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Article

Comparative Effectiveness and Persistence of Antimicrobial Durability in Dry and Wet States of a Novel Antimicrobial Surface Disinfectant

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Abstract: Aims: We evaluated a novel disinfectant (VR) and seven comparators (disinfectants A–G) against resistant pathogens common in healthcare settings. **Methods and Results:** VR at different dilutions, along with commercial disinfectants A–G, was tested against surrogate viruses, and resistant bacterial and fungal pathogens. Surrogate viruses had an initial concentration of ~1×10^8 mL^{-1}, and bacterial and fungal isolates had an initial concentration of ~1×10^6 mL^{-1} on Siliconee surfaces. After the application of VR or a comparator disinfectant, surfaces were tested for the reduction in microbial loads after 30 s and 5 min wet exposures, and after a 24 h dry residue exposure. Sterile deionized water was used as a control. The VR at a concentration of 4.68% was superior to all comparator disinfectants against most pathogens in wet and dry testing. The VR at 7.8% concentration showed the highest pathogen-reduction rate among all comparator disinfectants when tested against all pathogens. **Conclusions:** Overall, the novel VR disinfectant was the most effective disinfectant in both wet and dry residue states against the range of tested pathogens. **Significance and Impact of the Study:** VR is a broadly effective disinfectant combination for use in high-risk settings, particularly those in which intervals between applications of disinfectant can be lengthy or inconsistent.

Keywords: novel disinfectants; antimicrobial durability; PHMB; tetrasodium EDTA; dry and wet testing

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1. Introduction

Medical and other communal healthcare settings expose vulnerable patients and healthcare workers to the threat of infection from an increasing number of highly transmissible virulent pathogens. These include viruses, multidrug-resistant bacteria, and fungi [1]. Of significant note are RNA viruses with high adaptability, such as coronaviruses and noroviruses [2,3]. In addition, resistant *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus* (MRSA), and toxin-producing *Escherichia coli* bacteria are of great concern because they can survive on surfaces for prolonged durations, and cause infections that are both difficult to treat, and can also be associated with significant virulence [1]. The fungal pathogen *Candida auris* is of great concern because it is frequently azole-resistant and can be environmentally transmitted [4].

In response to these evolving microbiologic threats, multiple surface disinfectant technologies have emerged, including those based on quaternary ammonium compounds, peroxides, and alcohols [5]. Most of these are microbicidal on contact but have different results because the surfaces can dry, and the applied agents can evaporate when exposed to air and ambient humidity. Current facility-management practices and periodic staffing shortages present patient surges and other challenges to sustaining frequent and consistent wet disinfection of surfaces. Consequently, the durability of disinfecting activity on surfaces

following the application of the wet disinfectant is of critical practical importance when prolonged durations can elapse between repetitive applications [5,6].

The novel VR disinfectant contains mainly poly-hexamethylene biguanide hydrochloride (PHMB), ethylenediaminetetraacetic acid tetrasodium salt dihydrate (EDTA), trimethyl glycine (also known as betaine), and alkylpolyglucosides, known as Glucopon. PHMB is a well-known commercial bactericide that has been widely used in a variety of areas, such as the food industry and recreational water, because of its low toxicity and broad-spectrum bactericidal properties [7,8]. It also contains EDTA (as a biofilm disruptor), betaine, and Glucopon surfactants. However, VR as a novel disinfectant combination comprising a biguanide, biofilm disrupter, surfactants, and cleaners had not previously been tested for use against the resistant microbes that often occur in medical settings.

In this study, we evaluated VR for potential use in medical and communal healthcare settings. We tested VR both on contact (the wet state), and for residual antimicrobial activity in the dry state after 24 h of ambient drying. The comparator disinfectants were commercially available quaternary ammonium-based, hydrogen peroxide-based, and alcohol-based disinfectants for hospital and institutional use. The VR and comparator disinfectants were tested against the RNA surrogate viruses of greatest concern, as well as clinically relevant representative resistant bacterial and *C. auris* pathogens. Sterile deionized water was used as a control.

