

Chemical disinfection of human rotavirus-contaminated inanimate surfaces

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SUMMARY

Fomites may play a role in the transmission of rotavirus infections, and in view of this, 27 disinfectants were evaluated for their ability to inactivate human rotavirus (HRV) on contaminated non-porous inanimate surfaces. Disks of stainless steel, glass and two types of plastics were contaminated with about 10^7 plaque-forming units of HRV suspended in faecal matter. The inoculum was allowed to dry and an equal volume of the product under test was applied to the contaminated surface. After contact for 1 min, the action of the disinfectant was stopped by dilution. Surviving infectious virus on the disks was determined by plaque assay in MA-104 cells. A product was considered to be effective if it could reduce the virus titre by at least $3 \log_{10}$. Only 33.3% (9/27) of the formulations tested proved to be effective. Further testing of the effective products, which included antiseptics, instrument soaks and hard-surface disinfectants, showed that all of them could, in fact, reduce the virus titre on contaminated surfaces by at least $6 \log_{10}$. These findings show the relative resistance of HRV to a wide range of chemical disinfectants in common use, and also emphasize the need for a more thorough evaluation of the virucidal potential of formulations regularly employed in attempts to prevent and control outbreaks of rotaviral diarrhoea.

INTRODUCTION

The preceding paper (Springthorpe *et al.* 1986) investigated the efficacy of disinfectant formulations on human rotavirus (HRV) in suspension. This paper examines their activity in disinfecting contaminated non-porous surfaces.

Rotaviruses of human origin (HRV) have been shown to survive for prolonged periods in water (Raphael, Sattar & Springthorpe, 1985), air (Ijaz *et al.* 1985) and on environmental surfaces (Moe & Shirley, 1982; Sattar *et al.* 1986). Consumption of sewage-contaminated drinking water has resulted in outbreaks of rotavirus diarrhoea (Hung *et al.* 1984), and there is some evidence to suggest that faecally contaminated hands, fomites and environmental surfaces may act as vehicles in the spread of nosocomial rotavirus infections (Sattar *et al.* 1986).

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This is further substantiated by (a) the ability of these viruses to survive for several days on a variety of inanimate surfaces (Moe & Shirley, 1982; Sattar *et al.* 1986), (b) the recovery of infectious rotavirus particles from articles of common use in a day-care centre (Keswick *et al.* 1983), (c) the detection of rotavirus antigens in handwashings of attendants of patients with rotavirus diarrhoea (Samadi, Huq & Ahmed, 1983) and (d) the rapid spread of the disease by clustering, close contact or interaction with infected persons (Holzel *et al.* 1980). Apart from the direct contamination with infected faecal matter, indirect contamination of surfaces through contaminated waters or rotavirus-containing aerosols may also be important.

Earlier studies (Springthorpe *et al.* 1986) have shown that only a limited number of chemical disinfectants were effective in the inactivation of HRV in suspension tests. However, there is no published information on the efficacy of commercially available products in the proper disinfection of rotavirus-contaminated environmental surfaces, neither is there an established standard procedure for such tests.

This study was begun with the following objectives: (i) to develop a simple and efficient protocol for the assessment of surface disinfection; (ii) to evaluate the suspension test as a reliable pre-screen of disinfectants for use on surfaces and (iii) to identify which classes of chemical disinfectants are capable of efficient surface disinfection.

MATERIALS AND METHODS

Cells and virus

The Wa strain of human rotavirus (Wyatt *et al.* 1980) and the MA-104 line of embryonic rhesus monkey kidney cells were used throughout this study. The techniques used for cell culture and virus growth (Sattar *et al.* 1986) and virus quantitation (Raphael, Sattar & Springthorpe, 1985) have already been described.

