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Chemical disinfection of human rotaviruses: efficacy of commercially-available products in suspension tests

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SUMMARY

Suspension tests were conducted on 69 commercial and 7 non-commercial disinfectant formulations to determine which classes of chemicals were most active against human rotavirus (HRV). Virus samples, in the presence of varying levels of organic matter, were exposed to the disinfectants for 1 min. The levels of remaining infectious virus were determined by plaque assay. Products were rated by their ability to reduce the levels of infectious virus by more than 3 $\log_{10}$ in the presence or absence of tryptose phosphate broth (peptides and inorganic salts) or fecal matter.

Of the commercially-available products tested, only 25% were rated as highly and 7% as moderately effective. The remaining 68% were either effective only in the absence of any additional organic matter (48%) or were completely ineffective (20%). The majority (64%) of the moderately and highly effective products were further examined for their ability to inactivate > 6 $\log_{10}$ of infectious HRV in the presence of fecal matter or tryptose phosphate broth. With one exception, all these products were still effective. Products potentially suitable as topical antiseptics, hard surface disinfectants and instrument soaks were identified. The results emphasize the care that should be exercised in the selection of disinfectants for the control and prevention of rotaviral infections.

INTRODUCTION

At least 700 different types of viruses are known to infect man (Matthews, 1983), and many of these are responsible for a variety of contagious diseases. Chemical disinfectants and antiseptics are commonly used to prevent and control the spread of viral diseases in hospitals, laboratories and many other industrial, institutional or domestic settings. These chemicals are heavily relied upon in spite of a general lack of understanding of their virucidal potential. No standardized tests are available for assessing the virucidal efficacy of disinfectant or antiseptic products, and such data as are available have been generated with wide variations in test protocols and are, therefore, not easily compared.

Rotaviruses are well recognized as important human and animal pathogens, and

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are good examples of highly infectious agents which frequently cause disease outbreaks, even in settings where routine disinfection is practiced (Ryder *et al.* 1977; Hoh, Presser & Wigand, 1983; Noone & Banatvala, 1983). These infectious agents are excreted in large numbers in the faeces of infected individuals and are known to survive in faecal matter for prolonged periods (Woode & Bridger, 1975). Furthermore, the prevalence of unrecognized asymptomatic rotavirus infections (Wenman *et al.* 1979; Rossi *et al.* 1982; Champsaur *et al.* 1984), may result in additional cases or outbreaks of the disease (Keswick *et al.* 1983) because proper precautions, including disinfection, are not implemented. Hospitalization from both community- and nosocomially-acquired cases of rotaviral diarrhoea adds significantly to health-care costs (Ryder *et al.* 1977). Control of rotavirus transmission through proper disinfection practices would, therefore, have considerable importance for public health with resultant savings.

Preliminary work on animal rotaviruses, both in this laboratory (Sattar *et al.* 1983) and elsewhere (Snodgrass & Herring, 1977; Kurtz, Lee & Parsons, 1980; Brade, Schmidt & Gattert, 1981; Tan & Schnagl, 1981) has determined that these infectious agents are able to withstand many commercially-available disinfectants and antiseptics. Studies on the disinfection of human rotaviruses have so far been limited to chlorine-based disinfectants (Tan & Schnagl, 1983; Harakeh & Butler, 1984) and peracetic acid (Harakeh, 1984), and have shown varying results with different test protocols. Therefore this study was initiated to examine, under identical test conditions, a wider range of commercially-available disinfectant and antiseptic products to identify which types of chemicals show the most potential for disinfection of human rotaviruses.

MATERIALS AND METHODS

Virus growth and quantitation

The MA-104 line of rhesus monkey kidney cells was used for both the preparation of virus pools and quantitation of the virus by plaque assay. Techniques for the cultivation, maintenance and passage of these cells have already been described (Sattar *et al.* 1986).

The Wa strain of human rotavirus (HRV), kindly supplied by Dr R. G. Wyatt of the National Institutes of Health, Bethesda, MD, USA (Wyatt *et al.* 1980) was used to assess disinfectant activity. Methods for the preparation of virus pools are given in detail elsewhere (Sattar *et al.* 1986); virus pools thus prepared were suspended in either tryptose phosphate broth (TPB), faecal suspension or distilled water, as appropriate. Quantitation of HRV used the plaque assay described by Raphael, Sattar & Springthorpe (1985) and Ramia & Sattar (1979).

Virus suspending medium for disinfectant testing

In nature, viruses are usually shed in body secretions and excretions, which contain organic and inorganic matter providing the virus with both physical and chemical protection against disinfectant action. Use of purified virus to evaluate disinfectants may therefore give misleading results. The ideal organic load for testing virucidal efficacy of disinfectants against enteric viruses, such as HRV, is obviously faeces. However, undiluted faecal matter is usually toxic for cell cultures

used for virus assay, and high levels of particulate material and presence of antibodies and non-specific inhibitors interfere with virus quantitation.

The organic load used in the virus suspending medium here was either TPB, which is known to be non-inhibitory to rotaviruses (Ramia & Sattar, 1980) and contains a relatively large quantity of peptide material as well as inorganic salts, or a diluted faecal slurry prepared from a diarrhoeic infant stool. The latter was taken from a laboratory-confirmed case of rotaviral diarrhoea admitted to the Children's Hospital of Eastern Ontario (Ottawa) to simulate closely natural conditions, and screened for its inhibitory action against HRV. Indigenous rotaviruses present in the stool sample did not produce countable plaques in our assay system and, as a result, they were unable to interfere with the quantitation of the experimentally-added HRV. The stool sample was prepared as a 10% (w/v) suspension in normal saline and centrifuged at 1000 **g** for 15 min to remove gross faecal matter before storage of the supernatant at −70 °C.