2. Material and Methods

The novel antimicrobial surface disinfectant VR was compared with seven different commercial disinfectants that are commonly used (disinfectants A–G). The VR was tested at three different concentrations (2.34%, 4.68%, and 7.8%). The chemical constituents of VR and the comparator disinfectants are shown in Table 1. Disinfectants A–G are the products of Reckitt Benckiser LLC (A), Clorox (B), Seventh Generation, Inc. (C), Diversey (D), ECOLAB (E), Purell (F), and Microban (G). The VR and comparator disinfectants were tested against surrogate viruses (F-specific coliphage MS2, and feline calicivirus [FCV]), resistant clinical microbial MD Anderson hospital pathogens (carbapenem-resistant *E. coli*, multidrug-resistant *P. aeruginosa*, and MRSA), and Prevention Antibiotic Resistance Isolate Bank (ARIsolateBank) *C. auris*-AR 0387 [9].

Table 1. Chemical constituents of the novel antimicrobial surface disinfectant VR, and comparator disinfectants used in this study.

Product	Chemical Constituent
VR *	PHMB 7%, tetrasodium EDTA 1.5%, Dowanaol 5%, betaine 3%, Glucopon 10%
A	Alkyl (67% C12, 25% C14, 7% C16, 1% C8-C10-C18) dimethyl benzyl ammonium chloride 0.0860%, alkyl (50% C14, 40% C12, 10% C16) dimethyl benzyl ammonium chloride 0.0216%, and other ingredients (99.8924%)
B	Alkyl (C12 40%, C14 50%, C16 10%) dimethyl benzyl ammonium chloride 0.3% and other ingredients (99.7%)
C	Water, decyl glucoside (plant-derived cleaning agent), lauramine oxide (plant-based cleaning agent), sodium gluconate (plant-derived water softener), sodium carbonate (mineral-based alkalinity builder), benzothiazoline (synthetic preservative), methylisothiazolinone (synthetic preservative)
D	Hydrogen peroxide 8.0% and other ingredients (92.0%)
E	n-Alkyl (60% C14, 30% C16, 5% C12, 5% C18) dimethyl benzyl ammonium chlorides 0.105%, n-alkyl (68% C12, 32% C14) dimethyl ethylbenzyl ammonium chlorides 0.105%, hydrogen peroxide 8.0%, and inert ingredients (91.79%)
F	Ethyl alcohol 29.4% and other ingredients (70.6%)
G	Alkyl (50% C14, 40% C12, 10% C16) dimethyl benzyl ammonium chloride 0.200%, octyl decyl dimethyl ammonium chloride 0.150%, didecyl dimethyl ammonium chloride 0.075%, dioctyl dimethyl ammonium chloride 0.075%, and other ingredients (99.500%)

* VR 2.34% (3 fluid ounces of VR per gallon (125 fluid ounces of H_2O)), VR 4.68% (6 fluid ounces of VR per gallon (122 fluid ounces of H_2O)), or VR 7.8% (9 fluid ounces of VR per gallon (119 fluid ounces of H_2O)).

2.1. Viral Inoculum Preparation and Titration

MS2 bacteriophage (ATCC #15597-B1) testing was performed according to United States Environmental Protection Agency Methods 1601 and 1602 [10], modified as previously described [11] and according to the ATCC product sheet. MS2 was grown and titrated using an exponential culture of *E. coli* Famp–*E. coli* HS (pFamp) R (male-specific coliphage host, ATCC #700891) grown in tryptic soy broth containing ampicillin and streptomycin, and the count was calculated as plaque-forming units per milliliter (PFU mL^{-1}). FCV (strain F9-ATCC VR-782) testing was performed according to the ATCC product sheet and modified as previously described [12–15]. FCV was propagated and titrated in monolayers of Crandell–Rees feline kidney cells (ATCC #CCL-94; *Felis catus*), and the count was calculated as cytopathic effect per milliliter (CPE mL^{-1}).

2.2. Viral Inoculum Wet Testing

One milliliter of surrogate virus filtrate (approximately 10^8 PFU mL^{-1} or CPE mL^{-1}) was spread onto Silicone discs (1 mL for each disc). Discs were placed individually onto wells of a six-well microtiter plate (three discs were used for each disinfectant solution and control), dried at room temperature until visibly dry, sprayed with the control (sterile deionized water) or the experimental disinfectant solution, and exposed for 30 s or 5 min.

