Efficacy of Widely Used Hand Disinfectants Against Human Echovirus Type 11 in China

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Research

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Abstract

Background: In the summer of 2019 an outbreak of Nosocomial infections in a newborn nursery occurred in a Shunde hospital near Foshan, resulting in five babies deaths. Echovirus-11 was proven to be the responsible agent. Echovirus-11 infections are a significant health threat in the hospitals and occasionally cause severe neurological complications and even death in children. Although good hand hygiene is important for controlling infection, relevant data regarding the efficacy of widely used hand disinfectants against Echovirus-11 are still lacking.

Aim: To investigate the virucidal activity of widely used hand disinfectants against Echovirus-11.

Methods: The 13 widely used hand disinfectants from Guangdong hospitals were tested for virucidal activity against Echovirus-11 with the identification test of residual disinfectants removal and the suspension test with a 60 s exposure time based on Technical Standard For Disinfection (2002).

Findings: Both Disinfectant A and Disinfectant C achieved a mean log10 reduction factor in Echovirus-11 titre of ≥ 5.00, respectively, within 60 s. Disinfectant B, Disinfectant D, Disinfectant H and Disinfectant M had an effect on Echovirus-11 in a mean log10 reduction factor of ≥ 4.00.By contrast, Disinfectant E, Disinfectant F, Disinfectant G, Disinfectant I, Disinfectant J, Disinfectant K and Disinfectant L didn't improved the mean log10 reduction factor in Echovirus-11 titre with producing a factor of 4 after a 60 s exposure time.

Conclusions: 46.2% (6/13 products) of widely used hand disinfectants have effectiveness against Echovirus-11. In general, it is risky to rely too much on hand disinfectants. In case of epidemic season or definite enterovirus contamination, washing hands with soap and water is recommended for reducing viral contamination of enterovirus 11 in clinical practice, rather than hand hygiene with alcohol-based hand disinfectants alone.

Introduction

Human echovirus-11 is a member of the human enterovirus B species and is one of the most commonly isolated enteroviruses [1] and contains a non-enveloped capsid with a single-stranded genome. EV11 viral capsid protein 1 (VP1) sequences segregate into six or more genogroups [2]. Echovirus-11, like other Echoviruses, is mostly associated with asymptomatic or mild infections, such as aseptic meningitis, uveitis, hemorrhagic hepatitis, and myocarditis. Besides asymptomatic or mild infections, it is also responsible for more serious disorders, such as aseptic meningitis (AM), multisystemhemorrhagic disease of newborn, and uveitis. And also, Echovirus- 11 is a causative agents of hand, foot, and mouth disease (HFMD) [3–4]. More seriously, Echovirus – 11 was frequently associated with nosocomial infections.

In the past decade, the number of Echovirus-11 outbreaks has been increasing in many countries around the world. Notably, a large-scale outbreak of nosocomial infections caused by Echovirus-11 was reported in China in 2019 with 19 cases and 5 deaths. Since eradication of poliovirus, Echovirus-11, just like EV71 and CA16, has become the most important neurotropic enterovirus, which has increased public health concern.

Echovirus-11 is highly contagious and can be isolated from stool specimens, cerebrospinal fluid, and throat swabs of sick children. As a result, Echovirus-11 transmission may occur through direct contact with infected people or through contact with respiratory secretions or stools of an infected person.

Subsequently, the virus can be spread from one person to another through the faecal-oral route via contamination of fingers, formites, utensils and food. Due to the long periods of viral shedding in children, Echovirus-11 is frequently transmitted in families, kindergartens, and schools [5].A nosocomial outbreak of Echovirus-11 in a newborn nursery has also been reported [6].

Hand hygiene is the most important intervention to prevent the transmission of pathogenic microorganisms and has been shown to reduce infection rates [7], even among high-risk patient populations [8]. Echovirus-11 is thought to be resistant to low pH conditions and organic solvents and can survive at room temperature for several days. Moreover, some hand disinfectants have been ineffective against Echovirus-11 [9–10]. Therefore, a lack of hand washing or the improper use of hand disinfectants after caring for infected persons can pose a serious public health threat.