Two levels of organic load were used in the disinfectant testing: (i) the high added organic load (HOL) was TPB at 29·5 g/l (approx. 2% peptone) and (ii) the low added organic load (LOL) was either TPB at 14·75 g/l or 10% suspension of diarrhoeic faeces in normal saline. A 10% suspension of faeces in normal saline corresponded (Documenta Geigy, 1962) to the lower level of TPB (approx. 1% peptone) used as organic load. Tests were also conducted in the absence of added organic matter.

Disinfectant test procedure

In many disinfectant applications, virus-disinfectant contact is brief. Unless otherwise indicated, a 1 min contact time was selected for the test procedure because it was easily reproducible and allowed differentiation between truly reliable products and those with borderline efficiency: no attempt was made to study the kinetics of HRV inactivation. Further, earlier observations (Sattar *et al.* 1983) showed that the results obtained after 1 min were generally predictive of those after 30 min of contact.

Virus suspension (0·1 ml) containing TPB, faecal slurry or distilled water was mixed with 0·9 ml of the disinfectant under test; the organic load in LOL and HOL was thus reduced to 0·1 and 0·2% peptone, respectively. After a contact time of 1 min at room temperature (22–24 °C), 0·1 ml of the virus-disinfectant mixture was removed and immediately diluted 100-fold in Earle's Balanced Salt Solution (EBSS). This was done to reduce the cytotoxicity of the virus-disinfectant mixture and to stop the action of the disinfectant through dilution. It was subsequently subjected to four additional 10-fold dilutions (10^{-3} to 10^{-6}). Aliquots from the last three dilutions (10^{-4} to 10^{-6}) were plaque-assayed. Those reaction mixtures that remained cytotoxic to MA-104 cells after dilution were detoxified as described below. Control reaction mixtures contained 0·9 ml EBSS instead of disinfectant.

All disinfectant tests in this study were repeated at least three times. A disinfectant formulation was considered effective if it caused a 3 $\log_{10}$ (99·9%) or greater reduction in the plaque titre of the virus, when compared to the control.

Table 1. *Abbreviations for listed ingredients in disinfectant formulations*

AAD	acidic anionic detergent
BCP	*o*-benzyl-chlorophenol
BNP	2-bromo-2-nitropropane, 1-3-diol (bronopol)
BPP	butoxypolypropoxypolyethoxyethanol-iodine complex
CDP	2,4,4′,-trichloro-2′-hydroxydiphenyl ether (or 5-chloro-2(2,4-dichlorophenoxyl) phenol)
CET	cetrimide
CFT	chloramine-T
CHA	chlorhexidine acetate
CHG	chlorhexidine gluconate
CHX	4-chloro-3,5-xylenol
CIA	citric acid
CIT	sodium citrate
COO	coal tar oils
CRA	cresylic acid or phenolic homologues (a mixture of cresols, xylenols, etc.)
DBS	dodecylbenzenesulphonic acid
DOD	dodicin hydrochloride
EAL	ethyl alcohol
FOR	formaldehyde
GLT	glutaraldehyde
HCL	hydrochloric acid
HCP	hexachlorophene
IAL	isopropyl alcohol
KBR	potassium bromide
LAC	lactic acid
NAC	sodium chlorite
NAM	sodium metasilicate
NPE	nonylphenoxypolyethyleneethanol-iodine complex
NPP	nonylphenoxypolyethoxyethanol-iodine
NTR	nitrilotriacetic acid (as sodium salt)
OAA	organic acid activator
OPP	*o*-phenylphenol
OXL	oxalic acid
PDG	polymeric diguanide hydrochloride
PHA	phosphoric acid
PNP	polyethoxypolypropoxypolyethoxyethanol-iodine complex
POI	pine oil
POV	povidone-iodine complex
PRG	propylene glycol
SBA	sodium para-tertiary amyl phenate
SBC	sodium *o*-benzyl-*p*-chlorophenate
SCB	sodium carbonate
SDS	sodium dodecylbenzenesulphonate
SHC	sodium hypochlorite
SHO	sodium hydroxide
SOA	soap
SOP	sodium *o*-phenylphenate
SPC	sodium pentachlorophenate plus related chlorophenates
SSP	sodium sulphite
SUO	sulphonated oleic acid
SUX	sulphuric acid
TCN	sodium trochlosene
TEA	triethanolamine
TPP	*p*-tertiary amyl phenol
TRG	triethylene glycol
TSP	trisodium phosphate
TYT	tetrasodium ethylenediaminetetraacetate
WAL	methyl alcohol
XYL	xylenols

QUATERNARY AMMONIUM COMPOUNDS are given below with the substituents on the quaternary nitrogen shown as R (1–4)

	R1				R2	R3	R4
	C12%	C14%	C16%	C18%			
QAB	25	60	15	—	Methyl	Methyl	Benzyl
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
QAM		Methyldodecylbenzyl			Methyl	Methyl	Methyl
QAN	98	2	—	—	Methyl	Methyl	1-naphthyl methyl
QAO		Isobutylphenoxyethoxyethyl			As R1	Methyl	Methyl
QAQ	61	23	11	5(C8–18)	Methyl	Methyl	Benzyl
QAT		Stearyl			Stearyl	Methyl	Methyl
QDE		Octyl			Octyl	Methyl	Methyl
QDF		Octyl			Decyl	Methyl	Methyl

Procedure for removal of disinfectant when diluted virus-disinfectant mixture was cytotoxic for cell cultures

Disinfectant was removed from cytotoxic reaction mixtures by the gel filtration technique of Blackwell & Chen (1970). Prior testing of this system had shown that no HRV was retained by the LH-20 gel.

