like coronavirus, which has been shown to remain infectious on inert surfaces (Otter et al., 2016; van Doremalen et al., 2020). Therefore, prevention involves new protocols for the sanitization of ambulances and any facility that are frequented by potentially infected patients The emerging SARS-CoV-2 during the year 2020 exemplified the need for reliable, simple, and robust tools for viral disinfection of confined space for the next viral epidemic.

Hydrogen peroxide is well known for its virucidal activity, and its efficacy against a range of enveloped and non-enveloped viruses is well documented in the literature (Goyal et al., 2014; Tuladhar et al., 2012; Yeargin et al., 2016).

Otter et al. underlined that "no-touch disinfection systems" such as hydrogen peroxide nebulizers are gaining attention for surface decontamination in hospitals (Otter et al., 2013). Here, we aimed to determine the virucidal activity of a nebulizing system producing a hydrogen peroxide mist in a patient waiting room and an ambulance cabin, where disinfection procedures are often time consuming and incomplete. Innocuous MS2 bacteriophage and murine

Marie Estienney and Philippe Daval-Frerot have contributed equally to this work.

✉ Gaël Belliot
gael.belliot@u-bourgogne.fr

1 Laboratory of Virology, National Reference Centre for Gastroenteritis Viruses, University Hospital of Dijon, PBHU, CHU Dijon Bourgogne, 2 rue Angélique Ducoudray, BP37013, 21070 Cedex Dijon, France

2 UMR PAM A 02.102 Procédés Alimentaires et Microbiologiques, Université de Bourgogne Franche-Comté/AgroSup Dijon, 21000 Dijon, France

3 Epidemiology and Infection Control Unit, Public Hospital of Dijon, 21070 Dijon, France

4 Department of Infectious Diseases, University Hospital of Dijon, 21070 Dijon, France

5 Medical Transport Unit, University Hospital of Dijon, 21070 Dijon, France

6 Oxy'Pharm Ltd, 94500 Champigny-sur-Marne, France

norovirus (MNV) were used as surrogates for their robustness in the environment and for safety reasons since the assays were conducted outside of the laboratory.

Materials and Methods

Virus Preparation

The murine norovirus CW1 (MNV-1) strain was provided by Herbert W. Wirgin (Washington University, Saint Louis, MO) and the MS2 phage was obtained from ATCC (15597-B1). Viral stock and titration experiments for MNV-1 and MS2 were performed on RAW 264.7 cells (ATCC TIB-71) and *Escherichia coli* strain Hfr K12 (ATCC 23631), respectively. Growth conditions and titration procedures for both viruses have been described elsewhere (ISO/TC 147/SC 4Microbiological methods, 1995; Ogorzaly & Gantzer, 2006; Wobus et al., 2004). MNV genome detection was performed by RT-qPCR (Belliot et al., 2008). RT-qPCR were performed for MNV detection before and after the virucidal assay.

For each assay, 100 µl of viruses were aliquoted in five 20 µl drops on glass coverslips and desiccated in a controlled atmosphere chamber with 10% relative humidity (RH) at 23–25 °C (Colas de la Noue et al., 2014). A saturated solution of lithium chloride was used to reach 10% RH in the chamber. An electric fan was also placed in the chamber to speed up the drying process, which was reduced to 1 h. It is worth noting that minimal or no titer reduction was observed following the dehydration step (data not shown). For each assay, viruses were dried on a surface of 1 cm^2/assay. Each experiment was conducted in quadruplicate with 4 assays for the virucidal testing and 4 assays for the negative control. For both viruses, viral titers were estimated by plaque assay and titers were given in Log_{10} pfu.

Virus samples either destined for the assay or negative controls were dried at the same time. Negative controls and tests were put in the same locations during the assays. Negative controls were hermetically sealed in a petri dish for protection against the hydrogen peroxide mist.

