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A Review Article on Antimicrobial Efficiency of Complex-Type Chlorine-Based Disinfectant Cleaner Against Several Pathogenic Microorganisms

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Abstract

Antimicrobial efficacy of complex-type chlorine-based disinfectant cleaner RUBYSTA® (RST) consisting mainly of potassium peroxymonosulfate against pathogenic microoganisms such as methicillin-resistant *Staphylococcus aureus* (MRSA), multidrug-resistant *Pseudomonas aeruginosa* (MDRP), feline calicivirus (FCV) as a surrogate for norovirus, and bovine viral diarrhea virus (BVDV) as a surrogate for hepatitis C virus. In the suspension test, 1% RST caused 4 $\log_{10}$ or more reduction in MRSA, MDRP and FCV in 1 minute in the presence of 0.03% bovine serum albumin. The viral inactivation efficacy of RST was better than that of NaOCl at the same available chlorine concentration. In the immersion test using BVDV adhering to a stainless carrier, the viral inactivation efficacy of 0.1% NaOCl and 1% RST in 1 minute was 0.9 $\log_{10}$ reduction and 3.3 $\log_{10}$ reduction, respectively. Furthermore, in the wipe test, 1% RST immersion wipe caused 4.5 $\log_{10}$ or more reduction in FCV. The present findings suggest that RST is effective for daily disinfection and cleaning in the hospital environment.

Keywords: Potassium peroxymonosulfate; methicillin-resistant *Staphylococcus aureus* (MRSA); multidrug-resistant *Pseudomonas aeruginosa* (MDRP); feline calicivirus (FCV); bovine viral diarrhea virus (BVDV)

Introduction

Many microorganisms are present in the hospital environment. Among them, methicillin-resistant *Staphylococcus aureus* (MRSA) and norovirus, which cause healthcare-associated infections in susceptible patients, are important issues to be addressed in healthcare facilities [1]. The CDC guidelines [2] recommend intermediate or low-level disinfectants for environmental disinfection, and sodium hypochlorite (NaOCl) is widely used because of its broad anti-microbial spectrum. However, NaOCl has some challenges for daily use in environmental maintenance, such as metal corrosiveness and irritating chlorine odor. Environmental sanitizers and cleaners that contain potassium peroxymonosulfate as a main ingredient and surfactants are safe and material-compatible formulations, and have been approved by the United States Environmental Protection Agency (EPA) as a cleaning and disinfection product in the medical field for MRSA and norovirus control. For example, VIRKON® (VK) is registered as Multipurpose Disinfectant Cleaner.

VK was launched in Japan in 2012 under the name of RUBYSTA® (RST), and we could not find any data on short-time efficacy required in the medical field in Japan, although there have been many reports showing the microbicidal activity of VK [3]. Therefore, it was necessary to confirm the efficacy of RST in a short period by a suspension test, a carrier test, and a wiping test, targeting microorganisms that cause infections in medical facilities.

Literature Review

The efficacy against pathogenic microorganisms was tested on the following test products. RST is prepared by dissolving tablets in tap water. RST immediately after preparation was adjusted to a concentration of 1 w/v% RST at the time of contact for the test. Zero-point one v/v% sodium hypochlorite solution (0.1% NaOCl), Japanese Pharmacopoeia ethanol for disinfection (61.5-65.1% EtOH), benzalkonium chloride (0.1 v/v% BZC), and hydrogen peroxide water (0.5 v/v% H_2O_2) were used as comparison disinfectants. All concentrations are those at the time of contact.

