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Efficacy of alcohols and alcohol-based hand disinfectants against human enterovirus 71

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SUMMARY

Background: Human enterovirus 71 (HEV71) infections are a significant public health threat in the Asia–Pacific region 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 HEV71 are still lacking.

Aim: To investigate the virucidal activity of alcohols and alcohol-based hand disinfectants against HEV71.

Methods: A common alcohol-based hand disinfectant (0.5% chlorhexidine gluconate + 70% isopropanol) as well as different concentrations of isopropanol and ethanol were tested for virucidal activity against HEV71 using the suspension and the fingerpad tests.

Findings: In suspension tests, 85% and 95% ethanol achieved a mean $\log_{10}$ reduction factor in HEV71 titre of >3 and nearly 6, respectively, within 10 min. By contrast, 70% and 75% ethanol and any concentration of isopropanol (70–95%) produced a factor of <1 in this test after the same exposure time. In fingerpad tests, only 95% ethanol showed a mean $\log_{10}$ reduction factor of >4, while both 75% ethanol and a chlorhexidine gluconate-containing formula were ineffective against HEV71 with a mean $\log_{10}$ reduction factor of <1 after a 30 s exposure time.

Conclusions: Widely used alcohol-based hand disinfectants based on 70% ethanol or isopropanol have poor effectiveness against HEV71. Ninety-five percent ethanol is the most effective concentration, but still cannot fully inactivate HEV71 and may be impractical for use in many instances. Hand hygiene with alcohol-based hand disinfectants alone is not recommended for preventing HEV71 transmission.

Introduction

Human enterovirus 71 (HEV71) is a member of the *Enterovirus* genus in the Picornaviridae family and contains a non-enveloped capsid with a positive-sense RNA genome. Several enteroviruses are widespread causative agents of hand, foot, and mouth disease (HFMD).[1,2] HFMD caused by HEV71 is occasionally

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associated with severe neurological diseases, including aseptic meningitis, brainstem encephalitis, polio-like paralysis, and fatal neurogenic pulmonary oedema.[3] In the past decade, the number of HEV71 outbreaks has been increasing with deaths reported in many countries around the world.[4–12] The largest HFMD outbreak to date occurred in Taiwan in 1998, during which 129,106 cases of HFMD/herpangina (including 405 severe cases and 78 deaths) were reported.[12] Notably, a large-scale outbreak of HEV71 was reported in China in 2008 with nearly 500,000 HFMD cases and more than 120 deaths.[5] Since eradication of poliovirus, HEV71 has become the most important neurotropic enterovirus, representing an emerging public health concern, particularly in Asia–Pacific regions.[13]

HEV71 is highly contagious and can be isolated from throat swabs, rectal swabs, and stool specimens of sick children. Virus shedding can persist for nearly four weeks in the respiratory tract and for up to five weeks through stools.[14,15] As a result, HEV71 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 by contaminated hands or objects.[12] Due to the long periods of viral shedding in children, HEV71 is frequently transmitted in families, kindergartens, and schools.[16] A nosocomial outbreak of HEV71 in a newborn nursery has also been reported.[17]

Good personal hygiene practices, in particular hand washing with soap or other detergents, are extremely important for infection prevention and control. However, similar to other enteroviruses, HEV71 is thought to be resistant to low pH conditions and organic solvents and can survive at room temperature for several days.[18,19] Moreover, frequently used disinfectants have been ineffective at inactivating these viruses.[20] 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.

Alcohol-based hand disinfectants have broad-spectrum bactericidal activity and have been extensively used in healthcare settings for hand hygiene as recommended by the US Centers for Disease Control and Prevention guidelines.[21] However, the virucidal activity of these disinfectants has been shown to be poor against selected non-enveloped viruses.[22,23] Because of the increase in HEV71 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. Unfortunately, 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 echovirus.[24] In our study, different concentrations of isopropanol and ethanol, the main active ingredients in alcohol-based disinfectants, were analysed by an *in vitro* suspension test for anti-HEV71 activity. In addition, 75% and 95% (v/v) ethanol and a commercial alcohol disinfectant containing 70% isopropanol (v/v) and 0.5% chlorhexidine gluconate were also assessed for virucidal activities against HEV71 by an *in vivo* fingerpad test.

Methods

Virus propagation

HEV71 TW/72232/2004 and influenza virus A/72673/2007(H1N1) were obtained from the Clinical Virology Laboratory of Chang Gung Memorial Hospital. Human embryonic rhabdomyosarcoma (RD) cells and Madin–Darby canine kidney (MDCK) cells were inoculated with high-titre virus stocks for HEV71 and influenza A virus propagation, respectively. The viral growth medium for RD cells was Dulbecco's modified Eagle's medium (DMEM) with 2% fetal calf serum (FCS); that for MDCK cells was serum-free DMEM containing 2 μg/mL trypsin. The culture flasks were maintained at 35 °C and examined daily until a cytopathic effect was observed. For HEV71, the cells and supernatant were collected, frozen, and thawed thrice to release viruses from cells. The final culture fluid was harvested, divided into aliquots, and stored at −70 °C. The influenza A and HEV71 virus titres were determined by plaque assay and by 50% tissue culture infective dose ($TCID_{50}$), respectively.

Infectivity assay

To perform the plaque assay for influenza virus, MDCK cell monolayers were prepared in six-well plates. After the monolayers had been washed with phosphate-buffered saline, serial 10-fold dilutions of the virus were added on to cells and incubated for 1 h at 35 °C for virus adsorption. Unadsorbed virus was removed, and each well was covered with 3 mL agar medium. Following a 72 h incubation period, the monolayers were fixed with 10% formalin and stained with 0.1% Crystal Violet. Visible plaques were counted and viral titres of plaque-forming units (pfu)/mL were determined.

For titration of HEV71 by the $TCID_{50}$ assay, RD cells were prepared in 96-well plates. The confluent cell monolayers were adsorbed with serial dilutions of the virus or virus mixed with different concentrations of alcohols. After 1 h at 35 °C for virus adsorption, the cells were overlaid with 50 μL culture medium and incubated at 35 °C for 72 h. At the end of incubation, the cell monolayers were fixed with 10% formalin. 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.[25]

Hand disinfectant and chemicals

Hibisol (Panion & BF Biotech Inc., Taipei, Taiwan), which was the hand disinfectant used in this study, contains 70% isopropanol in combination with 0.5% chlorhexidine gluconate. Ethanol and isopropanol were purchased from Merck KGaA (Darmstadt, Germany) to determine effective concentrations of alcohol against HEV71; 70–95% ethanol and isopropanol (v/v) solutions were prepared in distilled water.

Suspension test

The suspension test was performed based on ASTM standard E-1052.[26] Virus suspension (0.5 mL) was mixed with 4.5 mL of the test chemicals in a 15 mL tube. The mixture was vortexed for 10 s and incubated at 20 °C for 10 min. Following the exposure period, 0.1 mL of the mixture was neutralized by serial 10-fold dilutions in DMEM supplemented with 2% FCS. The virus titre was determined by $TCID_{50}$. The mean $\log_{10}$ reduction factor was calculated by taking the mean of the log of the difference between the virus titre of each test and the virus titre of the virus control. Controls examining neutralization and cytotoxicity were performed at the highest concentrations