Disinfectants

More than 70 disinfectant formulations, including 69 commercial products, representing most major classes of disinfectants, were tested in this study. Product names have not been given to avoid the recommendation of a particular product when other similar formulations may be available but have not been tested. Furthermore, many disinfectants are marketed on a local or national rather than an international basis, and the same formulation may be sold under a variety of trade names in different countries. Additional chemicals were also tested where it was appropriate.

The chemical composition of the tested disinfectants are listed by active ingredients with the results of the tests. Active ingredients in the disinfectant formulations are represented by a three letter code given in Table 1. The disinfectants tested were grouped into the following categories: (*a*) acids, anionic surfactants and acidic anionic surfactants, (*b*) alcohols & aldehydes, (*c*) amphoteric compounds, chlorhexidene and polymeric biguanides, (*d*) halogen-based compounds (hypochlorites, mixed halides, chlorine dioxide and organochlorines; iodophores), (*e*) phenols, and (*f*) quaternary ammonium compounds. Many disinfectant formulations contain active components from more than one class of disinfectant; to simplify the presentation of results each disinfectant has been assigned to one category only.

The nature of the diluent can markedly affect the virucidal activity of a disinfectant (Sattar *et al.* 1983). In this study, all disinfectants were diluted according to the manufacturer's instructions. In the absence of specific recommendations, tap water was used as diluent. Unless otherwise stated, the percentage composition of disinfectant formulations is given on a weight for weight basis; dilutions are given as either parts by volume for liquid formulations or parts by weight for solid materials. Where the manufacturer has recommended more than one concentration of disinfectant for use in different applications, the highest recommended concentration was tested.

RESULTS

Based on the results obtained in this study, the disinfectants tested have been rated as follows: (A) highly effective; i.e. bring about a $> 3 \log_{10}$ reduction in virus titre in the presence of HOL; (B) moderately effective; i.e. brings about a $> 3 \log_{10}$ reduction in virus titre in the presence of LOL but not in the presence of HOL; (C) slightly effective; i.e. effective in the absence of added organic load but not when TPB or faecal matter is present; (D) ineffective; i.e. produces little or no reduction in virus titre even in the absence of added organic matter.

Table 2 gives the composition, recommended usage and rated efficacy for the

disinfectants tested in suspension. For the ease of the reader this table has been divided into convenient sections. Table 2*a* shows the results obtained for formulations based on acids and anionic surfactants.

Acid-containing disinfectants tested fall into two main groups. The first type (1–4) is used primarily for the disinfection of toilet bowls, urinals, etc. These disinfectants all contain at least one strong mineral acid, hydrochloric acid (HCl), and they may also contain quaternary ammonium compounds; the targets in this case are mostly ceramic surfaces. All were shown to be highly effective against HRV even in the presence of HOL. The second group, acidic anionic surfactants, are general purpose sanitizers widely used in the food and beverage industries: here phosphoric acid is the mineral acid of choice because of potential corrosion problems. These products (5–8) were ineffective against HRV in the presence of organic material. An anionic surfactant alone (9) was also ineffective against HRV.

Table 2*b* contains the results for alcohols and aldehydes. Many disinfectant formulations contain alcohols, not only for their disinfectant action, but also for their ability to potentiate the action of other active agents. They also evaporate readily and can be used to carry alcohol soluble components in sprayed disinfectants. However, for this study, only pure alcohols or their aqueous dilutions are considered in this class. In the suspension test, both ethanol (10) and isopropanol (11) were highly effective in the inactivation of HRV when their concentration was at least 70 %; they did not seem to be affected by the presence of organic matter. Dilution of ethanol to 35 %, on the other hand, rendered it completely ineffective in the inactivation of HRV.

There are two principal aldehydes that are used as disinfectants; formaldehyde and glutaraldehyde. The former is available as an aqueous solution of approximately 37 % (w/w). However, this solution is relatively unstable and undergoes polymerization to form the solid paraformaldehyde. It is, therefore, usually supplied in a stabilized form containing 10–15 % methyl alcohol. Formaldehyde gas, used for fumigation, and formaldehyde-releasing compounds for topical application were considered beyond the scope of this study. Glutaraldehyde is a dialdehyde in which both free aldehyde groups are reactive. In acidic aqueous solution it is relatively stable, but it rapidly loses biocidal activity in alkaline solution, probably due to irreversible polymerization. Commercial preparations of glutaraldehyde are usually supplied in acid solution, but many of them, including the one tested here (13), also contain corrosion inhibitors and alkaline buffered activators which provide disinfectant solutions with a limited stability (14–28 days). Glutaraldehyde solutions are primarily used for instrument sterilization.

Formaldehyde and glutaraldehyde are often considered as chemical sterilants because of their broad spectrum of activity. It is clear, however, from the results of this study that glutaraldehyde is the more reactive compound against HRV, since it was able to inactivate the virus effectively in the 1 min contact time in the presence of LOL or HOL; formaldehyde solutions with an approximately equivalent concentration of aldehyde groups required 30 min of virus contact to achieve the same effect.

The results of testing amphoteric and biguanide compounds against HRV in suspension tests are summarized in Table 2*c*. Amphoteric agents are surfactants containing amino acids substituted with long-chain alkyl amine groups. The only

amphoteric agent tested (15) was completely inactive against HRV at the manufacturer's recommended concentration.