Radio-labelled virus

¹⁴C-labelled HRV was prepared as follows. Two-day-old monolayers of MA-104 cells in 490 cm² plastic roller culture flasks (Corning Glass Works, Corning, NY, USA) were washed to remove all traces of FCS and were starved overnight in Earle's balanced salt solution (EBSS) containing one-twentieth the concentration of amino acids and vitamins usually present in MEM. The cells were then infected and, after a virus adsorption period of 3 h, maintained in EBSS supplemented with one-twentieth the normal MEM amino acid and vitamin concentrations, 1 μCi/ml of L-[U-¹⁴C]amino acids (Amersham, Oakville, Ontario, Canada), 0.1 μCi/ml D-[U-¹⁴C]glucosamine (Amersham) and 5 μg/ml of trypsin (NBC). After 72 h incubation, when > 90% of the cells had lysed, the labelled virus was harvested and concentrated as described previously (Sattar *et al.* 1986). The concentrate was then resuspended in tryptose phosphate broth (TPB), purified on an isopycnic CsCl gradient and the radioactive fractions corresponding to single rotavirus particles (density = 1.35–1.39 approx.) pooled and dialysed to remove any remaining low-molecular-weight ¹⁴C-labelled material.

Faecal samples

To simulate the conditions generally encountered in nature, the virus was suspended in four different faecal samples from laboratory-confirmed cases of

rotaviral diarrhoea. The faecal samples were screened and diluted as described previously (Sattar *et al.* 1986).

Surfaces tested as carriers

The following four types of carriers were selected as representative of non-porous inanimate surfaces found in areas of domestic and institutional settings where HRV contamination is most likely to occur, and are commonly subject to disinfection: (a) glass, (b) stainless steel, (c) smooth plastic (Milar) and (d) rough plastic (vinyl). Glass cover-slips (Chance Proper Ltd., Warley, England) of 1 cm diameter were purchased from Johns Scientific (Mississauga, Ontario, Canada). Sheets of the other three types of materials were obtained from local retail outlets and disks of 1 cm diameter were cut from them. Prior to contamination with the virus suspension, the disks were cleaned by sonication for 10 min in a detergent solution followed by thorough rinsing in running deionized water. They were then soaked for 10 min in 95 % ethanol and air dried before being placed individually in the wells of a 24-well plastic cell-culture plate (Costar).

Virus elution from disks

TPB was selected as an eluent to recover virus from the experimentally contaminated disks because it was non-toxic to MA-104 cells, harmless to rotaviruses (Ramia & Sattar, 1980) and helped to reduce the cytotoxicity of the disinfectants. Furthermore, experiments with radiolabelled rotavirus showed that TPB could consistently elute between 90 % and 100 % of the added virus from disks of the four surface types.

Disinfectants

Twenty-seven different disinfectant products and formulations were tested in this study. This selection was based on their performance in the suspension test (Springthorpe *et al.* 1986), the manufacturer's recommended use for surface disinfection, and/or the nature of their active ingredient(s). Each was numbered for convenience and their listed active ingredients indicated by three-lettered codes. The definition of these codes is given in Table 1, and their formulations in Table 2.

Test procedure

Each disk was contaminated by placing on its surface 20 μ l of the virus suspended in faeces and containing about 10^7 plaque forming units of HRV, strain Wa. The inoculum was then allowed to air-dry by keeping the disks for 2 h in a hood with a vertical laminar flow of HEPA-filtered air.

A 20 μ l volume of the disinfectant under test, at the manufacturer's recommended dilution, was applied directly over the entire surface of each virus-contaminated disk. The control disks received the same amount of TPB instead. After a contact time of 1 min at room temperature (22 ± 2 °C) a 980 μ l volume of TPB was added to each well containing the disks. This was done in order to stop the action of the disinfectant by dilution. Elution of the virus from the disks was achieved by placing the plates with the disks in a sonic bath (Branson 52; O. H. Johns Scientific, Toronto, Ontario, Canada) for 10 min. Reaction mixtures which were

