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SHORT COMMUNICATION

Antiviral activity of Ecasol against feline calicivirus, a surrogate of human norovirus

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Summary Human norovirus (NoV) is a major cause of acute gastroenteritis in closed settings such as hospitals, hotels and cruise ships. The virus survives on inanimate surfaces for extended periods of time, and environmental contamination has been implicated in its transmission. The disinfection of contaminated areas is important in controlling the spread of NoV infections. Neutral solutions of electrochemically activated (ECA)-anolyte have been shown to be powerful disinfectants against a broad range of bacterial pathogens. The active chemical ingredient is hypochlorous acid (HOCl), which is registered as an approved food contact surface sanitizer in the United States by the Environmental Protection Agency, pursuant to 40 CFR 180.940. We evaluated the antiviral activity of Ecasol (an ECA-anolyte) against feline calicivirus (FCV), a surrogate of NoV. FCV dried on plastic surfaces was exposed to Ecasol for 1, 2, or 5 min. After exposure to Ecasol, the virus titers were compared with untreated controls to determine the virus inactivation efficacy after different contact times. Ecasol was found to decrease the FCV titer by $>5 \log_{10}$ within 1 min of contact, indicating its suitability for inactivation of NoV on surfaces.

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Introduction

In recent years, there have been several outbreaks of acute gastroenteritis, predominantly in closed settings, including institutionalized housing, hotels and cruise ships [1]. Epidemiological investigations have confirmed that $>95\%$ of these outbreaks, especially on cruise ships, are caused by

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human norovirus (NoV) [2]. NoV is a non-enveloped, single-stranded RNA virus belonging to the family *Caliciviridae* and is one of the most common causes of acute gastroenteritis in humans. This virus is shed in high concentrations (up to 11 log₁₀ per gram of feces) and has a low infectious dose of <100 infectious virus particles [3]. Environmental contamination has been implicated in the transmission of NoV because the virus is able to survive for days to months on different types of surfaces [4].

Cleaning and disinfection of contaminated surfaces are important procedures for controlling outbreaks of NoV in hospital and community settings [4]. Although the use of alcohol-based hand rubs has been promoted to control the spread of infection, alcohol has a limited effectiveness in killing NoV [5]. Various virucides are commonly used to disinfect fomites and environmental contact surfaces implicated in NoV outbreaks. The material safety data sheets and labels for these virucidal compounds rarely allow for their aerosolization, spraying, or fogging due to their toxicity and adverse health effects for given exposure durations and concentrations. Many of these chemical compounds, such as sodium hypochlorite, chlorine gas, and glutaraldehyde, have been associated with occupational illnesses. For example, exposure to glutaraldehyde is associated with contact dermatitis in health workers, and the use of quaternary ammonium compounds has been found to cause occupational asthma in users [6,7]. For cases in which aerosolization is approved, the use of personal protective equipment and a self-contained breathing apparatus is required, which makes the use of these compounds difficult, especially in public places such as hospitals or schools.

Ecasol is a unique electrochemically activated (ECA)-neutral pH anolyte, which consists of an "activated" solution, produced by a process referred to as dilute brine electrolysis. Based on Faraday's laws of electrolysis, advanced continuous process ECA membrane cell manufacturing was pioneered in the 1970s in the former Soviet Union [8] and was then advanced to its current form by Trustwater (Clonmel, Ireland). Ecasol has been demonstrated as a powerful disinfectant and has been shown to be efficacious against a wide range of microorganisms in solution and when sprayed in the air [9,10]. Another significant benefit of Ecasol is its lack of toxicity at ready-to-use (RTU) concentrations. It is considered safe in food processing applications by the United States Food and Drug Administration [11]. In dental procedures, Ecasol has been shown to have no adverse effects on human oral tissues [12].

ECA technology involves the generation of electrochemically activated solutions by passing a carefully regulated electric current through a brine solution in specialized electrode compartments and separating the ions according to charge. Ecasol is a positively charged solution emerging from a Trustwater generator. It is a strong oxidizing solution, with a pH of 7.0, a redox potential of +1200 mV, and an active chlorine content of approximately ~700 mg L⁻¹. Hypochlorous acid (HOCL) is the major component of Ecasol, which also contains free radicals and a small amount of sodium chloride (NaCl). As the free radicals gradually lose energy and reform as water, HOCL dissociates into hydrogen and hypochlorite ions, which eventually revert to NaCl (<0.2%) and water (>99.8%). The water evaporates, leaving salt crystals that can be removed by routine cleaning.

We undertook this study to evaluate the effectiveness of Ecasol for decontaminating surfaces contaminated with NoV. Because NoV is currently non-cultivable in vitro, efficacy tests of disinfectants rely on the use of surrogates, e.g., feline calicivirus (FCV) or murine norovirus (MNV). In this study, we used FCV as a surrogate for NoV.

Materials and methods

Virus

Strain 255 of FCV was propagated in Crandell-Reese Feline Kidney (CRFK) cells, and aliquots of the virus were stored at -80 °C until use.

Preparation of ECA anolyte

The Ecasol anolyte solution was prepared on the day of the test using a fully automatic ECA device (Trustwater model AQ-50). This device electrolyzes dilute brine solution using a membrane-type electrolytic cell controlled to produce two metastabilized electrolyte streams in dynamic equilibrium. The catholyte stream (AversolTM by Trustwater) has a high pH and is classified as an amphoteric surfactant, having reduced surface tension and mild detergent-like properties. Trustwater's automated process uses this solution to maintain the Ecasol stream at a neutral pH. The new Ecasol solution was titrated at 700 ppm free available chlorine (FAC), with a pH of 6.7. The solution was delivered to the lab on the day of the experiment and was used immediately upon delivery, with a time from solution generation to lab experimentation of approximately 2 h. The stability of Ecasol depends

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