SARS-CoV, influenza A and syncitial respiratory virus resistance against common disinfectants and ultraviolet irradiation

Article *in* Journal of preventive medicine and hygiene · March 2004

CITATIONS
2

READS
8,446

9 authors, including:

Filippo Ansaldi
Università degli Studi di Genova
327 PUBLICATIONS **4,404** CITATIONS
SEE PROFILE

Paolo Durando
Università degli Studi di Genova
150 PUBLICATIONS **2,693** CITATIONS
SEE PROFILE

Laura Sticchi
Università degli Studi di Genova
91 PUBLICATIONS **1,237** CITATIONS
SEE PROFILE

Roberto Gasparini
Università degli Studi di Genova
293 PUBLICATIONS **3,450** CITATIONS
SEE PROFILE

SARS-CoV, influenza A and syncitial respiratory virus resistance against common disinfectants and ultraviolet irradiation

F. ANSALDI, F. BANFI, P. MORELLI, L. VALLE, P. DURANDO, L. STICCHI, S. CONTOS, R. GASPARINI, P. CROVARI
Department of Health Sciences, University of Genoa

Key words

SARS-CoV • Disinfectants • Virucidal efficacy

Summary

To evaluate the virucidal efficacy of six commonly used chemical disinfectants, and ultraviolet radiation (U.V.) against SARS-CoV and compare it to the resistance of other airborne viruses, such as Influenza and Respiratory Sincytial Virus, a salt solution containing a standard concentration of cell-grown viruses, was mixed with a larger volume of different disinfectants at their use dilution and the mixtures were held for a defined contact time. The virucidal efficacy of disinfectants and U.V. was evaluated by infectivity, detected by inoculation of samples in suitable cell culture and genome integrity, detected by nested RT-PCR. SARS-CoV as well as RSV seem to be sensible to the different disinfectants tested in our study and U.V. radiation, while influenza virus appear to be more resistant in particular to the action of chlorhexidine digluconate and benzalkonium-chloride. In consideration of the possible infectious role of SARS-CoV RNA, sodium hypochlorite 0.1% appear to be the more efficacious disinfectant for surfaces and hands potentially contaminated with respiratory viruses and SARS-CoV.

Background

In late 2002 a new acute and often severe respiratory illness (SARS) emerged in Southern China and spread to distant areas within a very short period of time [1]. The aetiological agent for SARS is a novel Coronavirus called SARS-CoV, that H has never been described both in human and animals previously [2-4]. CDC in according to WHO, reports that a total of 8,098 people in 32 countries became sick with this new illness during 2003 outbreak. Of these 774 died, with a case-fatality rate of about 10% that reach 50% in > 64 year age group [5]. On 5 July 2003 WHO announced that SARS epidemic was over. During outbreak the major percentage of cases was among health-care workers because the main way of transmission seems to be by close person to person contact during the peak of viral shedding that occurs about ten days after onset of symptoms. Since epidemic was over there have been a number of laboratory confirmed SARS-CoV infections resulting from laboratory accidents and from exposure to animal sources or environmental contamination [6].

In consideration of the high efficiency in nosocomial transmission and the occurrence of infection in laboratory, to know the resistance of this new virus in environment and towards common disinfectants appear to be a fundamental tool to choose the correct control measures. The aim of this work is to evaluate the virucidal efficacy of chemical disinfectants, used to clean surfaces, and ultraviolet radiation (U.V.) against SARS-CoV and compare it to the resistance of other airborne enveloped viruses, such as Influenza and Respiratory Sincytial Virus (RSV).