Virucidal Experiments and Virus Recovery

The ambulance used for the study was a Master L2H2 van from Renault SA (Boulogne-Billancourt, France), equipped as a mobile intensive care unit for pediatrics by Petit-Picot SAS (Joué-les-Tours, France). The vehicle was therefore equipped with an ambulance stretcher (Fig. 1). The virus samples were positioned on the floor beneath the stretcher, on the stretcher itself, and on built-in shelves at the top of the cabin. The machine used for the experiment was a nebulizer under the brand Nocospray® from Oxy'Pharm® (Champigny-sur-Marne, France). The nebulizer produces a hydrogen peroxide mist with an average particle diameter of 5 µm. The nebulizer was positioned on the floor in the corner of the cabin. The nebulizer was used following manufacturer's recommendations, while the ambulance was parked outside the garage. The average temperature in the cabin during the assay was 25 °C at mid-day. The nebulization time recommended by the manufacturer to reach 5 g/m^3 hydrogen peroxide for the volume of the cabin (14 m^3) was 4.5 min followed by 1 h of exposure before opening the vehicle. One-hour contact allowed the hydrogen peroxide to fully decompose into water and oxygen. Color-based test strip chemical indicators were placed all over the cabin before starting the spraying process. The test strips were used to determine whether the hydrogen peroxide was standing long enough in the cabin. If so, the test strip turned from brown to dark blue. Surface sampling was performed on the stirring bar, knob, and handles before and after nebulization. Each swab was rinsed with 2 ml of SARS-CoV-2 inactivating buffer prior to detection of the SARS-CoV-2 genome using RT-qPCR using the SARS-CoV-2 real-time fluorescent RT-PCR kit from BGI (Shenzhen, PRC). Similar virucidal experiments were performed in a waiting room in the infectious disease department following the same procedure described above. Air vents were shut off for better treatment efficiency. Control and virucidal assays were placed on the floor, on a tabletop, and suspended near the ceiling. The nebulization time was set to 13 min for a total volume of 44 m^3 including the restroom in the back of the waiting room.

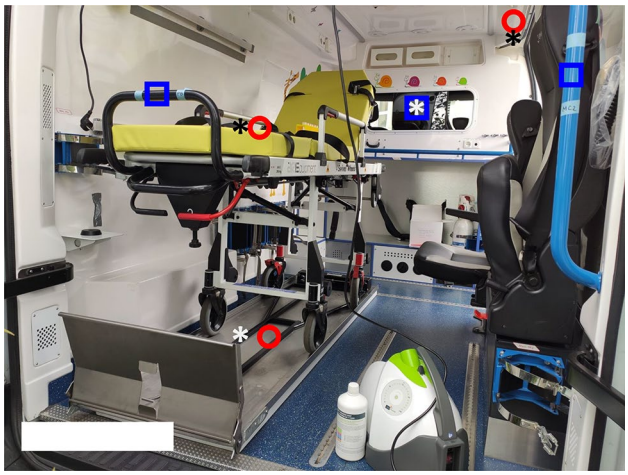

Fig. 1 Cabin interior of an emergency pediatric ambulance with nebulizer located at the bottom right corner. Viral surrogates were placed on top shelf, under, and beneath the stretcher (red circle). Blue square indicates the sweep for the detection of SARS-CoV-2 genome. White and black asterisks show locations of the test strips. Certain spots designated for test strips and sweep are not visible on the picture

Swabbing of the room was performed for the detection of SARS-CoV-2 genome before and after the treatment.

Results

Genome Detection of SARS-CoV-2 and MNV

The RT-qPCR used for the SARS-CoV-2 detection is a duplex system targeted against the ORF-1ab gene of SARS-CoV-2 and the detection of a reference gene as an internal control. Swab samples on which the Cq (quantification cycle) was above 38 were considered negative following manufacturer's recommendations. No trace of SARS-CoV-2 genome was detected in environmental samples before and after nebulization. However, traces of human DNA were logically detected on the steering wheel of the ambulance (Cq 29.4). The detection of human DNA validated our sampling method for nucleic acid recovery. For the waiting room, fragments of human DNA were detected on the doorknob of the entry door (Cq 32.0) and the window handle (Cq 29.87) before nebulization and on the restroom door handle (Cq 27.4) and the tabletop (Cq 31.8) after nebulization. These data suggest that the concentration of hydrogen peroxide used might be too low for the complete degradation of the nucleic acid. To determine whether viral genomes persisted following hydrogen peroxide treatment, we performed MNV-specific RT-qPCR. The MNV genome target was detected in samples taken from the upper, middle, and lower areas of the waiting room and the ambulance with Cq comprised between 18 and 22 (data not shown). The presence of MNV genome confirmed that hydrogen peroxide treatment was not deleterious for viral RNA and was not a good indicator of the infectious status of the viral particles although low Cq values suggest the presence of infectious particles (Kampf et al., 2021; Richards, 1999).

Infectivity Assay for MNV and MS2 by Plaque Assay

In the last part of the study, the virucidal performance of hydrogen peroxide was assessed using MS2 and MNV, which have been tittered by the plaque assay method. Here, we performed virucidal assays in settings (i.e., hospital waiting room and cabin ambulance) likely to be contaminated by viruses during a sanitary crisis, like the COVID-19 epidemic. For both settings, the virucidal efficacy of the nebulization was less effective on the floor than on the top shelf, tabletop, and stretcher (Table 1). A 4 $\log_{10}$ titer reduction of the MS2 was only observed for the stretcher in the ambulance, while titer reductions ranged between 2.47 ± 0.22 and 3.24 ± 0.15 $\log_{10}$, with the control titers at 9.14 ± 0.11 and 8.89 ± 0.10 $\log_{10}$ pfu/ml for ambulance and waiting room, respectively. A 4 $\log_{10}$ titer reduction of the MNV was only observed for the stretcher and the top shelves in the ambulance, while titer reduction was not higher than 3.82 ± 0.17 $\log_{10}$ pfu/ml with control titers at 6.25 ± 0.39 and 6.06 ± 0.20 log pfu/ml for the ambulance and waiting room, respectively.