Table 1. Abbreviations for listed ingredients in disinfectant formulations

ACE	acetone	NPE	nonylphenoxypolyethylenecethanol-iodine complex
BCP	<i>o</i> -benzyl-chlorophenol	NPP	nonylphenoxypolyethoxyethanol-iodine
BPP	butoxypolypropoxypolyethoxyethanol-iodine complex	NTR	nitrotriacetic acid (as sodium salt)
CDP	2,4,4'-trichloro-2'-hydroxydiphenyl ether (or 5-chloro-2(2,4-dichlorophenoxy) phenol)	OAA	organic acid activator
CET	cetrimide	OPP	<i>o</i> -phenylphenol
CFT	chloramine-T	PDG	polymeric diguanide hydrochloride
CHA	chlorhexidine acetate	PER	peracetic acid
CHG	chlorhexidine gluconate	PHA	phosphoric acid
CHX	4-chloro-3,5-xyleneol	PNP	polyethoxypolypropoxypolyethoxyethanol-iodine complex
CIT	sodium citrate	POV	povidone-iodine complex
DBS	dodecylbenzenesulphonic acid	PRG	propylene glycol
EAL	ethyl alcohol	SBC	sodium <i>o</i> -benzyl- <i>p</i> -chlorophenolate
GLT	glutaraldehyde	SDS	sodium dodecylbenzenesulphonate
HCL	hydrochloric acid	SHC	sodium hypochlorite
HPO	hydrogen peroxide	SHO	sodium hydroxide
IAL	isopropyl alcohol	SUX	sulphuric acid
LAC	lactic acid	TPP	<i>p</i> -tertiary amyl phenol
NAC	sodium chlorite	TRG	triethylene glycol
NAM	sodium metasilicate	TYT	tetrasodium ethylenediaminetetraacetate
		WAL	methyl alcohol

	R1				R2	R3	R4
	C12 %	C14 %	C16 %	C18 %			
QAC	40	50	10	—	Methyl	Methyl	Benzyl
QAE	50	30	17	3	Methyl	Methyl	Ethylbenzyl
QAF	68	32	—	—	Methyl	Methyl	Ethylbenzyl
QAI	50	30	17	3	Methyl	Methyl	Benzyl
QAK		100 % C10			C10	Methyl	Methyl
QAL	5	60	30	5	Methyl	Methyl	Benzyl
QAO	Isobutylphenoxyethoxyethyl				As R1	Methyl	Methyl

Quaternary ammonium compounds are given below with the substituents on the quaternary nitrogen shown as R (1-4)

toxic to MA-104 cells were filtered through Sephadex LH-20 (Pharmacia, Uppsala, Sweden), as described by Blackwell & Chen (1970). The reaction mixtures were diluted in EBSS and plaque-assayed.

Whenever a disinfectant required dilution, tap water was used as a diluent. All tests in this study were repeated at least six times. A disinfectant was considered effective if it could bring about a $3 \log_{10}$ (99.9%) or greater reduction in the plaque titre of the virus when compared to the control. Products found effective were further tested for their capacity to cause at least a $6 \log_{10}$ reduction in virus titre.

RESULTS

The results of the carrier tests for the chemical disinfection of rotavirus-contaminated surfaces are summarized in Table 2. For comparison, the performance rating of each product or formulation in the suspension tests (Springthorpe *et al.* 1986) is also listed. An additional section of the table gives the results on those disinfectants that were not examined by the suspension test.

In initial experiments, the virucidal capacity of representative products was assessed in triplicate tests on each of the four different types of surfaces. Since no variation in their disinfection efficacy was observed among the surfaces, all further tests were carried out on the stainless-steel disks only.

Of the formulations rated A in the suspension test (Springthorpe *et al.* 1986), 8 of the 12 further evaluated in our carrier test were able to reduce the infectious HRV titre by $\geq 3 \log_{10}$. Surprisingly, high concentrations (70%, v/v) of alcohols on their own (5 and 6) were ineffective in this second test. However, alcohols at concentrations $> 40\%$ in combination with other active ingredients (3 and 7), showed marked rotavirucidal activity. The other formulations which were effective against HRV include 2% glutaraldehyde (8), 2.5% chloramine T (9), 10% povidone-iodine with 1% available iodine (10), a phenolic compound containing an anionic surfactant (12), our modification of a quaternary ammonium-based product (4) and an acid (HCl) in combination with a quaternary ammonium compound (2). One acid-containing product (1), which was ineffective in disinfecting rotavirus-contaminated surfaces, contained a combination of acids (including HCl) and alcohol, but at very low concentrations at its in-use dilution. The only other ineffective product in this category was a combination of three different phenolics with an in-use dilution of 1:80.