Chlorhexidine is a cationic biguanide, available as the dihydrochloride, diacetate or gluconate salts, with a wide spectrum of action against gram negative and gram positive bacteria. It is mainly used as a topical antiseptic in either aqueous or alcoholic solution because it combines rapid bactericidal properties with persistence on the skin surface, and also has a low oral and percutaneous toxicity. When chlorhexidine compounds were tested in aqueous solution, alone (16) or with a quaternary ammonium compound (17, 18), they were either completely inactive against HRV even in the absence of an organic load (16, 17) or effective only in the absence of organic matter (18). This is in marked contrast to the results obtained with a chlorhexidine and quaternary ammonium containing formulation which was diluted in ethanol (19).

Polymeric biguanides are now also available, and are mainly used in conjunction with quaternary ammonium compounds for general surface disinfection. Formulation 20 contains a molecule of this type as well as a quaternary ammonium compound and 20% ethanol; it was found to be highly effective against HRV in the suspension test.

The results of testing halogen-based disinfectants are shown in Table 2*d*. Chlorine has a potent disinfectant action against most vegetative bacteria. For this reason, and because of the relatively low residual toxicity and low cost of manufacture, chlorine compounds, particularly hypochlorites, are among the most widely used of all disinfectants, particularly in the food and dairy industries, and for the terminal treatment of potable water. Aqueous solutions of chlorine compounds produce both hypochlorous acid (HOCl) and hypochlorite ions. It is generally considered that the lethal action of chlorine on organisms results from the oxidation and or chlorination of cell proteins by unionized hypochlorous acid.

Hypochlorites are sold primarily as aqueous solutions containing either 6 or 12% w/v chlorine. It can clearly be seen that hypochlorite solutions, alone or buffered (21–23) are relatively ineffective in the inactivation of HRV; the chlorine concentrations in these formulations range from 500 to 5000 p.p.m.

Mixed halogen compounds (24, 25) were somewhat more effective than sodium hypochlorite, and were able to inactivate HRV in the presence of LOL but not HOL. However, chlorinated trisodium phosphate (26) was completely ineffective against HRV.

Chlorine dioxide is a powerful oxidising agent and has not been available for general use as it must be freshly generated. Commerical formulations for its preparation have now become available (27, 28). This compound was unable to inactivate HRV in the absence of an organic load (27) or to reduce the titre of HRV in the presence of faecal material on a consistent basis (28). It is interesting to note that, in spite of the inefficacy of product 27 when used as directed, the acid activator (part B alone), which contained low levels of HCl and organic acids, was highly active against HRV.

Although chloramines may have a direct antimicrobial action, they are also hydrolysed in solution and slowly release HOCl. Formulation 29 contains chloramine-T (67%, w/w) and is recommended for use at various concentrations depending on the application. We were particularly interested in chloramine-T as

a possible substitute for hypochlorite solutions in laboratory disinfection, and it was therefore tested at a range of concentrations, some of which exceeded those recommended by the manufacturer for routine disinfection. As can be seen, many of the concentrations tested were able to give complete inactivation of HRV even in the presence of HOL. Chloramine-T alone (30) showed the same activity against HRV, but, because it lacked the unspecified bulking agent present in 29, it tolerated more dilution before it became ineffective.

Halane, 1,3-dichloro-5,5-dimethyl hydantoin (31), can be an effective bactericide, but its efficacy is very dependent on the specific formulation, concentration and pH. The concentration of halane tested here was very dilute, but was nevertheless effective in the absence of added organic matter. No higher concentrations were tested in this study, but this, and similar organic chlorine compounds, deserve further investigation.

In many respects iodine-based disinfectants are analogous to those of chlorine, but iodophores have largely replaced aqueous or alcoholic iodine solutions. These compounds are neutral polymers which form loose complexes with elemental iodine or triiodide and release the available iodine into solution when diluted. They have surface active properties and some ability to cut through organic or fatty soil on surfaces. Iodine-based compounds have a wide spectrum of action, and are mainly used for topical antisepsis and the disinfection of inanimate surfaces.

The rated efficacy for the iodophores tested (32–37) varied widely, and may reflect the total available iodine present during virus contact. Formulations 32–35 contain 1·60–1·75% of titratable iodine and are diluted from 1 : 100 to 1 : 640; all of these products received either a C or D rating. Product 36 contained 3·5% titratable iodine and was tested at the highest recommended concentration (1 : 160; for use on porous or hard to clean surfaces). This dilution could consistently inactivate HRV in faecal matter, but not in the presence of HOL. The only iodophore which received the A rating (37) was used undiluted in accordance with the manufacturer's recommendation; in this case the available iodine concentration during virus contact was much higher (1%) than with the other iodophores tested.

Phenol and phenolic derivatives are a diverse group of substances which have been widely used as hard surface or topical disinfectants. The results of testing phenol-based disinfectants against HRV are shown in Table 2*e*. Of the 17 such products tested, 13 were relatively ineffective and were rated as either C or D. However, 4 of the tested formulations were effective in inactivating HRV even in the presence of HOL. Two of these four were hexachlorophene-based disinfectants (53 and 54), and are no longer available because of their high toxicity for humans. The other two effective phenolics (40 and 48) are not similar and contain different substituted phenols; one of them (48) also includes an anionic surfactant. The only property which these two formulations share is an extremely high pH value (pH 12·5 approx.), and this in itself may have contributed to their virucidal efficacy.

