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Short communication

Efficacy of chemical disinfectant compounds against human norovirus

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ABSTRACT

The purpose of this study was to evaluate the efficacy of various disinfectant compounds against human norovirus (NoV). We investigated the disinfection effects of ethanol, sodium hypochlorite, hydrogen peroxide, quaternary ammonium compounds, and iodine using an anti-NoV GII.4 monoclonal antibody-conjugated immuno-magnetic separation (IMS) technique combined with quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR). Ten-minute treatments of samples containing NoV GII.4 with 10–70% ethanol resulted in mean $\log_{10}$ reductions in genomic copies/μL of less than 1. In contrast, 10-min treatments with sodium hypochlorite at 200, 500, and 1000 ppm resulted in mean $\log_{10}$ reductions of 1.55, 1.85, and 2.45, respectively; however, 50 and 100 ppm sodium hypochlorite had no disinfection effect, as shown by the $\log_{10}$ reductions of less than 1. Treatment with hydrogen peroxide (200–1000 ppm), quaternary ammonium compounds (100–2000 ppm), and iodine (25–500 ppm), also exhibited no disinfection effect. The results of this study show that NoV GII.4 is remarkably resistant to most disinfectants and suggests that new disinfectant compounds are needed to inactivate foodborne bacteria and viruses, especially NoV GII.4.

1. Introduction

Human noroviruses (NoVs) are recognized as a major cause of foodborne illnesses worldwide. NoV genotype GII, more precisely GII.4, is the leading cause of NoV infections, representing more than 75% of confirmed cases. NoV transmission occurs mostly via the fecal-oral route, through direct person-to-person transmission, and from food handlers with poor personal hygiene practices. In addition, spread by air-borne droplets of infected vomitus is also possible (Tian et al., 2008). The impact of foodborne viral pathogens such as NoV on human health can be substantial (Appleton, 2000).

Among the treatments that effectively reduce pathogens, chemical disinfectants are considered to be key in stopping transmission and are recognized as an effective strategy for controlling the spread of pathogens. Ethanol, chlorine, quaternary compounds, and hydrogen peroxide are the most commonly used disinfectants due to their convenience, safety, low cost, residual biocide effect, and superior efficacy against various bacteria and viruses. In previous studies, ethanol has been used against feline calicivirus (FCV) (Gehrke, Steinmann, & Goroncy-bermes, 2004), and chlorine (Duizer et al., 2004; Malik & Goyal, 2006) and quaternary ammonium (Jimenez et al., 2006) have been used to inactivate FCV and murine NoV (MNV). However, only a limited number of studies have reported the effectiveness of chemical disinfectants against human NoV due to the considerable difficulties in detecting, identifying, and recovering viruses from food and environmental samples.

Human volunteer studies have been performed to estimate inactivation techniques for NoV; however, such studies are expensive to carry out and logistically complicated, limiting their use and the amount of data that can be obtained (Leon et al., 2011). As a result, most studies of NoV inactivation typically use surrogates such as culturable FCV (Tree, Adams, & Lees, 2005; Hudson, Sharma, & Petric, 2007), MNV (Kingsley, Holliman, Claci, Chen, & Flick, 2007; Lim, Kim, & Ko, 2010; Kim et al., 2012), and Tulane virus (Li, Ye, Neetoo, Golovan, & Chen, 2013).

However, although the above-mentioned viruses are surrogates, the chemical resistance characteristics of FCV and MNV have been shown to differ from those of NoV (Doultree, Druce, Birch, Bowden, & Marshall, 1999; Cannon et al., 2006; Park, Linden, & Sobsey, 2011; Sattar, Ali, & Tetro, 2011; Kingsley, Vincent, Meade, Watson, & Fan, 2014). Recently, several studies have determined the *in vitro*

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efficacy of various disinfectant treatments against FCV, MNV, or NoV using molecular methods such as RNA extraction and real-time reverse transcription polymerase chain reaction (RT-PCR). The presence of organic and inorganic RT-PCR inhibitors in environmental samples and insufficient concentrations of specific virus particles for subsequent PCR amplification are two major obstacles for accurate recovery of NoV using these methods. Several methods based on ultracentrifugation (Cheong, Lee, Choi, & Kim, 2009; Scherer et al., 2010), polyethylene glycol precipitation (Scherer et al., 2010), immuno-magnetic separation (IMS) (Tian et al., 2008; Morton, Jean, Farber, & Mattison, 2009; Yang et al., 2011), and adsorption-elution (Morales-rayas, Wolffs, & Griffiths, 2009) have been used to concentrate specific virus particles. Recently, a porcine gastric mucin-magnetic bead (PGM-MB) binding assay has also been evaluated as an alternative method for assessment of NoV inactivation (Dancho, Chen, & Kingsley, 2012; Kingsley et al., 2014). In this study, we investigated the efficacy of various chemical disinfectants against NoV in suspension using an IMS procedure combined with quantitative real-time RT-PCR (qRT-PCR).

2. Materials and methods

2.1. Virus origin

NoV-positive stool samples, containing genotype GII.4 viral particles, were kindly provided by Kim Laboratories, Inc. (Rantoul, IL, USA). The presence of GII.4 NoV was confirmed by RT-PCR and sequencing. Stool samples were diluted in RNase-free water (Quanta Biosciences; Gaithersburg, MD, USA) to obtain a 40% suspension, vortexed briefly, and clarified by centrifugation at 550 × *g* for 2 min to remove the solids, and then the supernatant was stored in 1-mL aliquots at −80 °C until use (Ha et al., 2015).