Tested microorganisms included *Staphylococcus aureus* IID 1677 (MRSA), multidrug-resistant *Pseudomonas aeruginosa* GTC14659 (MDRP), feline calicivirus, F-9, ATCC VR-782 (FCV) as human norovirus surrogate, and bovine viral diarrhea virus, NADL, ATCC VR-549 (BVDV) as hepatitis C virus surrogate were used. Since there was no established method in Japan for evaluating the efficacy of environmental disinfectants on non-living subjects, the suspension test and the carrier test were conducted based on the procedures of test standards overseas. The suspension test is a basic microbicidal activity evaluation test of disinfectants. Five minutes of contact time was reduced to 1 minute with reference to the suspension test (phase 1/step 1) [4,5] in the medical field of European Norm (EN). Hard water (375 ppm) was used for preparation of test product solutions in the medical field of EN, but was changed to sterile distilled water because the quality of water in Japan greatly differs from that of hard water. Bovine Serum Albumin (BSA) was added as a loading material to be added with the test microorganisms at a final concentration of 0.03 w/v% (clean conditions).

Suspension tests against FCV and BVDV were also tested in dirty conditions [4,5]. Contaminants in dirty conditions included 0.3 w/v%BSA and 0.3 v/v% sheep blood cells washed in phosphate buffered saline (PBS).

The carrier test, which assumes immersion of the apparatus, was based on the procedure of AOAC official method 955.14 [6]. The duration of contact was reduced from 10 minutes to 1 minute, and JS hard water [7] (53.58 mg/L in terms of $CaCO_3$), which assumes the quality of tap water in Japan, was used for the preparation of the test product solutions. The microbicidal activity was evaluated when MRSA and FCV were attached to a stainless steel carrier, dried, and immersed in the test product solution for 1 minute. The effectiveness of RST-impregnated wipes, one of the uses of RST, was evaluated after one wipe of a stainless steel-plate surface with MRSA and FCV adhering to it, based on the Japanese industry standard, "Test Method for Sterilization Performance of Wet Wipers" [8]. SCDLP medium (Eiken Chemical) was used as the neutralizer of test product. SCDLP medium with 0.3 w/v% sodium thiosulfate solution was used for inactivation in virus tests, and SCDLP medium with 0.1 v/v% catalase was used for inactivation of 0.5% H_2O_2. Each inactivation medium was used after confirming in advance that it exhibited complete inactivation against each test product.

Suspension test (clean conditions) results showed that the number of cfu/ml of MRSA and MDRP was reduced by more than 6 $\log_{10}$ from the initial number of cfu/ml after 1 minute of contact for all test products except 0.5% H_2O_2. Zero-point five percent H_2O_2 showed a reduction of only 0.2 $\log_{10}$ after 3 minutes of contact for MRSA or 0.5 $\log_{10}$ for MDRP.

The results of the suspension tests (clean and dirty conditions) on FCV showed that 1% RST and 0.1% NaOCl were effective at 1 minute of contact in both clean and dirty conditions. In FCV, 1% RST and 0.1% NaOCl were effective in a 1-minute contact, with a reduction of more than 4 $\log_{10}$. EtOH decreased FCV infection titer by 2.7 $\log_{10}$ in clean conditions and by 3.0 $\log_{10}$ in dirty conditions after 3 minutes of contact, while 0.1% BZC and 0.5% H_2O_2 showed only a slight decrease from the initial infection titer after 3 minutes of contact.

All products were evaluated against MRSA and MDRP by carrier tests according to the AOAC method. Evaluation was considered valid when all carriers were negative. All test products except 0.5% H_2O_2 were found to be bactericidal in 5 minutes for MRSA. On the other hand, 1% RST and 0.1% NaOCl were negative in all test tubes after 1 minute of contact against MDRP, indicating bactericidal efficacy. EtOH and 0.1% BZC showed bactericidal effect after 5 minutes of contact, while 0.5% H_2O_2 showed no bactericidal effect after 5 minutes of contact, as all test tubes were positive.

For the carrier test against viruses, the sterilizing effects of a 1-minute immersion in 1% RST and 0.1% NaOCl were analyzed for FCV and BVDV as test viruses. The results showed that 1% RST was more effective than 0.1% NaOCl in inactivating both viruses. The decrease in infectious titer of FCV was 3.5 $\log_{10}$ for 1% RST and 3.2 $\log_{10}$ for 0.1% NaOCl. The decrease in infectious titer of BVDV was 3.3 $\log_{10}$ for 1% RST and 0.9 $\log_{10}$ for 0.1% NaOCl. These results confirm that 1% RST has the same performance as 0.1% NaOCl and is superior in inactivation against both viruses in the carrier test.