The hand disinfectants have broad-spectrum bactericidal activity and have been extensively used for hand hygiene as recommended by Chinese CDC, the US CDC and ECDC. However, the virucidal activity of these disinfectants has been shown to be poor against selected non-enveloped viruses. Because of the increase in ECHOVIRUS-11 epidemics with high mortalities in many countries, the need to verify the expected virucidal activity of hand disinfectants against this virus has also significantly increased.

Nevertheless, most of our knowledge regarding the sensitivity of this virus to disinfectants is based on previous studies in closely related viruses, such as poliovirus and enterovirus [11] and few studies has so far been published on the virucidal activity of hand disinfectants against Echovirus-11 in detail.

In the present study, different hand disinfectants were analysed by an in vitro suspension test for anti-Echovirus-11 activity to offer an added advantage of improving prevention and control of hospital infection.

Methods

Virus propagation

Human Echovirus-11 were obtained from the Guangdong Provincial Center for Disease Preverntion and Control, China. Human embryonic rhabdomyosarcoma (RD) cells were inoculated with high-titre virus stocks for Echovirus-11. The viral growth medium for RD cells was modified Eagle medium (MEM) (Gibco) with 2% fetal calf serum (FCS) (Gibco); The culture flasks were maintained at 37℃ in 5 percent

CO_2 and examined daily until a cytopathic effect was observed. The viruses were released from infected cells by freezing and thawing the culture flasks three times. To eliminate cell debris, the suspensions were centrifuged at 4,000 rpm for 30 minutes. The final upper culture fluid was harvested, divided into aliquots, and stored at -70℃. The Echovirus-11 virus titres were determined by 50% tissue culture infective dose ($TCID_{50}$).

Infectivity assay

For titration of Echovirus-11 by the $TCID_{50}$ assay, RD cells were grown to 95 percent confluence in flat-bottom ninety-six-well plates (Corning ninety-six-well plates). Then, virus samples were diluted over a tenfold dilution series in MEM supplemented with 2% FCS. The medium in each well was discarded and replaced with 150 μL of a diluted virus sample. Infected plates were then incubated for 7 days, after which inverted microscopy was used to differentiate infected from non-infected wells. The highest dilution of virus suspension that produced a cytopathic effect in 50% of cell monolayers was determined under microscope observation. $TCID_{50}$ was calculated by the Reed and Muench method.

Test hand disinfectants

There are 13 widely-used hand disinfectants for testing from the hospitals in Guangdong. The active components and contents are shown as Table 1.

Table 1
The active component and content for hand disinfectant products

Disinfectant	The active component content
A	Sodium hypochlorite(effective chlorine, 0.037%)
B	Ethanol(75%-85%, v/v) and triclosan (0.4%-0.5%, w/v)
C	Ethanol(63%-77%, v/v) and PHMB (0.45%-0.55%, v/v)
D	Ethanol (63.1%-77%,w/v) and Chlorhexidine glucanate (0.45%-0.55%, v/v)
E	Ethanol (63%-77%, w/v) and Chlorhexidine glucanate (0.45%-0.55%, v/v)
F	Ethanol(50%-60%, v/v) and triclosan (0.48%-0.58%, w/v)
G	Chlorhexidine glucanate (2500 mg/L ± 250 mg/L) and PHMB (2500 mg/L ± 250 mg/L)
H	Ethanol (63%-77%) and PHMB (1050 mg/L ± 105 mg/L)
I	DOAC (3000 mg/L-3500 mg/L) and hydrogen peroxide(1800 mg/L-2200 mg/L)
J	Ethanol (60%±7%, w/w) and isopropanol(10%±1%, w/w)
K	Ethanol(50%-60%, w/w) and PHMB·HCl(0.36%-0.44%, w/w)
L	Ethanol(70%±7%)
M	Ethanol (72%-82%, v/v) and hydrogen peroxide(0.1%-0.14%, w/v)