Quaternary ammonium compounds are a large group of cationic surfactants where the hydrogen atoms in the ammonium group are replaced by alkyl and/or aryl substituents. Typically, at least one of the alkyl groups is a long hydrophobic carbon chain. Because of their bactericidal activity against both gram-positive and gram-negative bacteria, these compounds have gained wide acceptance in hospitals, restaurants, dairies, food plants and laundries. Although dilute solutions are used

Table 2*a*. *Rating of disinfectants tested for their efficacy against human rotavirus in the suspension test*

Disinfectant formulation no.	Listed composition (W/W)	Dilution tested	Recommended use	Rated efficacy
Acids, anionic surfactants and acidic anionic surfactants				
1	PHA 15·00% IAL 18·00% DBS 14·55% HCL 4·70% SUX 0·07%	1:128	Toilet and urinal cleaner	A
2	HCL 23·00% QAC 0·40%	Undiluted	Germicidal bowl and urinal cleaner	A
3	HCL 7·00% QXL 2·00% QAF 0·10% QAL 0·10%	Undiluted	Toilet bowl cleaner	A
4	HCL 8·50% QAO 0·25%	Undiluted	Toilet bowl cleaner	A
5	PHA 18·75% SUX 19·20% IAL 5·00%	1:160	Cleaning/sanitizing of hard non-porous surfaces	C
6	PHA 22·00% SDS 2·75%	1:320	Sanitizing dairy and food processing equipment	C
7	AAD NA	1:320	General sanitizing in dairy, beverage and food industry	C
8	PHA 28·50% SUO 4·00%	1:320	Sanitation of food preparation equipment	D
9	SDS 0·60%	Undiluted	Constitutent of disinfectant mixtures	D

NA, Not available.

Table 2b. *Rating of disinfectants tested for their efficacy against human rotavirus in the suspension test*

Disinfectant formulation no.	Listed composition (W/W)	Dilution tested	Recommended use	Rated efficacy
Alcohols				
10	EAL 95·00 %	Undiluted	General disinfection of contaminated fluids and surfaces in laboratories	A
	70·00 %	1 : 0·35		A
	35·00 %	1 : 1·70		D
11	IAL 70·00 %	Undiluted	General disinfection of contaminated fluids and surfaces in laboratories, can also be used for hands	A
Aldehydes				
12	FOR 37 %	1 : 10	Equipment sterilization, general disinfectant for farm buildings	A*
	WAL 15 %			
13	FOR 37 %	1 : 10	Sterilization of contaminated objects, fixation of infected cells and tissues	A*
	WAL 10–15 %			
14	GLT 2 %	Undiluted	Disinfection of instruments for gastrointestinal examinations	A

* Effective only after 30 min of contact.

Table 2c. *Rating of disinfectants tested for their efficacy against human rotavirus in the suspension test*

Disinfectant formulation no.	Listed composition (W/W)	Dilution tested	Recommended use	Rated efficacy
Amphoteric compounds				
15	DOD 3·50 %	1 : 100	General purpose disinfectant	D
	QAC 2·80 %			
	TYT 1·60 %			
	TEA 1·00 %			
Chlorhexidine and polymeric biguanides				
16	CHA 2·00 %	Undiluted	Skin cleanser	D
17	QAK 0·75 %	1 : 2	Antiseptic hand soap for hospitals and food processing	D
	CHG 0·50 %			
18	CHG 1·50 %	1 : 200	General purpose disinfectant	C
	CET 15·00 %			
19	CHG 1·50 %	1 : 30 diluted in EAL	Topical and instrument disinfectant	A
	CET 15·00 %			
20	QAC 0·30 %	Undiluted	Spray for general purpose hard surface disinfection	A
	PDG 0·75 %			
	EAL 20·00 %			

Table 2*d*. *Rating of disinfectants tested for their efficacy against human rotavirus in the suspension test*

Disinfectant formulation no.	Listed composition (W/W)	Dilution tested	Recommended use	Rated efficacy
Hypochlorites, mixed halides, chlorine dioxide and organochlorines				
21	SHC 6·00%	1:100	Hard surface disinfectant	C
		1:9		C
22	SHC 6·00%	1:9	Possible sporicide	
			Diluted in phosphate buffer pH 7·5	C
			Diluted in citrate/phosphate buffer pH 6·0	C
23	SHC 10·80%	1:160	General sanitizer for dairy and food processing equipment	C–D
24	TCN 28·00%	1:100	General purpose sanitizer	B
	KBR 8·00%			
	SCB 30·00%			
25	SHC 3·15%	1:110	General purpose cleaner/sanitizer for food processing	B
	KBR 0·10%			
26	SHC 3·40%	1:200	Disinfectant cleaner for food processing plants	D
	TSP 43·00%			
27*	Part A	1:50	Industrial and municipal use for general disinfection of equipment and environmental surfaces, also for swimming pools	D
	NAC 7·80%			
	SHO 0·70%			
	Part B			
	HCL 1·86%			
	LAC 2·13%			
	CIA 2·24%			
	CIT 2·13%			
	* two component germicide, 30 min allowed after mixing before use as specified by manufacturer.			
	Part A alone	1:50	—	D
	Part B alone	1:50	—	A
28	OAA 15·10%	1:12	General purpose hard surface disinfectant	C
	NAC 2·73%			

29	CFT 67·00 ? %	1 : 8	General purpose sanitizer for food and beverage handling equipment	A
		1 : 16		A
		1 : 40		A
		1 : 56		B
		1 : 80		D
		1 : 800		D
30	CFT 100·00 %	1 : 40		A
		1 : 56		A
		1 : 80		A
		1 : 800		D
31	DDH 100·00 %	1 : 140,000	Toilet bowl sanitizer	C
Iodophores				
32	BPP 15·50 % PHA 6·50 % titratable I_2 1·75 %	1 : 133	Sanitizing dairy and food processing equipment	C
33	NPE 18·76 % PHA 15·96 % titratable I_2 1·76 %	1 : 167	Inanimate surfaces	C
34	NPP & NPE to give titratable I_2 1·75 % PHA 18·75 %	1 : 640	Cleaning, sanitizing or disinfecting food and beverage equipment	D
35	NPP 8·74 % PNP 9·10 % titratable I_2 1·60 %	1 : 100	General purpose germicide for industrial and institutional use on inanimate surfaces	C
		1 : 200		D
36	NPP 27·60 % PHA 9·0 % titratable I_2 3·50 %	1 : 160	General purpose detergent/germicide	B
37	POV 10·00 % titratable I_2 1·00 %	Undiluted	All purpose skin disinfectant	A