2.3. Viral Inoculum Dry Testing

The discs were sprayed with the control or the experimental solution, and dried using a fan-air drier until visibly dry. Discs were placed individually onto wells of a six-well microtiter plate, inoculated with 1 mL of the virus filtrate (approximately 10^8 CPE mL^{-1}), dried at room temperature using a fan-air drier until visibly dry, and tested at 24 h.

2.4. Counting Procedures for Both Wet and Dry Testing of Viral Inocula

Each disc was immersed in a tube containing 5 mL of D/E neutralizing broth and shaken gently to ensure thorough coverage. For viral elution, the discs were transferred to tubes containing 5 mL of a 3% beef extract solution (pH 8.5) and shaken vigorously to ensure complete elution of the virus. For MS2 viral elution, serial dilution was done for each beef extract tube containing a disc (10^0 to 10^{-6}), and 1 mL of each dilution was mixed with 200 µL of an exponential culture of *E. coli* and 5 mL of tryptic soy broth containing 0.75% agar (45–55 °C). The mixture was poured on top of a solidified bottom agar layer (tryptic soy broth with 1.5% agar contained in a petri dish) and allowed to solidify. The plates were then inverted and incubated at 37 °C for 24 h. The plaques were counted on plates with 30 to 300 plaques, and the titer was recorded as PFU mL^{-1}. For FCV viral elution, serial dilution was performed for each beef extract tube containing a disc (10^0 to 10^{-6}), and 0.1 mL of diluted solution was inoculated onto 96-well tissue culture plates with 90% confluent monolayers of Crandell–Rees feline kidney cells, then incubated at 37 °C with 5% CO_2. Plates were observed daily under an inverted microscope, and CPEs were enumerated. CPE for each dilution was recorded and calculated as CPE mL^{-1}. Positive controls (inoculated and treated only with sterile deionized water) were processed in each experimental run.

2.5. Bacterial and C. auris Inoculum Preparations

Bacterial and *C. auris* pathogens were performed according to previous publications [16–19].

2.6. Bacterial and C. auris Wet Testing

One milliliter of ~10^6 mL^{-1} inoculum was spread onto Silicone discs. Three Silicone discs, each with a 3 cm diameter, were used for each disinfectant and positive control. Discs were placed in individual wells of a six-well microtiter plate. The inoculum was dried at room temperature until visibly dry. After drying, the discs were sprayed with control (sterile deionized water) or experimental disinfectant solution, and exposed for 30 s and 5 min.

2.7. Bacterial and C. auris Dry Testing

The discs were sprayed with the experimental or control solution, and dried using a fan-air drier until visibly dry. Discs were left for 24 h and placed individually onto wells of a six-well microtiter plate. One milliliter of ~10^6 mL^{-1} inoculum was spread onto each of the Silicone discs, and the inoculum was dried at room temperature using a fan-air drier until visibly dry.

2.8. Counting Procedures for Both Wet and Dry Testing of Bacteria and C. auris

Each Silicone disc was carefully transferred to a corresponding 14 mL tube containing 5 mL of sterile saline and sonicated in a sonicating water bath for 15 min. Serial dilution for each tube from 10^0 to 10^{-5} was made for positive control, and from 10^0 to 10^{-3} for tested tubes. One hundred microliter of the appropriate dilution was plated onto trypticase soy agar +5% sheep blood for bacteria (or on Sabouraud agar for *C. auris)* and spread with sterile glass beads. The plates were incubated at 37 °C for up to 72 h. Colonies were counted on plates containing between 30 and 300 colonies, and the titer was recorded as colony-forming units per milliliter (CFU mL^{-1}).

2.9. Data Analysis

We evaluated the comparative effectiveness and persistence of antimicrobial durability in dry and wet states for VR and the seven comparator disinfectants using SAS version 9.4 software (SAS Institute Inc., Cary, NC, USA). Original count values (PFU, CPE, or colony-forming units per milliliter) were $\log_{10}$-transformed, and *t* tests were employed to compare count values between VR and comparator disinfectants. $p \leq 0.05$ was considered statistically significant.