Neither of the disinfectants (13 and 14) with a B rating in the suspension test was effective in disinfecting rotavirus-contaminated surfaces. Eight formulations which received a C or D rating in suspension tests were further examined in the carrier test. Their inclusion in this study helped to assess the predictive value of the suspension test. None of these formulations was found to be capable of reducing the HRV titre by $\geq 3 \log_{10}$ in the carrier test (Table 2).

Five compounds or formulations, which had not been previously examined in the suspension test, were also tested for their disinfectant ability. Methanol (23), as a 70% solution, was found to be no more effective than ethanol and isopropanol. Peracetic acid has been reported to inactivate several enteric viruses found in sewage effluents (Harakeh, 1984). Sporckenbach, Wieggers & Dernick (1981) have also shown that peracids and compounds which generate them can disinfect a

Table 2. *Efficacy of disinfectant formulations against human rotavirus in the carrier test*

Disinfectant formulation	Listed composition	Dilution tested	Recommended use	Suspension test rating*	> 99.9% loss in p.f.u.†
1	PHA 15.00% IAL 18.00% DBS 14.55% HCL 4.70% SUX 0.07%	1:128	Toilet and urinal cleaner	A	No
2	HCL 8.50% QAO 0.25%	Undiluted	Toilet-bowl cleaner	A	Yes
3	IAL 45.00% PRG 2.00% TRG 3.00% QAC 0.30%	Undiluted	Sanitize air and deodorize environmental surfaces	A	Yes
4	QAE 0.05% QAL 0.05% EAL 19.68% NAM 2.50%	Undiluted	—	A	Yes
5	EAL 95.00% EAL 70.00%	Undiluted Undiluted	General disinfection of contaminated fluids and surfaces in laboratories	A A	No No
6	IAL 70.00%	Undiluted	—	A	No
7	CHG 1.50% CET 15.00%	1:30 diluted in EAL Undiluted	Topical and instrument disinfectant	A	Yes
8	GLT 2.00%	Undiluted	Disinfection of instruments for gastrointestinal examinations	A	Yes
9	CFT 67.00%	1:40	General-purpose sanitizer for food and beverage handling equipment	A	Yes
10	POV 10.00% (1% available I ₂)	Undiluted	All-purpose skin disinfectant	A	Yes
11	OPP 1.70% BCP 2.50% TPP 0.90%	1:80	Disinfection in hospitals, clinics and surgical areas	A	No
12	SBC 0.50%	Undiluted	General domestic use, especially bathroom	A	Yes

	WAL NAM	V10% 0.23%		washable surfaces		
14	QAC NAM TYT	1.60% 2.50% 1.50%	1:20	Disinfection and cleaning of water washable inanimate surfaces	B	No
15	OAA NAC	15.10% 2.73%	1:12	General purpose hard surface disinfectant	C	No
16	QAE QAL EAL	0.05% 0.05% 19.68%	Undiluted	Disinfection of water washable inanimate surfaces	C	No
17	SHC	6.00%	1:9	Hard surface disinfectant	C	No
18	NPE PHA	18.76% 15.96%	1:167	Inanimate surfaces	C	No
19	NPP PNP	8.74% 9.10%	1:100	General purpose germicide for industrial or institutional use on inanimate surfaces	C	No
20	CHX IAL	4.80% 9.40%	1:40	Topical and general disinfectant	C	No
21	CDP NTR	0.50% 0.10%	Undiluted	Antibacterial hand-soap	C	No
22	CHG CET	1.50% 15.00%	1:200	General purpose disinfectant	D	No
23	WAL	70.00%	Undiluted	Disinfectants and chemicals not tested in the suspension tests		
24	PER	35.00%	1:100 1:1000	Disinfection of instruments and hard surfaces	N.T. N.T.	No Yes Yes
25	ACE	100.00%	Undiluted	Ophthalmological instruments	N.T.	No
26	QAL QAF	2.25% 2.25%	1:64	Cleaner/disinfectant for environmental surfaces	N.T.	No
27	HPO	30.00%	1:100	Plastic surgical implants and contact lens	N.T.	No

N.T., Not tested.