Table 2*e*. *Rating of disinfectants tested for their efficacy against human rotavirus in the suspension test*

Disinfectant formulation no.	Listed composition (W/W)	Dilution tested	Recommended use	Rated efficacy
Phenolics				
38	BCP 2·25%	1:80	Surface disinfectant	D
39	BCP 3·50%	1:55	General cleaning in institutions	C
	IAL 6·00%			
	TYT 1·33%			
40	OPP 1·70%	1:80	Disinfection in hospitals, clinics and surgical areas	A
	BCP 2·50%			
	TPP 0·90%			
41	OPP 2·80%	1:100	General disinfection of inanimate surfaces	C
	BCP 2·70%			
	XYL 1·50%			
	EAL 1·85%			
	TYT 0·78%			
42	BCP 4·50%	1:100	General disinfectant	C
	POI 5·00%			
43	BCP 5·20%	1:64	General purpose hard surface disinfectant	C
	POI 5·70%			
	IAL 16·75%			
	TYT 0·39%			
44	OPP 3·40%	1:80	Hard surface disinfectant/cleaner	D
	BCP 1·70%			
	TYT 2·00%			
	EAL 4·50%			
	WAL 0·50%			

45	CHX 4·80% IAL 9·40%	1 : 40	Topical and general disinfectant	C
46	SBC 3·80% SOP 1·20%	1 : 80	General purpose disinfectant/cleaner	C
47	SBC 17·00% IAL 5·00% SPC 4·40% SBA 2·80% SDS 2·10% TYT 0·80% SSP 0·50%	1 : 230	General purpose hard surface cleaner	C
48	SBC 0·50% SDS 0·60%	Undiluted	General domestic use, especially bathroom surfaces	A
49	CDP 0·50% BNP 0·10%	Undiluted	Antibacterial hand soap	C
50	CDP 0·50% NTR 0·10%	Undiluted	Antibacterial hand soap	C
51	CDP 0·90% PRG 0·70%	1 : 3	Antibacterial hand soap	C
52	CRA 16·00% COO 54·00% SOA 17·00%	1 : 120	Disinfectant for general farm use	C?
53	HCP 0·75%	1 : 3	Hand cleanser	A
54	IAL 70·00% HCP 0·10%	Undiluted	Disinfectant for hands and instruments	A

?, Variable results.

Table 2*f*. *Rating of disinfectants tested for their efficiacy against human rotavirus in the suspension test*

Disinfectant formulation no.	Listed composition (W/W)	Dilution tested	Recommended use	Rated efficacy
Quaternary ammonium compounds				
55	QAC 10·00 %	1 : 250	Hard surfaces, fabric sanitation, handwashing	C
56	QAC 10·00 %	1 : 125	Precleaned inanimate surfaces	C
57	QAC 3·90 %	1 : 80	Inanimate hard surfaces	C
58	QAC 0·13 %	Undiluted	Skin, hands and instruments	C
59	QAC 9·00 % TYT 0·40 %	1 : 160	General hard surface sanitizer	C
60	QAC 2·70 % TYT 1·00 %	1 : 33	Cleaner disinfectant for washable surfaces	D
61	QAC 5·00 % TSP 2·00 % TYT 0·60 %	1 : 83	General purpose cleaner/disinfectant	C
62	QAC 5·00 % QAK 1·88 % QDE 1·88 % QDF 3·75 % EAL 1·00 % IAL 3·00 % TYT 3·42 %	1 : 250	General purpose disinfectant/cleaner	C
63	QAC 0·80 % IAL 4·00 % TYT 1·10 % NAM 1·00 %	1 : 10	Disinfection of floors	C
64	QAC 1·60 % NAM 2·50 % TYT 1·50 %	1 : 20	Disinfection and cleaning of water washable inanimate surfaces	B
65	QAK 1·44 % QDE 1·44 % QDF 2·88 % EAL 1·15 %	1 : 80	Cleaning and disinfection of inanimate surfaces	D
66	QAL 1·60 % QAF 1·60 % TSP 1·30 % TYT 1·10 %	1 : 40	Germicidal cleaner for institutional use	D

67	QAF 0·10% QAL 0·10% NAM 0·23%	Undiluted	General purpose cleaner for all water washable surfaces	B
68	QAL 2·26% QAF 2·26% EAL 1·12% PHO 1·80% NAM 2·00%	1 : 80	One-step cleaning disinfecting of hard surfaces	C
69	QAF 10·76% QAL 10·76% IAL 16·40%	1 : 500	General farm and dairy disinfectant	C
70*	QAF 10·76% QAL 10·76% IAL 16·40% NAM 0·50%	1 : 500		C
71*	QAF 10·76% QAL 10·76% IAL 16·40% NAM 2·50%	1 : 500		A
72	QAI 0·09% QAL 0·09% EAL 19·68%	Undiluted	Disinfection of water washable inanimate surfaces	C
73*	QAI 0·09% QAL 0·09% EAL 19·68% NAM 2·50%	Undiluted		A
74*	QAI 0·09% QAL 0·09% EAL 19·68% NAM 5·00%	Undiluted		A
75	IAL 27·30% PRG 4·40% TRG 4·70% QAM 0·20%	Undiluted	Reduce airborne bacteria and viruses and deodorise environmental surfaces	A
76	IAL 45·00% PRG 2·00% TRG 3·00% QAC 0·30%	Undiluted	Sanitize air and deodorise environmental surfaces	A

* Laboratory modification of commercial product.

as topical antiseptics, the most widespread use of quaternary ammonium compounds is as hard surface disinfectants.