3. Results

Table 2 presents the $\log_{10}$ reductions in microbial load for three different dilutions of VR concentrate and comparator disinfectants A–G relative to the positive control for viral pathogens. Table 3 presents the corresponding $\log_{10}$ reduction comparisons of VR 4.68% and VR 7.8% relative to comparator disinfectants (A–G) when tested against viral pathogens for 30 s wet contact testing, 5 min wet contact testing, and 24 h dried residue testing. Table 4 presents the $\log_{10}$ reductions in microbial load for three different dilutions of VR concentrate and comparator disinfectants A–G relative to the positive control for bacterial pathogens. Table 5 presents the corresponding $\log_{10}$ reduction comparisons of VR 4.68% and VR 7.8% relative to comparator disinfectants (A–G) when tested against bacterial pathogens for 30 s wet contact testing, 5 min wet contact testing, and 24 h dried residue testing. Table 6 presents the $\log_{10}$ reductions in microbial load for three different dilutions of VR concentrate and comparator disinfectants A–G relative to the positive control for Candida auris. Table 7 presents the corresponding $\log_{10}$ reduction comparisons of VR 4.68% and VR 7.8% relative to comparator disinfectants (A–G) when tested against Candida auris for 30 s wet contact testing, 5 min wet contact testing, and 24 h dried residue testing.

Table 2. Inactivation efficacy of VR and the comparator disinfectants compared with the positive control against the surrogate viruses F-specific coliphage MS2, and feline calicivirus.

Disinfectant	Mean ± Standard Deviation $\log_{10}$ Reduction *		
	30 s Wet Contact	5 min Wet Contact	24 h Dry Residue
	F-specific coliphage MS2		
VR 2.34%	2.56 ± 0.37	3.54 ± 0.17	1.30 ± 0.09
VR 4.68%	2.62 ± 0.15	3.74 ± 0.10	1.44 ± 0.08

Table 2. *Cont.*

Disinfectant	Mean ± Standard Deviation log_{10} Reduction *		
	30 s Wet Contact	5 min Wet Contact	24 h Dry Residue
VR 7.8%	2.91 ± 0.25	4.48 ± 0.23	1.62 ± 0.10
A	1.28 ± 0.05	2.17 ± 0.09	0.40 ± 0.17
B	1.47 ± 0.12	2.64 ± 0.16	0.72 ± 0.09
C	0.87 ± 0.24	2.43 ± 0.24	0.49 ± 0.24
D	1.09 ± 0.13	2.37 ± 0.06	0.74 ± 0.05
E	1.27 ± 0.11	3.35 ± 0.08	0.25 ± 0.14
F	1.34 ± 0.15	3.29 ± 0.11	0.45 ± 0.24
G	1.65 ± 0.06	2.80 ± 0.01	0.56 ± 0.14
	Feline calicivirus		
VR 2.34%	3.00 ± 0.37	3.59 ± 0.05	0.58 ± 0.17
VR 4.68%	3.19 ± 0.27	4.95 ± 0.66	0.95 ± 0.06
VR 7.8%	4.44 ± 1.02	5.39 ± 0.33	1.57 ± 0.10
A	1.88 ± 0.18	2.59 ± 0.09	0.33 ± 0.10
B	2.31 ± 0.15	3.18 ± 0.06	0.40 ± 0.16
C	1.06 ± 0.09	2.05 ± 0.15	0.19 ± 0.11
D	2.27 ± 0.09	3.19 ± 0.08	0.28 ± 0.11
E	3.88 ± 1.40	3.97 ± 0.01	0.25 ± 0.17
F	3.06 ± 0.15	4.05 ± 0.09	0.16 ± 0.13
G	2.18 ± 0.60	3.21 ± 0.01	0.25 ± 0.02

* Mean ± standard deviation of three independent exposures for each agent under each set of conditions is shown. Log_{10} reduction indicates a reduction in log_{10} value of viral count compared with positive control.

Table 3. Antimicrobial performance of comparator disinfectants relative to VR 4.68% and VR 7.8% against surrogate viruses.