Discussion

The virucidal activity of the nebulization system is rather disappointing if we consider that a 4 $\log_{10}$ reduction titer is required by norm EN14476. However, the inactivation of 99.99% of the viral load largely depends upon the initial titer of the virus used in the assay. It is thus more difficult to inactivate 99.99% of a viral load that represents over a billion of viral particles, as exemplified for MS2. We then determined the virucidal efficacy of hydrogen peroxide for a surface of 1 cm^2. More than 10^6 infectious MNV particles per cm^2 were destroyed in both settings. More than 10^8 and 10^9 infectious MS2 particles per cm^2 were inactivated in the waiting room and the ambulance, respectively. Despite the fact that we did not observe a 4 $\log_{10}$ titer reduction, we assume that the virucidal activity of a hydrogen peroxide mist would fit the need

Table 1 Virucidal activity of hydrogen peroxide mist

	MS2[a]		MNV[a]	
	Reduction in titer [$\text{Log}_{10} \pm$ SD pfu/ml (% reduction)]	Titer reduction per cm^2 ($\text{Log}_{10} \pm$ SD pfu/cm^2)	Reduction in titer [$\text{Log}_{10} \pm$ SD pfu/ml (% reduction)]	Titer reduction per cm^2 ($\text{Log}_{10} \pm$ SD pfu/cm^2)
Floor (ambulance)	2.47 ± 0.22 (99.66)	9.23 ± 0.02	4.33 ± 0.41 (> 99.99)	6.33 ± 0.41
Stretcher (ambulance)	4.22 ± 0.15 (> 99.99)	9.14 ± 0.12	4.08 ± 0.33 (> 99.99)	6.20 ± 0.50
Top shelf (ambulance)	2.79 ± 0.32 (99.84)	9.04 ± 0.04	3.87 ± 0.41 (99.98)	6.21 ± 0.35
Floor (waiting room)	3.24 ± 0.15 (99.94)	8.86 ± 0.03	3.82 ± 0.17 (99.98)	6.24 ± 0.03
Tabletop (waiting room)	3.18 ± 0.33 (99.93)	9.01 ± 0.07	3.42 ± 0.15 (99.96)	6.09 ± 0.09
Ceiling (waiting room)	2.66 ± 0.09 (99.78)	8.79 ± 0.04	2.94 ± 0.13 (99.89)	5.85 ± 0.18

SD standard deviation

for surface decontamination, reducing the risk of transmission in hospital settings, provided the room is confined (sealed door and sealed air vent) and the access is forbidden for 1.5 to 2 h. Given the promising results we obtained with sturdy viruses, such as MS2 and MNV, we believe that similar treatment will also be very efficient at destroying other more fragile viruses. We acknowledge that it would have been more pertinent to study the virucidal efficacy of hydrogen peroxide against SARS-CoV-2 or other human coronavirus surrogate in this time of COVID epidemic. That being said, we chose to use MS2 and MNV models rather than the coronavirus to avoid a potential risk of contamination with other coronaviruses at the time of the study when SARS-CoV-2 was heavily circulating in the population (Pastorino et al., 2020).

In summary, the main advantage of the nebulization system is the efficient treatment of areas that are unattainable with a regular cleaning procedure. Because hydrogen peroxide is unstable and decomposes into water and oxygen, the risk of oxidizing fragile medical equipment is minimal. One-hour contact with hydrogen peroxide allows complete decomposition of the active compounds, thus reducing the risk of exposure for patients and health professionals. It should be taken into account that a mist nebulization system is not as efficient as a vapor production system in laboratory settings, especially for bacterial contamination (Fu et al., 2012; Holmdahl et al., 2011), but nebulization apparatuses are more affordable and offer the best efficacy/cost ratio, as previously stated (Otter et al., 2013).

Conclusion

Efficacy, cost, fire safety, and the risk of damaging medical equipment (i.e., corrosion, premature wearing of plastic surfaces) should be taken into account when choosing a disinfection system (Otter et al., 2020). Therefore, a nebulization system is particularly advantageous for the disinfection of an ambulance cabin or any confined and cluttered space, provided all apertures like air vents are hermetically closed. Further studies in real-life conditions will be required to optimize the position of the nebulizer and to determine how often disinfection should be performed and to establish the overall benefits for staff.