Results of disinfectant testing from this group are shown in Table 2*f*. The substitutents on the quaternary ammonium nitrogen are shown in Table 1. A total of 22 formulations were examined under this category; 18 were commercially-available products and 4 were our laboratory modifications of 2 of these. The majority (15/22 : 68 %) of quaternary ammonium compounds tested received either a C or D rating in the suspension test; this indicates that this class of disinfectants is relatively ineffective against HRV in the presence of any additional organic matter. The remaining 32 % (7/22) which received either an A or B rating, contained either sodium metasilicate or relatively high proportions of alcohol. Several of the quaternary ammonium-based formulations tested here contained either ethanol or isopropanol with concentrations ranging from 1 to 20 % for ethanol and 4–45 % for isopropanol. None of the ethanol-containing products received higher than a C rating, and the only isopropanol-containing products which were highly effective (75 and 76) were those intended as air sanitisers. These two products contained 44·5 % and 27·3 % of isopropanol respectively, and in addition they contained both propylene glycol and triethylene glycol.

Four of the commercial formulations tested contained sodium metasilicate as one of the listed ingredients; two rated B (64 and 67) and two rated C (63 and 68). In order to test if sodium metasilicate itself played a part in HRV inactivation in the presence of organic matter, it was tested alone (results not tabulated) and added at two different levels to two separate quaternary ammonium-based disinfectant formulations which had only a C rating in previous tests (69, 72). Concentrations of sodium metasilicate from 0·5 to 5 % were effective against HRV. Formulation 71, 73 and 74 received an A rating when tested against HRV in suspension. Altough it is recognized that the sodium metasilicate concentration in 73 and 74 was considerably higher than was present in the commercial formulations tested, it was comparable with the sodium metasilicate levels tested against other viruses (Herniman *et al.* 1973; Blackwell, Graves & McKercher, 1975). The final concentration of sodium metasilicate in 71 was only 0·005 % (w/v), much lower than that in any of the commercial formulations tested. It is possible that the quaternary ammonium compounds, the isopropanol and the sodium metasilicate were acting in an additive or synergistic fashion to produce the results obtained with this formulation.

The ability of disinfectants to reduce the titre of HRV by 6 log_{10}

Because of the large number of rotavirus particles in faeces, the ability to reduce the virus titre by 3 log_{10} may mean that a considerable amount of infectious virus remains. To test whether the effective disinfectants (A or B rating) could also reduce the virus titre by 6 log_{10} (the detection limit of our test system), a series of disinfectant tests were conducted in which the disinfectant was treated as a cytotoxic agent and was removed on the LH-20 column as described earlier. This enabled the virus titration to be performed to show a 6 log_{10} reduction in virus titre. However, it should be noted that use of the column method of disinfectant removal prevented termination of the virus-disinfectant reaction at exactly 1 min. The results of these tests, performed with an organic load of either 10 % faecal slurry or HOL, are summarized in Table 3.

Table 3. *Results of testing rotavirucidal agents for their ability to reduce the titre of human rotavirus by 6 log_{10}*

Formulation no.	Rating in suspension test	6 log_{10} reduction in HRV titre	
		10 % faeces	HOL
1	A	Yes	Yes
2	A	Yes	Yes
3	A	Yes	Yes
10	A	Yes	Yes
29	A (1/40)	Yes	Yes
	B (1/56)	Yes	ND
36	B	Yes	ND
40	A	Yes	ND
48	A	Yes	Yes
64	B	Yes	ND
67	B	Yes	ND
71	A	Yes	Yes
73	A	Yes	Yes
75	A	No	No
76	A	Yes	Yes

ND, Not done.

It can clearly be seen that, with the exception of 75, all formulations tested which could bring about a 3 log_{10} in HRV titre could also reduce the infectious virus by at least 6 log_{10}. In addition to those listed below, sodium metasilicate alone (0·5 and 2·5 %) could also reduce the infectious virus titre by 6 log_{10} in the presence of HOL.

DISCUSSION

Testing a large number of products under identical conditions against HRV allowed a comparative evaluation of different disinfectant classes. In general, products with a similar composition of listed active ingredients had the same effect on HRV under our test conditions and the results were consistent with those from other studies on rotavirus disinfection (Snodgrass & Herring, 1977; Kurtz, Lee & Parsons, 1980; Brade, Schmidt & Gattert, 1981; Tan & Schnagl, 1981, 1983; Harakeh & Butler, 1984). Although many of the commercially-available disinfectants were unable to inactivate HRV in the presence of additional organic matter, some formulations were identified which have a high potential for effective disinfection of HRV-contaminated material in a variety of natural situations: it should be emphasized, however, that these results do not confirm the efficacy of similar products under in-use conditions. A large proportion of HRV-virions produced by an infected cell remain membrane-associated or in large aggregates (Williams, 1985), and any effective antirotaviral agent must be able to penetrate organic matter including cell membranes. Moreover, indigenous rotavirus present in undiluted stool specimens would be even more protected from disinfectant action than virus particles externally added to faecal matter. Addition of substances with surface active or lipophilic properties appeared to enhance the activity of many of the disinfectants in this study.

HRV can contaminate materials directly through stool contact or indirectly from aerosols (Ijaz *et al.* 1985) such as are generated by toilet flushing (Gerba, Wallis & Melnick, 1975). Bathroom surfaces are among those most likely to be contaminated and the toilet bowl cleaners tested (1–4) clearly have potential for effective HRV disinfection on corrosion-resistant inanimate surfaces. The mechanism of differential sensitivity of HRV and other viruses (Herniman *et al.* 1973), to mineral acids is unknown. However, if insoluble salts such as phosphates and sulphates are formed on the virion surface, they may inhibit disinfectant action.