Materials and methods

Study design

A known quantity (1ml) of salt solution containing a standard concentration of cell-grown virus, was mixed with a larger volume (2 ml) of different disinfectants under evaluation at their use dilution. The mixtures were held for a defined contact time (30 seconds, 1 minute, 2 min, 5 min, 15 min, 30 min) at controlled temperature (18°C) and humidity (40%). The virucidal activity of the test products was arrested immediately by adding a neutralizer (Sodium thiosulphate for sodium hypoclorite; sodium hydroxide for acid peracetic) or diluting the germicide-mixture. For evaluate the physical effects of U.V. irradiation (40mW/cm^2), the salt solutions containing viruses, were distributed on a plate of 20 cm^2 and exposed to U.V. irradiation for above-mentioned contact time. A positive control containing a standard concentration of cell-grown virus without disinfectant was used in every run. Preliminary tests were made to evaluate the sensibility of cells against disinfectants and neutralizer/disinfectant solution for selecting the right dilution that did not damage the cells.

Disinfectant

In this study SARS-CoV, Influenza virus and RSV were tested with six common disinfectants at the following dilutions:

– Sodium Hypochlorite 0.01% – 0.05% – 0.1%;
– Ethanol 70%;
– Benzalkonium-chloride 1%;
– Chlorhexidine digluconate 1%;

– 2-benzil-chlorophenol 2%;
– Peracetic acid 0.035%.

Outcomes

To evaluate the virucidal efficacy of disinfectants and U.V. we considered two parameters: (i) infectivity, detected by inoculation of samples in suitable cell culture, i.e. Vero E6 for SARS-CoV, MDCK for influenza virus, HEp2c for RSV [7]; (ii) genome integrity, detected by nested RT-PCR for SARS-CoV and multiplex nested RT-PCR.

Laboratory methods

All practice of experiment with SARS-CoV were made in laboratory BSL3 as recommended by WHO [6].

Isolation culture

Conventional viral culture was performed by inoculating 0.3 ml of each specimen (virus-disinfectant mixture) into Vero E6, MDCK and HEp-2 cells for SARS-CoV, influenza and RSV isolation, respectively [7]. Virus detection was performed by PCR indirect immuno fluorescence and hemagglutination test for SARS-CoV RSV, and influenza, respectively [8].
RNA was extracted using QIA techniques, following the manufacturer's instructions (Rneasy Minikit, Qiagen, Valencia, CA). Amplification of specific RSV and Influenza virus sequence was performed by multiplex RT-nested PCR (Influenza/RSV multiplex, Amplimedical S.p.A., BIOLINE, Italy). SARS-CoV was detected by a commercial RT-PCR kit (SARS-CoV RNA polymerase, Amplimedical S.p.A., BIOLINE, Italy).

Results

The results of preliminary test of citotoxicy showed that 0.2 ml ethanol 70%, benzalkonium-chloride 1%, chlorhexidine digluconate 1% in 2 ml cell culture medium did not significantly damage the cell monolayer. Sodium hypochlorite 10% and peracetic acid 0.035% determined a strong cytotoxic effect on cells, that disappeared when disinfectant-neutralizer mixture was used. Also 2-benzil-chlorophenol 2% showed a strong cytotoxic effect on cells that declined and disappeared when a 1:3 and 1:10 dilution was inoculated, respectively. For this reason after the end of contact time the mixture virus-disinfectant was diluted 1:10 before inoculation.
In Table I last virus-disinfectant contact times resulting positive by cell culture and PCR are reported. Peracetic acid 0.035%, ethanol 70% and sodium hypochlorite 0.05% showed to inhibit viral replication in cell culture after < 2' contact time, while viral genome seems to be intact after prolonged exposition (30').
Sodium hypochlorite 0.1%, 2-benzil-chlorophenol 2% and U.V. have a stronger virucidal effect: they inhibit completely viral replication and damage viral genome after < 2 minutes of exposition. In particular, sodium hypochlorite 0.1% showed the more rapid action: after 1' of contact, influenza virus, RSV and SARS-CoV were not able to replicate and their genome integrity was lost. This data is confirmed by electronic microscopy: the viral structures appear completely destroyed with lost of spikes and envelope integrity after 1' of exposition (data not shown).
Chlorhexidine digluconate 1% and benzalkonium-chloride 1% showed a similar pattern of virucidal efficacy: they inhibited RSV replication after 1' of contact, while prolonged exposition (30') did not affect infectivity of influenza virus. SARS-CoV replication was inhibited by 5' exposition to benzalkonium-chloride 1%, while all experimental samples collected at different time resulted culture negative after contact with chlorhexidine digluconate 1%. Influenza virus, RSV and SARS-CoV RNA is still detectable after 30 minutes of contact time

Tab. I. Last contact time resulting positive by cell culture and PCR.