* The disinfectants evaluated in the suspension test studies (Springthorpe *et al.* 1986) have been rated as follows. (A) Highly effective, i.e. brings about a > 3 log₁₀ reduction in virus titre in the presence of high organic load (29.5 g/l of TPB/l). (B) Moderately effective, i.e. brings about a > 3 log₁₀ reduction in virus titre in the presence of low organic load (14.75 g TPB/l) but not in high organic load (29.5 g TPB/l) or 10% suspension

variety of viruses in the suspension test. Although peracetic acid is unlikely to be widely used because of its instability and its possible cocarcinogenic properties, the results with pure peracetic acid (24), at either 0.1 % or 1.0 %, show that it could be an effective disinfectant, reducing the HRV titre on virus contaminated surfaces by $\geq 3 \log_{10}$.

Acetone (25) was included because it is frequently used for fixing virus-infected monolayers and has been suggested as a suitable virucide for disinfecting ophthalmological instruments (Drews, 1977). However, against HRV dried on to surfaces it was not adequate. These results also indicated that acetone fixation of rotavirus-infected cell monolayers may not make them non-infectious and they should therefore be handled subsequently with care. Formulation 26 is another quaternary ammonium-based disinfectant intended for general disinfection of hard surfaces. It was found to be ineffective.

In view of the successful disinfection of HRV-contaminated carriers by peracetic acid, the action of hydrogen peroxide in the carrier test was also evaluated. Hydrogen peroxide at 0.3 % (27) was not effective, but the use of hydrogen peroxide concentrations ranging from 3 to 6 % has been suggested for the surface disinfection of surgical implants (Turner, 1983). Further tests with higher concentrations of this substance may, therefore, be required.

Because large numbers of HRV particles are usually found in the faeces of infected individuals, the validity of a $3 \log_{10}$ reduction in virus titre for disinfectant efficacy has been questioned. Therefore, the disinfectant formulations producing a $\geq 3 \log_{10}$ reduction were re-tested and all were shown to produce a greater than $6 \log_{10}$ reduction in infectivity of HRV in the carrier tests. This strongly indicates that if a disinfectant could reduce the HRV titre by $3 \log_{10}$, it could be considered capable of bringing the virus titre to an undetectable level.

DISCUSSION

Despite the magnitude of the global problem with rotavirus infections, very little was known about the virucidal activity of disinfectants used routinely to control spread of human rotaviruses. Springthorpe *et al.* (1986), using only the suspension test, examined the rotavirucidal potential of a wide range of chemical disinfectants. However, this study is the only published report on the chemical disinfection of HRV-contaminated surfaces.

Whatever the use to which a disinfectant will be put, it is contaminated surfaces which must be disinfected. Therefore, the suspension test is useful only for prescreening and formulations should always be further evaluated by a carrier test before their use can be recommended.

Environmental contamination by pathogenic viruses generally occurs in the presence of body secretions and excretions and a product can only be regarded as reliable if it can rapidly inactivate virus on a contaminated surface in the presence of organic and inorganic matter. In this study HRV suspended in faeces was used to simulate closely the problem occurring under natural conditions. The carrier tests were done on clean disks of non-porous surfaces, although in nature surfaces may be coated with dust, dirt and a variety of residues capable of interfering with the rotavirus inactivating capacity of chemical disinfectants.

Rotavirus particles in faeces are often found as large aggregates (Narang & Codd, 1981) or as clumps embedded in tissue fragments (Williams, 1985). This can protect the indigenous virus even more against the action of chemical disinfectants, some of which were also made ineffective by such interfering factors. Preliminary experiments in this laboratory have shown this to be particularly true with stools containing high concentrations of lipids. Whether this is due to greater inaccessibility of the virus or enhanced neutralization of the disinfectant is not known.