Alcohol and alcohol-containing products are frequently used for both topical antisepsis and hard-surface disinfection. Their efficacy here, and in other studies of HRV disinfection (Kurtz, Lee & Parsons, 1980; Brade, Schmidt & Gattert, 1981; Tan & Schnagl, 1981) remains to be assessed on surfaces and under in-use conditions. The difference in effectiveness between aqueous (18) and alcoholic (19) cetrimide and chlorhexidine gluconate-containing formulations may be particularly important in the control of nosocomial HRV transmission because of their widespread use for handwashing by hospital personnel. Similar observations have been made for the bactericidal efficacy of these products (Jarvis *et al.* 1979; Gardner & Gray, 1983). In some effective formulations (e.g. 20) a low concentration of alcohol may be acting in an additive or synergistic manner with the other active ingredients.

Glutaraldehyde is often used as an instrument soak and the results here confirm that it is active against HRV. The slower reaction of formaldehyde is in accordance with previous work (Brade, Schmidt & Gattert, 1981; Tan & Schnagl, 1981; Sattar *et al.* 1983) and suggests that formaldehyde fixation of HRV-containing laboratory specimens requires at least 30 min.

This study, and kinetic data on rotavirus disinfection by chlorine (Grabow *et al.* 1983*a*, *b*; Berman & Hoff, 1984), show the interference by organic matter with the action of chlorine compounds on HRV. Harakeh & Butler (1984) compared the effect of chlorine on different rotaviruses and concluded that simian rotavirus was less resistant to inactivation than HRV, but the organic content of the two virus preparations was not comparable. The resistance of rotaviruses to chlorine is emphasized by comparison with polioviruses prepared and held under identical conditions: the low chlorine residual present in potable water reduced infectious poliovirus by $> 99{\cdot}9\,\%$ while having little or no effect on the titre of rotaviruses (Raphael, Sattar & Springthorpe, 1986). This may be highly significant in view of the use of chlorine for the terminal disinfection of drinking waters and sewage as well as the disinfection of swimming and wading pools. Neither buffered hypochlorites (Death & Coates, 1979) nor chlorine dioxide showed improved efficacy over hypochlorite solutions against HRV. The data from chloramine-T (1–2 %) suggest that chlorine-based disinfectants with free chlorine levels of 10000–20000 p.p.m. or greater may be effective against HRV; this remains to be confirmed in undiluted fecal material and on surfaces. The greater stability to temperature and sunlight and the lower odour associated with chloramine-T solutions may make them preferable to hypochlorites under certain conditions, but the higher concentrations necessary make them less cost effective.

Both halogen mixtures and iodophores appear to be more effective against HRV than chlorine compounds with an equivalent halogen concentration. In the former

case oxidation of potassium bromide (Cheremisinoff, Cheremisinoff & Trattner, 1981) may be the mechanism responsible whereas in the latter, iodine may react much more slowly than the lower halogens with interfering organics such as peptides (Gottardi, 1976).

Wide variations in the activity of phenolic compounds emphasise the caution that should be exercised in selecting a product of this type for HRV-disinfection. One of the two effective commercially-available phenolics identified (40) contains a substituted phenol not found in the other products tested (p-tertiary amyl phenol). Such para-substituents of short alkyl chains are known to have increased bactericidal properties (Suter, 1941) but similar studies on their virucidal efficacy have not been conducted. The other (48) contained a relatively high concentration of an anionic surfactant, which was completely ineffective against HRV when tested alone (9) at the same concentration. It remains to be determined if these phenolics are equally effective on HRV-contaminated surfaces.

There are probably now more commercially-available quaternary ammonium-based disinfectants than all other disinfectant classes combined. As a group quaternary ammonium compounds were relatively inactive against HRV in the presence of additional organic matter and no differential effect of various quaternary ammonium substituents against HRV was observed. However, certain formulations containing quaternary ammonium compounds in addition to active ingredients such as HCl, alcohols or sodium metasilicate were effective. Although these additional ingredients were all shown to be effective alone against HRV, their inclusion in quaternary ammonium based products may have resulted in disinfectants with wide applicability, and hence they deserve further investigation.

All studies of rotavirus disinfection have so far been conducted in suspension tests. Such tests are relatively simple to perform but it is vital to evaluate potential hard surface disinfectants, instrument soaks and topical antiseptics on contaminated surfaces as well. Disinfection and cleaning of environmental surfaces has been shown to limit transmission of animal rotaviruses (McNulty & Logan, 1983). Available circumstantial evidence on the transmission of HRV (Sattar *et al.* 1986) indicates that effective disinfection of environmental surfaces and the hands of attendant personnel may have a role to play in interrupting the transmission of human rotaviruses as well. Comparisons of suspension and carrier tests on antiviral agents are very limited, but where these have been made (Kirchhoff, 1969; Nakao *et al.* 1978; Klein & Deforest, 1983; Schurmann & Eggers, 1983) disinfection of contaminated surfaces appears much more difficult to achieve. We have examined the ability of the suspension test to act as a screening tool for surface disinfection of HRV in the accompanying paper (Lloyd-Evans, Springthorpe & Sattar, 1986). Selection of suitable chemical disinfectants for use in the control of rotavirus spread should not be based solely on the results of suspension tests reported here, but should also take into account how the disinfectants which were 'effective' in suspension tests performed on surfaces contaminated with HRV in faecal matter (Lloyd-Evans, Springthorpe & Sattar, 1986).

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