Comparators	VR 4.68%			VR 7.8%		
	Log_{10} Reduction versus 4.68% (*p*-Value) *			Log_{10} Reduction versus 7.8% (*p*-Value) *		
	30 s Wet	5 min Wet	24 h Dry	30 s Wet	5 min Wet	24 h Dry
	F-specific coliphage MS-2					
A	1.34 (<0.001)	1.56 (<0.0001)	1.03 (0.002)	1.08 (0.002)	2.30 (<0.001)	1.21 (0.001)
B	1.15 (0.001)	1.09 (0.001)	0.71 (0.001)	0.89 (0.006)	1.83 (<0.001)	0.89 (0.001)
C	1.76 (<0.001)	1.31 (0.002)	0.94 (0.006)	1.54 (0.001)	2.05 (0.001)	1.12 (0.004)
D	1.53 (≤0.001)	1.37 (<0.0001)	0.69 (<0.001)	1.27 (0.002)	2.11 (<0.001)	0.87 (<0.001)
E	1.35 (<0.001)	0.39 (0.013)	1.19 (<0.001)	1.09 (0.002)	1.13 (0.003)	1.37 (<0.001)
F	1.28 (0.001)	0.45 (0.013)	0.99 (0.006)	1.02 (0.005)	1.19 (0.003)	1.17 (0.003)
G	0.97 (<0.001)	0.93 (0.006)	0.88 (0.002)	0.72 (0.008)	1.67 (0.009)	1.06 (0.001)
	Feline calicivirus (FCV)					
A	1.30 (0.005)	2.35 (0.035)	0.62 (0.002)	2.55 (0.07)	2.79 (<0.001)	1.24 (<0.001)
B	0.88 (0.015)	1.76 (0.06)	0.55 (0.011)	2.13 (0.09)	2.20 (0.001)	1.17 (0.001)

Table 3. *Cont.*

Comparators	VR 4.68%			VR 7.8%		
	Log_{10} Reduction versus 4.68% (*p*-Value) *			Log_{10} Reduction versus 7.8% (*p*-Value) *		
	30 s Wet	5 min Wet	24 h Dry	30 s Wet	5 min Wet	24 h Dry
C	2.12 (<0.001)	2.89 (0.004)	0.76 (0.001)	3.37 (0.042)	3.33 (<0.001)	1.38 (<0.001)
D	0.92 (0.01)	1.76 (0.06)	0.67 (0.002)	2.17 (0.09)	2.20 (0.001)	1.29 (<0.001)
E	0.29 (0.39)	0.97 (0.17)	0.70 (0.006)	0.55 (0.67)	1.42 (0.004)	1.32 (0.001)
F	0.13 (0.58)	0.90 (0.19)	0.79 (0.002)	1.38 (0.19)	1.34 (0.005)	1.41 (<0.001)
G	1.01 (0.007)	1.73 (0.06)	0.70 (<0.001)	2.26 (0.09)	2.17 (0.001)	1.32 (<0.0001)

* $p \leq 0.05$ indicates statistically significant difference. A positive log10 reduction value indicates that the VR had fewer surviving microbes than the comparator disinfectant following; negative value indicates that the microbial count of the comparator disinfectant was lower than that of the VR disinfectant.

Table 4. The inactivation efficacy of VR and the comparator disinfectants compared with the positive control against the bacterial pathogens carbapenem-resistant *Escherichia coli*, multidrug-resistant *Pseudomonas aeruginosa*, and methicillin-resistant *Staphylococcus aureus*.