Removal of residual disinfectant

The removal of residual disinfectant were performed according to the guidelines laid down by Ministry of Health of China for Technical Standard For Disinfection(2002) and Test method for bactericial effect of disinfectant in Laboratory(GB/T 38502 – 2020) with a modification to the evaluation of virucidal activity by means of the dilution–ultracentrifugation method. The exposure period is 1 min. The test was divided into 6 groups: Virus suspension (0.2 mL) was mixed with 0.8 mL of the test hand disinfectant in a 50 mL tube;Virus suspension (0.2 mL) was mixed with 0.8 mL of the test hand disinfectant in a 30 mL tube. Following the exposure period, 29 mL deionized water was added;Virus suspension (0.2 mL) was mixed with 29.8 mL deionized water in a 15 mL tube;The test hand disinfectant (0.8 mL) was mixed with 29 mL deionized water in a 50 mL tube and then 0.2 mL virus suspension was added;virus suspension (0.2 mL) is combined with 0.8 deionized water;RD cells was as the negtive control. The mixture was vortexed for 10 s and incubated at 20°C for 1 min. The mixture (2 mL) was ultracentrifugated by 10000 rpm at 4°C for 2.5 h. The supernatant was discarded and the precipitates resuspended with 1 mL MEM. The mixture (1 mL) was diluted by serial 10-fold dilutions in MEM supplemented with 2% FCS. The virus titre from to was determined by $TCID_{50}$.

Suspension test

The suspension test was performed using a modification of ASTM standard E-1052 [12]and Regulation of Disinfection Technique in China (2002). Virus suspension (0.2 mL) was mixed with 0.8 mL of the test hand disinfectant in a 15 mL tube. The mixture was vortexed for 10 s and incubated at 20°C for 1 min. Following the exposure period, 0.1 mL of the mixture was neutralized by ultracentrifugation-dilution method as previously mentioned. The virus titre was determined by $TCID_{50}$. A reduction of infectivity of $\geq 4 \log_{10}$ steps (inactivation 99.99%) was regarded as evidence for sufficient virucidal activity against the tested virus [13–14].The mean log10 reduction factor was calculated by taking the mean of the log of the difference between the virus titre of each hand disinfectant and the virus titre of the virus control.

Statistical Analyses

All experiments were repeated at least three times, and data are expressed as the mean ± one standard deviation around the mean (SD). The one-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used to make pairwise comparisons between the treatment group means. The difference was considered statistically significant at $P < 0.05$.

Results

Identification of removal of residual disinfectant

A total of 13 kinds of hand disinfectants were carried out. All the tests were in full compliance with the judgment requirement in suspension quantitative test for identification of neutralization effect There was low virus titer in the 1st group. The virus titer in the 1st group was low; The virus titer in the 2nd group was more than that in the 1st group, but less than that in the 3rd, 4th or 5th groups; The virus titers in the third, fourth and fifth groups were similar, which indicated that the dilution method could effectively terminate the residual effect of disinfectant on Echovirus-11. All groups meet the requirement from Technical Standard For Disinfection in China (2002). The dilution method had no effect on the virus or cells (Table 2).

Table 2
Test results of removal of residual disinfectant by ultracentrifugation-dilution method(mean ± SD)