Disinfectant	Virus					
	Influenza virus		RSV		SARS-CoV	
	Culture	PCR	Culture	PCR	Culture	PCR
Peracetic acid 0.035%	–	30′	–	30′	–	30′
Ethanol 70%	2′	30′	–	30′	–	30′
Sodium Hypochlorite 0.01%	30′	30′	30′	30′	30′	30′
Sodium Hypochlorite 0.05%	30′	30′	1′	2′	1′	30′
Sodium Hypochlorite 0.1%	–	30î	1′	1′	–	30î
Chlorhexidine digluconate 1%	30′	30′	1′	30′	–	30′
2-benzil-chlorophenol 2%	–	–	–	2′	–	2′
Benzalkonium– chloride 1%	30′	30′	1′	30′	5′	30′
UV irradiation	1′	1′	1′	1′	2′	2′

Legend: – negative after 30′ contact time.

with chlorhexidine digluconate 1% and benzalkonium-chloride 1%.

Discussion

Since when SARS epidemic appeared, SARS-CoV has shown high efficiency in nosocomial transmission and high risk of virus spread in laboratory setting. The new outbreak and the emergence of new respiratory viruses such as avian influenza virus and human metapneumovirus, underline the needs to improve the knowledge about the resistance of these microorganisms in environment and towards common disinfectants for choosing the correct control measures.

Studies on SARS-CoV demonstrated that the virus is more stable at room temperature than the previously known human Coronavirus [9 10]. Preliminary study of WHO laboratory network showed that the virus survive for up to 48 hours on plastic surfaces and up to 4 days in diarrhoea. Nevertheless, the virus loses infectivity after exposure to different commonly used disinfectants and fixatives [9 10]. Our study confirmed that SARS-CoV is quite sensible to common disinfectants. In fact, SARS-CoV appears to be completely inactivated by disinfectants such as acid peracetic, ethanol 70%, sodium hypochlorite 0.05% and 0.1%, chlorhexidine digluconate 1% and 2-benzil-chlorophenol 2% after < 1' exposition, while a longer contact time with benzalkonium-chloride 1% is necessary to inhibit the replication in culture. PCR findings are of interest, not only to demonstrate the complete destruction of viral structure, but also as RNAs of some Coronaviruses are infectious [11 12]. As regards as human infecting SARS-CoV, this biological property has not been demonstrated yet. Only sodium hypochlorite 0.1% and 2-benzil-chlorophenol 2% are rapidly efficacious in destruction of viral RNA at an undetectable level after < 2' contact time, while prolonged exposition with other disinfectants seems not alter RNA integrity.

RSV showed a resistance pattern rather similar to SARS-CoV and appears to be completely inactivated by disinfectants such as acid peracetic, ethanol 70%, sodium hypochlorite 0.05% and 0.1%, chlorhexidine digluconate 1% and 2-benzil-chlorophenol 2% and benzalkonium-chloride 1% after < 1' exposition. The RSV weakness was confirmed by on-field studies on disinfection and survival studies [13 14].

Influenza can not replicate after short exposure to acid peracetic, ethanol 70%, sodium hypochlorite 0.05% and 0.1%, and 2-benzil-chlorophenol 2%, while it showed high stability after contact with chlorhexidine digluconate 1% and benzalkonium-chloride 1%. The complete inefficacy of benzalkonium-chloride 1% is not completely unexpected as quaternary ammonium were shown to be very effective against herpes virus but totally ineffective against non-enveloped virus and influenza virus, as demonstrated also Moldenhauer [15]. On the other hand, chlorhexidine showed to be totally ineffective against rotavirus, enveloped virus such as influenza virus [16].