Disinfectant	Mean ± Standard Deviation log_{10} Reduction *		
	30 s Wet Contact	5 min Wet Contact	24 h Dry Residue
	E. coli		
VR 2.34%	4.76 ± 1.20	6.45 ± 0.00	2.65 ± 0.08
VR 4.68%	6.45 ± 0.00	6.45 ± 0.00	3.52 ± 0.32
VR 7.8%	6.45 ± 0.00	6.45 ± 0.00	4.34 ± 0.29
A	2.30 ± 0.44	4.35 ± 1.49	1.36 ± 0.16
B	3.54 ± 0.38	6.45 ± 0.00	1.96 ± 0.10
C	1.86 ± 0.15	3.17 ± 0.19	1.38 ± 0.11
D	2.70 ± 0.25	6.45 ± 0.00	2.28 ± 0.51
E	2.64 ± 0.20	3.70 ± 0.10	1.55 ± 0.10
F	4.19 ± 0.40	6.45 ± 0.00	2.01 ± 0.10
G	3.06 ± 0.28	6.45 ± 0.00	2.11 ± 0.09
	P. aeruginosa		
VR 2.34%	2.19 ± 0.33	3.90 ± 1.05	1.41 ± 0.20
VR 4.68%	2.70 ± 0.28	4.64 ± 1.03	2.38 ± 0.95
VR 7.8%	4.07 ± 0.93	5.36 ± 0.00	2.65 ± 0.21
A	1.37 ± 0.52	2.38 ± 0.49	0.57 ± 0.47
B	5.36 ± 0.00	5.36 ± 0.00	1.21 ± 0.24
C	0.86 ± 0.45	2.68 ± 0.49	1.03 ± 0.45
D	2.28 ± 0.08	5.36 ± 0.00	1.12 ± 0.03
E	2.56 ± 0.57	5.36 ± 0.00	1.12 ± 0.58
F	2.28 ± 0.18	5.36 ± 0.00	0.85 ± 0.43
G	1.51 ± 0.27	3.74 ± 1.15	1.23 ± 0.50
	S. aureus		
VR 2.34%	3.37 ± 0.20	5.63 ± 0.00	2.37 ± 0.77
VR 4.68%	4.96 ± 0.94	5.63 ± 0.00	3.03 ± 0.27
VR 7.8%	5.63 ± 0.00	5.63 ± 0.00	5.22 ± 1.13
A	3.36 ± 0.51	5.63 ± 0.00	1.05 ± 0.28
B	5.63 ± 0.00	5.63 ± 0.00	1.15 ± 0.10
C	1.01 ± 0.46	1.67 ± 0.59	0.16 ± 0.08

Table 4. *Cont.*

Disinfectant	Mean ± Standard Deviation log_{10} Reduction *		
	30 s Wet Contact	5 min Wet Contact	24 h Dry Residue
D	3.18 ± 0.25	5.63 ± 0.00	1.19 ± 0.25
E	2.67 ± 0.10	4.50 ± 0.80	0.92 ± 0.32
F	3.31 ± 0.44	5.63 ± 0.00	0.20 ± 0.14
G	3.09 ± 0.26	5.63 ± 0.00	0.29 ± 0.11

* Mean ± standard deviation of three independent exposures for each agent under each set of conditions is shown. Log_{10} reduction indicates a reduction in log_{10} value of viral count compared with positive control.

Table 5. The antimicrobial performance of the comparator disinfectants relative to VR 4.68% and VR 7.8% against the bacterial pathogens carbapenem-resistant *Escherichia coli*, multidrug-resistant *Pseudomonas aeruginosa*, and methicillin-resistant *Staphylococcus aureus* isolates.