Disinfectant	Echovirus-11					
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
A	0.00 ± 0.00	0.00 ± 0.00	6.67 ± 0.66	7.00 ± 0.94	7.00 ± 0.38	0.00 ± 0.00
B	1.50 ± 0.06	2.33 ± 0.19	6.67 ± 0.59	6.40 ± 0.77	7.00 ± 0.42	0.00 ± 0.00
C	1.50 ± 0.12	1.67 ± 0.22	6.33 ± 0.72	6.50 ± 0.65	7.00 ± 0.32	0.00 ± 0.00
D	2.00 ± 0.10	2.33 ± 0.19	6.23 ± 0.77	6.50 ± 0.78	7.00 ± 0.44	0.00 ± 0.00
E	3.50 ± 0.18	3.00 ± 0.25	6.00 ± 0.69	6.23 ± 0.96	7.00 ± 0.35	0.00 ± 0.00
F	5.00 ± 0.22	5.33 ± 0.33	6.50 ± 0.83	6.67 ± 1.03	6. 67 ± 0.56	0.00 ± 0.00
G	4.50 ± 0.21	5.00 ± 0.48	6.50 ± 0.98	6.55 ± 0.88	6.67 ± 0.39	0.00 ± 0.00
H	1.67 ± 0.09	2.67 ± 0.27	6.00 ± 0.67	6.00 ± 0.82	6.67 ± 0.40	0.00 ± 0.00
I	4.33 ± 0.19	4.67 ± 0.36	6.33 ± 0.76	6.50 ± 0.65	6.67 ± 0.65	0.00 ± 0.00
J	4.50 ± 0.22	5.22 ± 0.51	6.50 ± 0.58	6.72 ± 0.73	6.67 ± 0.34	0.00 ± 0.00
K	4.67 ± 0.30	5.33 ± 0.51	6.50 ± 0.43	6.00 ± 0.66	6.50 ± 0.58	0.00 ± 0.00
L	4.00 ± 0.31	5.23 ± 0.45	6.44 ± 0.69	6.39 ± 0.49	6.50 ± 0.49	0.00 ± 0.00
M	1.50 ± 0.12	2.33 ± 0.31	6.33 ± 0.60	6.44 ± 1.08	6.50 ± 0.50	0.00 ± 0.00

Virucidal activities of 13 widely-used hand disinfectant products against Echovirus-11 in the suspension test

The effectiveness of different hand disinfectant products against Echovirus-11 was determined by in vitro suspension test using Regulation of Disinfection Technique in China (2002).The virucidal activities of the hand disinfectants were assessed at stock solution with an exposure time of 1 min. As shown in Table 2, 0.037% sodium hypochlorite (Disinfectant A), was most effective against Echovirus-11, produced the mean $\log_{10}$ reduction factor 6.50 in virus titre against Echovirus-11. Disinfectant B, C, D, H and M could improve the mean log10 reduction factor in Echovirus-11 titre with producing the factors of 4.17, 4.83, 4.00 and 4.00, respectively. Conversely, Disinfectant F, G, I, J, K and L had shown less effective against Echovirus-11 with a mean log10 reduction factor of 1.50 (Table 3).

Table 3
Activity of thirteen hand disinfectants against Echovirus-11 in the test(mean ± SD)

	Viral tire in positive control($TCID_{50}$)	1 min	
		Viral tire($TCID_{50}$)	Mean log10-reduction of viral infectivity
A	6.50 ± 0.00	0.00 ± 0.00	6.50
B	6.50 ± 0.00	2.33 ± 0.54	4.17
C	6.50 ± 0.00	1.67 ± 0.19	4.83
D	6.50 ± 0.00	2.50 ± 0.18	4.00
E	6.50 ± 0.00	3.67 ± 0.26	2.83
F	6.50 ± 0.00	5.00 ± 0.44	1.50
G	6.50 ± 0.00	5.00 ± 0.53	1.50
H	6.50 ± 0.00	2.50 ± 0.29	4.00
I	6.50 ± 0.00	5.00 ± 0.69	1.50
J	6.50 ± 0.00	5.00 ± 0.79	1.50
K	6.50 ± 0.00	5.00 ± 1.09	1.50
L	6.50 ± 0.00	5.00 ± 0.72	1.50
M	6.50 ± 0.00	2.50 ± 0.33	4.00

Discussion

Echovirus-11 is the main pathogenic virus in neonatology, especially in neonatal intensive care unit (NICU), which was a serious threat to newborns. Transmission via contaminated hands may be a contributing factor in causing nosocomial infection by Echovirus-11. Therefore, hand hygiene is an important measure to prevent and control nosocomial infections including Echovirus-11. Effective prevention and control against Echovirus-11, was not only to improve the medical staff hand hygiene compliance, but also need to determine whether hand disinfectants has inactivation effect on Echovirus-11.