It was planned to include field strains of HRV in this study, but faecal samples with sufficiently high titres of infectious rotavirus could not be obtained. Those faecal samples that were made available contained no more than 4×10^3 rotavirus infective units/ml of a 10% (w/v) suspension, whereas a minimum of 10^5 infective units/ml was needed to demonstrate properly a 99.9% reduction in the virus titre. However, the validity of the data with the laboratory-adapted strain of HRV is supported by similar results obtained using simian rotavirus SA-11 (Sattar *et al.* 1983) and calf rotavirus (Sattar *et al.* unpublished data).

Although the number of products tested in this study was much smaller than that tested in suspension (Springthorpe *et al.* 1986), a close examination of each class of the effective disinfectants suggests that their ability to successfully inactivate HRV is related to certain combinations and/or minimum concentrations of specific ingredients. For example, quaternary ammonium compounds in combination with alcohols at concentrations > 40% (3 and 7) or HCl (2) showed high rotavirucidal activity. The effectiveness of formulation 4, our modification of an otherwise ineffective quaternary ammonium-based product (16), further strengthens these observations.

A comparison of these results with those from the suspension tests (Springthorpe *et al.* 1986) shows that disinfection of HRV from contaminated surfaces is more difficult to achieve than in suspension. This agrees with the findings of other workers using different viruses (Kirchoff, 1969; Klein & Deforest, 1983; Nakao *et al.* 1978; Schurmann & Eggers, 1983).

Our results confirm that a formulation ineffective in the suspension test was likely to be incapable of disinfecting HRV-contaminated surfaces. However, formulations that could effectively inactivate the virus in suspension did not always do so on contaminated surfaces.

Depending on the application, the contact time between virus and disinfectant can vary from a few seconds, in the case of hand-washing and routine general purpose use, to several hours in the case of instrument soaks. However, it is generally agreed that an efficient disinfectant should produce a pronounced virucidal effect after a minimal contact time. Our selection of a contact time of 1 min not only gave a reproducible time interval, but gave a more realistic picture of the usual practices of routine surface disinfection. Furthermore, previous work in this laboratory has shown that, in the suspension test, disinfectants (with the exception of formaldehyde) that were not effective within the 1 min contact time were not significantly more effective after 30 min of virus contact (Sattar *et al.* 1983). We believe that a contact time of 1 min has allowed us to identify the reliable products from among those which show only marginal efficacy.

A surprisingly large proportion of chemical disinfectants and antiseptics com-

monly used in the institutional, professional and domestic environment was shown to be unsatisfactory for the proper disinfection of rotavirus-contaminated materials. Available evidence strongly incriminates improperly disinfected fomites in the nosocomial spread of rotaviral gastroenteritis (Ryder *et al.* 1977; Halvorsrud & Ørstavik, 1980). Therefore, because of the highly contagious nature of rotaviruses and the large volume of copious watery stools that can result from such infections, the use of products which have little or no capacity for proper disinfection of HRV on surfaces may contribute to rotavirus transmission. Furthermore, improperly decontaminated objects and surfaces may pose a significant but unrecognized health hazard to persons handling them. Therefore, wherever possible, only those products which have been shown to be effective in the inactivation of rotaviruses as well as other pathogenic viruses and bacteria should be used. It is unreasonable to expect a single product to be suitable for use on all surfaces, instruments as well as on hands, but those products which could inactivate HRV by a $\geq 6 \log_{10}$ on our carriers were formulations that could be suitable for these categories of use. Of those products deemed effective, two are favoured for topical use (7 and 10), one for instrument soaks (8) and the remaining six are recommended for general use on hard surfaces.

In summary, neither cationic nor anionic surfactants were sufficient to inactivate HRV when acting alone and many conventional disinfectants such as alcohols and phenols were also inadequate. The following disinfectant classes or combinations proved effective in this study: glutaraldehyde; quaternary ammonium compounds in combination with (a) alcohols at $> 40\%$, or (b) some acids, e.g. HCl, or (c) some bases, e.g. sodium metasilicate; phenols in combination with strong anionic surfactants; chlorine-based disinfectants with free chlorine of > 20000 p.p.m.; iodophores with > 10000 p.p.m. iodine. Although caution should be exercised in extrapolating to untested formulations, the findings of this investigation will help in selecting suitable disinfectants and antiseptics for the prevention and control of outbreaks of rotaviral diarrhoea.

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