Comparators	VR 4.68%			VR 7.8%		
	Log_{10} Reduction versus 4.68% (*p*-Value) *			Log_{10} Reduction versus 7.8% (*p*-Value) *		
	30 s Wet	5 min Wet	24 h Dry	30 s Wet	5 min Wet	24 h Dry
			E. coli			
A	4.15 (0.006)	2.09 (0.19)	2.17 (0.001)	4.15 (0.006)	2.09 (0.19)	2.98 (<0.001)
B	2.91 (0.009)	0.0 (NA)	1.56 (0.003)	2.91 (0.009)	0.0 (NA)	2.38 (<0.001)
C	4.59 (<0.001)	3.28 (0.002)	2.14 (0.001)	4.59 (< 0.001)	3.28 (0.002)	2.95 (<0.001)
D	3.75 (0.002)	0.0 (NA)	1.24 (0.044)	3.75 (0.002)	0.0 (NA)	2.06 (0.008)
E	3.81 (0.001)	2.74 (0.001)	1.97 (0.001)	3.81 (0.001)	2.74 (0.001)	2.78 (<0.001)
F	2.26 (0.015)	0.0 (NA)	1.51 (0.003)	2.26 (0.015)	0.0 (NA)	2.32 (<0.001)
G	3.39 (0.003)	0.0 (NA)	1.41 (0.004)	3.39 (0.003)	0.0 (NA)	2.23 (<0.001)
			P. aeruginosa			
A	1.33 (0.03)	2.25 (0.049)	1.81 (0.07)	2.7 (0.023)	2.98 (0.013)	2.08 (0.005)
B	−2.66 (0.005)	−0.73 (0.42)	1.17 (0.17)	−1.29 (0.19)	0.0 (NA)	1.44 (0.003)
C	1.84 (0.008)	1.96 (0.07)	1.35 (0.14)	3.21 (0.012)	2.68 (0.016)	1.61 (0.01)
D	0.42 (0.10)	−0.73 (0.42)	1.27 (0.20)	1.79 (0.11)	0.0 (NA)	1.53 (0.009)
E	0.14 (0.77)	−0.73 (0.42)	1.27 (0.18)	1.51 (0.12)	0.0 (NA)	1.53 (0.025)
F	0.42 (0.15)	−0.06 (0.96)	1.53 (0.11)	1.79 (0.056)	0.67 (0.42)	1.80 (0.006)
G	1.19 (0.012)	0.90 (0.46)	1.15 (0.20)	2.56 (0.02)	1.63 (0.18)	1.41 (0.021)
			S. aureus			
A	1.60 (0.10)	0.0 (NA)	1.98 (0.002)	2.27 (0.024)	−0.23 (0.42)	4.17 (0.007)
B	−0.67 (0.42)	0.0 (NA)	1.89 (0.001)	0.0 (NA)	−0.23 (0.42)	4.07 (0.035)
C	3.95 (0.006)	3.96 (0.011)	2.87 (<0.001)	4.62 (0.005)	3.72 (0.002)	5.06 (0.024)

Table 5. *Cont.*

Comparators	VR 4.68%			VR 7.8%		
	Log_{10} Reduction versus 4.68% (*p*-Value) *			Log_{10} Reduction versus 7.8% (*p*-Value) *		
	30 s Wet	5 min Wet	24 h Dry	30 s Wet	5 min Wet	24 h Dry
D	1.78 (0.06)	0.0 (NA)	1.85 (0.002)	2.45 (0.005)	−0.23 (0.42)	4.04 (0.008)
E	2.29 (0.07)	1.13 (0.18)	2.11 (0.002)	2.96 (0.001)	0.9 (0.22)	4.3 (0.007)
F	1.65 (0.09)	0.0 (NA)	2.83 (<0.001)	2.32 (0.017)	−0.23 (0.42)	5.02 (0.023)
G	1.87 (0.054)	0.0 (NA)	2.75 (<0.001)	2.54 (0.005)	−0.23 (0.42)	4.94 (0.024)

* $p \leq 0.05$ indicates statistically significant difference. A positive log_{10} reduction value indicates that VR had fewer surviving microbes than the comparator disinfectant following; negative value indicates that the microbial count of the comparator disinfectant was lower than that of VR disinfectant.

Table 6. The inactivation efficacy of VR and the comparator disinfectants compared with the positive control against *Candida auris isolate*.

Disinfectant	Mean ± Standard Deviation log_{10} Reduction *		
	30 s Wet Contact	5 min Wet Contact	24 h Dry Residue
VR 2.34%	2.59 ± 0.05	3.47 ± 0.19	0.65 ± 0.14
VR 4.68%	3.19 ± 0.08	4.84 ± 1.05	0.86 ± 0.27
VR 7.8%	3.64 ± 0.13	5.58 ± 1.03	3.83 ± 1.79
A	0.76 ± 0.06	1.61 ± 0.03	0.09 ± 0.14
B	0.88 ± 0.04	2.00 ± 0.18	0.17 ± 0.10
C	0.63 ± 0.07	1.22 ± 0.16	0.06 ± 0.02
D	0.64 ± 0.08	1.79 ± 0.15	0.05 ± 0.10
E	2.60 ± 0.02	4.91 ± 1.03	0.33 ± 0.08
F	2.38 ± 0.11	3.87 ± 0.13	0.11 ± 0.10
G	0.71 ± 0.04	2.03 ± 0.16	0.17 ± 0.09

* Mean ± standard deviation of the three independent exposures for each agent under each set of conditions is shown. Log_{10} reduction indicates a reduction in the log_{10} value of viral count compared with the positive control.