This study has evaluated the effectiveness of 13 hand disinfectants used in Guangdong hospitals against Echovirus-11.The suspension test were performed to determine the effectiveness of these hand disinfectants. In our study, thirteen kinds of hand disinfectants widely used in Guangdong hospitals were selected with a 60 s exposure time. Considering that the choice of the suitable neutralizer for 13 hand disinfectants is time-consuming and laborious, the study has employed a modification dilution-method based ultracentrifugation[15]. According to the test results, disinfectant A (0.037%, effective chlorine) has the best virucidal effect on Echovirus-11, which can be used as the first choice of the hand disinfectant for clinically virucidal activity against Echovirus-11, involving in the mechanism to destroy the local fragment of nuclear acid to achieve the virucidal effect [16].

The disinfectant B and disinfectant F contain the same active ingredients. The disinfectant B (ethanol 75%-85% and trichlorohydroxydiphenyl ether 0.4%-0.5%) can exhibit high virucial activity against echovirus-11 in 60 s, conversely, disinfectant F produced the poor effect on echovirus-11, which may be related to the different ethanol concentration or the enhancement of virucidal effect by adding other components into disinfectant [17–18].

The disinfectant D and disinfectant E containing chlorhexidine dextrinate and ethanol have broad-spectrum antimicrobial activity against bacteria, fungi, and enveloped viruses. Their major antimicrobial activity can be attributed to the ability of the alcohol to denature proteins [19].Addition of chlorhexidine dextrinate to ethanol can provide substantial residual activity over ethanol alone [20].However, both of them had shown different virucidal effect on echovirus-11. The disinfectant D can produce $\geq$ 4.00 reduction(high effectiveness)in echovirus-11 titre in 60 s, however, which is less effective in E than that in D. The hand disinfectant C with ethanol (63%～77%) and polyhexamethylene biguanide (0.45%～0.55%) can exhibit virucidal effect on echovirus-11, however, its effect on other enteroviruses is unknown and needs further study, which should be cautious in clinical application.

All of the disinfectants G (chlorhexidine gluconate 2250 ± 250 mg/L, polyhexamethylene biguanide 2250 ± 250 mg/L), H(ethanol 63%-77%,1050 ± 105 mg/L polyhexamethylene biguanide), I(3000–3500 mg/L bis octadecyl dimethyl ammonium chloride, 1800–2200 mg / L hydrogen peroxide), J (ethanol 60% ± 7%, isopropanol 10% ±1%), and K (polyhexamethylene biguanidine hydrochloride 0.36%) can not exhibit virucidal activity (a $\log_{10}$ reduction of 4.00) at the set exposure time. The virucidal effect of these disinfectants depend heavily on pH value and ethanol content. These results are in agreement with previous studies in which a higher concentration of ethanol exhibited better virucidal activity against non-enveloped virus than did lower concentration [21].Therefore, these hand disinfectants were not suitable for clinical use to inactivate echovirus-11.

hand disinfectants are widely use in hand hygiene; however, their effectiveness is largely dependent on the type and concentration of active ingredients. Disinfectants A(effective chlorine 370 mg/L), B (ethanol 75%-85%, trichlorohydroxydiphenyl ether 0.4%-0.5%) and C (polyhexamethylene biguanide 0.45%-0.55%, ethanol 63%.) can exhibit virucidal activity against echovirus-11 with 60 s exposure time, about 23.1% in 13 hand disinfectants, indicating that there was a great risk of over-reliance on hand disinfectants in clinical hand hygiene to block the enterovirus transmission. Our results is consistent with the World Health Organization recommendations that hand washing with soap and running water is the main hand hygiene method[22–23]. Therefore, in epidemic season or the existence of enterovirus pollution, the preferred method of hand hygiene is washing with soap and running water.

Declarations

Conflict of interest statement

None declared

Authors' contributions

Z.L., J.X. Z.J.and Z.Y. conceived and designed the experiments. Z.L. and J.X. performed most of the experiments. P.T., H.C. and Z.X. provided support for virus propagation. Z.Y., X.W. and Z.X. contributed technical assistance for suspension test. Z.L. and J.X. analyzed the data and drafted the manuscript.

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