U.V. radiation, instead, damage nucleic acid because RNA adsorb UV radiation of germicidal wavelengths and is the major targets of the powerful antimicrobal effects of these type of electromagnetic radiation. In according to this, irradiation of UV for few minutes (1'-2') on the virus in culture medium resulted in the destruction of viral infectivity.

In conclusion, SARS-CoV as well as RSV seem to be sensible to the different disinfectants tested in our study and U.V. radiation, while influenza virus appear to be more resistant in particular to the action of chlorhexidine digluconate and benzalkonium-chloride. In consideration of the possible infectious role of SARS-CoV RNA, sodium hypochlorite 0.1% appear to be the more efficacious disinfectant for surfaces and hands potentially contaminated with respiratory viruses and SARS-CoV.

References

1 World Health Organization (WHO). *Chronology of travel recommendations, areas with local transmission*. Wkly Epidemiol Rec 2003;78:258-9.

2 Peiris JSM, Lai ST, Poon LLM, Guan Y, Yam LYC, Lim W, et al. *Coronavirus as a possible cause of severe acute respiratory syndrome*. Lancet 2003;361:1319-25.

3 Drosten C, Gunther S, Preiser W, van der Werf S, Brodt H-R, Becker S, et al. *Identification of a novel Coronavirus in patients with severe acute respiratory syndrome*. New Engl J Med 2003;348:1967-76.

4 Ksiazek TG, Erdman D, Goldsmith CS, Zaki SR, Peret T, Emery S, et al. *A novel Coronavirus associated with severe acute respiratory syndrome*. New Engl J Med 2003;348:1953-66.

5 World Health Organization. *Update 95-SARS: Chronology of the serial killer*. http://www.who.int/csr/don/2003_07_04/en/.

6 World Health Organization. *WHO SARS International Reference and Verification Laboratory Network: Policy and Procedures in the Inter-Epidemic Period*. http://www.who.int/resources/publications/en/SARSreferencelab.pdf.

7 Zambon M. *Laboratory diagnosis and techniques*. In: Nicholson KG, Webster RG, Hay AJ, eds. *Textbook of influenza*. London: Blackwell Science Ltd 1998:291-313.

8 Manuguerra JC, Hannoun C. *Grippe et autres viroses respiratoires. Surveillance et diagnostic de Laboratoire*. Paris: Institut Pasteur, 1999:208-237.

9 Berger A, Drosten C, Doerr HW, St¸rmer M, Preiser W. *Severe acute respiratory syndrome (SARS)-paradigm of an emerging viral infection*. J Clin Virol 2004;29:13-22.

10 Duan SM, Zhao XS, Wen RF, Huang JJ, Pi GH, Zhang SX, et al. *Stability of SARS Coronavirus in human specimens and environment and Its sensitivity to heating and U.V. irradiation*. Biomed Environ Sci 2003;16:246-55.

11 Schochetman G, Stevens RH, Simpson RW. *Presence of infectious polyadenylated RNA in coronavirus avian bronchitis virus*. Virology 1977;77:772-82.

[12] Lomniczi B. *Biological properties of avian coronavirus RNA*. J Gen Virol 1977;36:531-3.

[13] Platt J, Bucknall RA. *The disinfection of respiratory syncytial virus by isopropanol and a chlorhexidine-detergent handwash*. J Hosp Infect 1985;6:89-94.

[14] Hall CB, Douglas RG, Geiman JM. *Possible transmission by fomites of respiratory syncytial virus*. J Infect Dis 1980;141:98-102.

[15] Moldenhauer D. *Quantitative evaluation of effects of disinfectants against viruses in suspension experiments*. Zentralbl Mikrobiol Hyg 1984;179(6):544-54.

[16] Bellamy K, Alcock R, Babb JR. *A test for the assessment of hygienic hand disinfection using rotavirus*. J Hosp Infect 1993;24:201-10.

■ Correspondence: Filippo Ansaldi, Department of Health Sciences, University of Genoa, Via Pastore 1, 16132 Genoa, Italy. Tel. +39 010 3533002. E-mail: filippo.ansaldi@unige.it