2.2. Sanitizing solutions

Ethanol (99% fermented ethanol, anhydrous; Fisher Scientific; Waltham, MA, USA) was diluted with deionized, sterile water to 10%, 30%, 50%, and 70% and was adjusted to pH 7.2. 3000 ppm Sodium hypochlorite (Kirbychlor; Schering-Plough, Ltd.; Kenilworth, England) was diluted with deionized, sterile water to 50, 100, 200, 500, and 1000 ppm and was adjusted to pH 7.0. Alkyl dimethyl benzyl ammonium chloride (40%)/alkyl dimethyl ethyl benzyl ammonium chloride (40%) solution, containing quaternary ammonium compounds (Mason Chemical Co.; Arlington Heights, IL, USA) was diluted with deionized, sterile water to 200, 1,000, and 2000 ppm. Hydrogen peroxide (49.9% Huwa-san TR-50; Oam Chemical NV; Houthalen, Belgium), was diluted with deionized, sterile water to 100–2000 ppm and was adjusted to pH 6.8 as a chemical disinfectant. Iodine (99.99% trace metal basis; Sigma–Aldrich; St. Louis, MO, USA) was diluted with deionized, sterile water to 25, 100, 250, and 500 ppm.

2.3. Evaluation of chemical disinfectants

The efficacy of the test chemical disinfectants was estimated using a modified European CEN EN 1276 method (Dilution-Neutralization Method) based on quantitative suspension testing (Agrahar–murugkar & Subbulakshmi, 2005; Koivunen & Heinonen-Tanski, 2005). The Korean Food and Drug Administration (KFDA) uses the CEN EN 1276 as an official method. In this study, 20 μL of a 40% NoV GII.4 stool sample suspension (containing approximately 10^5 NoV GII.4 genome copies) was suspended in each virucidal disinfectant solution. After exposure, the number of NoV genome copies was determined by the IMS method combined with qRT-PCR. Suspension tests of the virucidal activity of the disinfectants were performed as described previously by Liu, Yuen, Hsiao, Jaykus, and Moe (2010). Briefly, 20 μL of the 40% NoV GII.4 stool suspension was mixed with 180 μL of either a test disinfectant solution, PBS (negative control), or 50% Clorox bleach (positive control; containing 20,000 ppm sodium hypochlorite). The virus-disinfectant (or control) mixture was quickly vortexed and incubated for 10 min at room temperature as indicated in the CEN EN 1276 procedure. Immediately following the exposure period, 40 mL of PBS was added to the mixture to neutralize the disinfectant reaction (Fig. 1). Fig. 1 shows a flow diagram of the in suspension test of disinfectants against NoV GII.4 using the IMS/qRT-PCR method.

2.4. IMS procedure combined with RT-qPCR

In order to concentrate the NoV GII.4, IMS technique combined with qRT-PCR was prepared and conducted as described previously (Ha et al., 2015). The qRT-PCR standard curves generated using these serial dilutions of the GII.4 standard RNA transcript were linear, with a slope of −3.2318 and a coefficient of determination (R^2) >0.9975. In this study, the entire mixture, containing 40 mL of PBS (as a neutralizing agent) and 200 μL of test disinfectant solution, was mixed with 10 μL of IMS beads and incubated for 1 h at room temperature with constant shaking to capture NoV GII.4. Beads with captured NoV GII.4 were collected with the Poly-ATtract® System 1000 (Promega; Madison, WI, USA) and washed with PBS. Finally, the beads were suspended in 140 μL of 1 × PBS and transferred to a 50-mL sterile conical tube.

2.5. Statistical analysis

Triplicate samples were used, and the experiments were repeated twice. One-way analysis of variance was performed using SPSS software, and Duncan's multiple range test was used to compare differences among mean values. *P*-values less than 0.05 were considered significant. The results are expressed as $\log_{10}$ genomic copies/10 μL, and the results were plotted using SigmaPlot software (ver. 7.0).

3. Results and discussion

In this study, we applied an IMS/qRT-PCR method to evaluate the efficacy of various chemical disinfectants against NoV GII.4 in suspension tests. The detection and quantification method combines an IMS method described by Tian et al. (2008) to determine viral concentration with a real-time RT-PCR technique that was developed by Schultz, Saadbye, Hoorfar, and Nørrung (2007) for the quantitative assessment of virus in inoculated shellfish. Since then, IMS has been used successfully by some researchers to replace enrichment in selective media (Coleman, Chick, & Nye, 1995). The IMS/qRT-PCR method has also been used to concentrate the virus and minimize the PCR inhibitors present in a food matrix and other materials. Several studies using the IMS/qRT-PCR method have reported that IMS beads can capture intact and infectious viruses and efficiently remove RT-PCR inhibitors (Morton et al., 2009; Tian et al., 2008).

Alcohols, when used as a disinfectant, is usually in the form of ethyl or isopropyl alcohol. Both of these chemical compounds rapidly and effectively inactivate foodborne pathogenic bacteria such as *Bacillus cereus*, *Cronobacter sakazakii*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* Typhimurium, and *Listeria monocytogenes* (LeguerineL & Mafart, 2001; Ölmez & Aran, 2005). The effects of ethanol treatment on NoV in the suspension test are shown in Table 1. In this test, PBS solution was used as the negative control, and a 50% Clorox solution was used as the positive control. The titer of the NoV GII.4 sample treated with the PBS solution was