As a wiping test, the disinfection effect of wiping with 1% RST-impregnated wipes was compared with that of wiping with distilled water-impregnated wipes. MRSA and FCV adhered to a stainless steel-test plate were wiped off with wipes, and the number of microorganisms remaining on the stainless steel-plate after wiping was determined. The results showed that 1% RST for MRSA showed a 3.9 $\log_{10}$ reduction after one wipe each way, a difference of 2.8 $\log_{10}$ compared to 1.1 $\log_{10}$ for wipes impregnated with distilled water. One percent RST-impregnated wipes showed a reduction of more than 4.5 $\log_{10}$ after one wipe each way against FCV, and a reduction of more than 3.0 $\log_{10}$ compared to 1.5 $\log_{10}$ for distilled water-impregnated wipes.

These results confirm that RST has microbicidal activity against MRSA and FCV, a norovirus surrogate, which are important healthcare-related infectious microorganisms for infection control.

Discussion

It is widely known that the healthcare environment is a transmission route for pathogenic microorganisms, and it has been proposed that disinfection of the healthcare environment, focusing not only on the disinfection of the hands of healthcare workers but also on high frequency contact surfaces, is useful in infection control. Among them, MRSA is the most frequent healthcare-associated infectious organism and an important target for environmental disinfection. There were 15,595 patients of new cases of infection caused by antibiotic-resistant bacteria in 2022 in Japan, of which about 95% were MRSA-infected patients, according to the Japanese Agency for Nosocomial Infection Surveillance (JANIS) conducted by the Ministry of Health, Labour and Welfare (MHLW), Japan. Recently, the issue of antimicrobial resistance, AMR, has been viewed as internationally important. In addition to the proper use of antibiotics, basic infection control measures are indispensable. We believe that routine environmental maintenance must be implemented without fail. The results of this study will provide concrete information for implementing appropriate environmental remediation. RST is the same ingredient as VK [9], which is on the EPA list as a formulation applicable to norovirus, which is highly resistant to disinfectants, as well as MRSA, VRE, and hepatitis B and C viruses. RST was launched in Japan in 2012. RST has been listed as an environmental maintenance product in infection control guidelines for the dialysis, medical devices, operating rooms, and dental fields since 2015. In this study, RST was found to have the same or stronger anti-microbial activity against MRSA and other test microorganisms than the NaOCl formulation, with a short duration of contact. The NaOCl formulation, which has a broad anti-microbial spectrum, has some problems for daily use in environmental maintenance. Practically, (i) its high concentration decreases with storage, (ii) concentration before dilution is not constant, so it is not easy to prepare a solution with an accurate concentration, in addition, (iii) its metal corrosiveness and irritating chlorine odor. On the other hand, RST is available in tablets and pre-measured individual packages, the adjusted solution is light red in color, and the color tone changes in proportion to the decrease in effective chlorine, so that the estimated expiration date can be estimated by the color change. It has also been confirmed that the component contains surfactant, which has better permeability than 0.1% NaOCl and has less effect on metal and resin materials [10], and is expected to be used for immersion disinfection of instruments.

Conclusion

The antimicrobial activity against bacteria (MRSA and MDRP) and viruses (FCV and BVDV) was examined in the suspension tests and carrier tests in a short time. The results showed that RST had

carrier tests in a short time. The results showed that RST had antimicrobial activity against all microorganisms comparable to orgreater than NaOCl, with no decrease in effectiveness against FCV and BVDV under conditions of high organic load. In a wipe test using a nonwoven fabric impregnated with 1% RST for actual use, RST was found to be effective against MRSA and FCV, and its excellent contact against environmental microorganisms and high safety profile make it an option for daily hygiene management in hospital environments.

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