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Data Availability The data are available upon reasonable request.

Declarations

Conflict of interest Romain Rouleau is an employee of Oxy'Pharm, which manufactures and sells the nebulizer under the name "Nocospray." The other authors declare no conflict of interest.

References

Belliot, G., Lavaux, A., Souihel, D., Agnello, D., & Pothier, P. (2008). Use of murine norovirus as a surrogate to evaluate resistance of human norovirus to disinfectants. *Applied and Environmental Microbiology, 74*, 3315–3318.

Cohen, J., & Normile, D. (2020). New SARS-like virus in China triggers alarm. *Science, 367*, 234–235.

Colas de la Noue, A., Estienney, M., Aho, S., Perrier-Cornet, J. M., de Rougemont, A., Pothier, P., Gervais, P., & Belliot, G. (2014). Absolute humidity influences the seasonal persistence and infectivity of human norovirus. *Applied and Environment Microbiology, 80*, 7196–7205.

Fu, T. Y., Gent, P., & Kumar, V. (2012). Efficacy, efficiency and safety aspects of hydrogen peroxide vapour and aerosolized hydrogen peroxide room disinfection systems. *Journal of Hospital Infection, 80*, 199–205.

Goyal, S. M., Chander, Y., Yezli, S., & Otter, J. A. (2014). Evaluating the virucidal efficacy of hydrogen peroxide vapour. *Journal of Hospital Infection, 86*, 255–259.

Holmdahl, T., Lanbeck, P., Wullt, M., & Walder, M. H. (2011). A head-to-head comparison of hydrogen peroxide vapor and aerosol room decontamination systems. *Infection Control and Hospital Epidemiology, 32*, 831–836.

ISO/TC 147/SC 4 Microbiological methods. 1995. Water quality—Detection and enumeration of bacteriophages-part 1: Enumeration of F-specific RNA bacteriophages. In I.O.f. (Ed.), *Standardisation*. https://www.iso.org/committee/52944.html

Kampf, G., Lemmen, S., & Suchomel, M. (2021). Ct values and infectivity of SARS-CoV-2 on surfaces. *Lancet Infectious Diseases, 21*, e141.

Ogorzaly, L., & Gantzer, C. (2006). Development of real-time RT-PCR methods for specific detection of F-specific RNA bacteriophage genogroups: Application to urban raw wastewater. *Journal of Virological Methods, 138*, 131–139.

Otter, J. A., Donskey, C., Yezli, S., Douthwaite, S., Goldenberg, S. D., & Weber, D. J. (2016). Transmission of SARS and MERS coronaviruses and influenza virus in healthcare settings: The possible role of dry surface contamination. *Journal of Hospital Infection, 92*, 235–250.

Otter, J., Yezli, S., Barbut, F., & Perl, T. M. (2020). An overview of automated room disinfection systems: When to use them and how to choose them. In J. Walker (Ed.), *Decontamination in hospitals and healthcare* (2nd ed., pp. 323–369). Public Health England.

Otter, J. A., Yezli, S., Perl, T. M., Barbut, F., & French, G. L. (2013). The role of "no-touch" automated room disinfection systems in infection prevention and control. *Journal of Hospital Infection, 83*, 1–13.

Pastorino, B., Touret, F., Gilles, M., de Lamballerie, X., & Charrel, R. N. (2020). Prolonged infectivity of SARS-CoV-2 in fomites. *Emerging Infectious Diseases, 26*(9), 2256.

Richards, G. P. (1999). Limitations of molecular biological techniques for assessing the virological safety of foods. *Journal of Food Protection, 62*, 691–697.

Tuladhar, E., Terpstra, P., Koopmans, M., & Duizer, E. (2012). Virucidal efficacy of hydrogen peroxide vapour disinfection. *Journal of Hospital Infection, 80*, 110–115.

van Doremalen, N., Bushmaker, T., Morris, D. H., Holbrook, M. G., Gamble, A., Williamson, B. N., Tamin, A., Harcourt, J. L., Thornburg, N. J., Gerber, S. I., Lloyd-Smith, J. O., de Wit, E., & Munster, V. J. (2020). Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1. *New England Journal of Medicine, 382*, 1564–1567.

Wobus, C. E., Karst, S. M., Thackray, L. B., Chang, K. O., Sosnovtsev, S. V., Belliot, G., Krug, A., Mackenzie, J. M., Green, K. Y., & Virgin, H. W. (2004). Replication of Norovirus in cell culture reveals a tropism for dendritic cells and macrophages. *PLoS Biol, 2*, e432.

Yeargin, T., Buckley, D., Fraser, A., & Jiang, X. (2016). The survival and inactivation of enteric viruses on soft surfaces: A systematic review of the literature. *American Journal of Infection Control, 44*, 1365–1373.