Table 7. The antimicrobial performance of the comparator disinfectants relative to VR 4.68% and VR 7.8% against *Candida auris* isolate.

Comparators	VR 4.68%			VR 7.8%		
	Log_{10} Reduction versus 4.68% (*p*-Value)			Log_{10} Reduction versus 7.8% (*p*-Value) *		
	30 s Wet	5 min Wet	24 h Dry	30 s Wet	5 min Wet	24 h Dry
			Candida auris			
A	2.43 (<0.0001)	3.22 (0.049)	0.77 (0.025)	2.88 (<0.0001)	3.97 (0.032)	0.93 (0.043)
B	2.32 (<0.0001)	2.84 (0.02)	0.69 (0.028)	2.76 (<0.0001)	3.58 (0.008)	0.85 (0.0502)
C	2.56 (<0.0001)	3.62 (0.037)	0.80 (0.053)	3.01 (<0.0001)	4.36 (0.024)	0.96 (0.08)
D	2.55 (<0.0001)	3.04 (0.052)	0.81 (0.017)	2.99 (<0.0001)	3.79 (0.033)	0.97 (0.035)

Table 7. *Cont.*

Comparators	VR 4.68%			VR 7.8%		
	Log_{10} Reduction versus 4.68% (*p*-Value)			Log_{10} Reduction versus 7.8% (*p*-Value) *		
	30 s Wet	5 min Wet	24 h Dry	30 s Wet	5 min Wet	24 h Dry
E	0.59 (<0.001)	−0.07 (0.95)	0.53 (0.06)	1.04 (0.006)	0.67 (0.55)	0.69 (0.14)
F	0.81 (0.001)	0.97 (0.32)	0.74 (0.023)	1.25 (<0.001)	1.71 (0.14)	0.91 (0.043)
G	2.48 (<0.0001)	2.81 (0.06)	0.69 (0.028)	2.93 (<0.0001)	3.55 (0.037)	0.85 (0.051)

* $p \leq 0.05$ indicates statistically significant difference. A positive log_{10} reduction value indicates that VR had fewer surviving microbes than the comparator disinfectant following; negative value indicates that the microbial count of the comparator disinfectant was lower than that of VR disinfectant.

3.1. Viral Pathogens

Table 2 presents the efficacy of tested disinfectants against the F-specific coliphage MS2, and FCV, at three different exposure conditions. Results are presented as log_{10} reduction in microbes relative to a positive control viral load of 1.02×10^6 PFU mL^{-1} for MS2, and 6.76×10^5 CPE mL^{-1} for FCV. Our results for applied inocula relative to positive control (10^8 PFU mL^{-1}) are consistent with those of Wyrzykowska-Ceradini et al. (2019) [20], who reported similar reductions in MS2 when disinfectants were applied to non-grimed, soft, porous surfaces. In 30 s wet contact testing, the log_{10} reductions of MS2 after treatment with VR 2.34% were nearly 1.0 higher than all tested comparators. Similar results were found in 30 s wet contact testing against FCV, except for comparator disinfectants E and F, which led to similar reductions in FCV to those observed for VR 2.34%. In 5 min wet contact testing, the difference between VR 2.34% and the comparators was narrower for both viruses. All VR dilutions after 5 min wet contact led to greater than 3.5 log_{10} reductions in viral inocula, and the highest concentration of VR, 7.8%, led to a greater than 4.0 log_{10} reduction in viral inocula. Comparator disinfectants A and C were less effective than the other disinfectants when tested against FCV, yielding less than 3.0 log_{10} reductions after 5 min wet contact. The performance of all disinfectants diminished in 24 h dried residue testing; however, VR at all concentrations led to a greater than 1.0 log_{10} reduction in MS2, whereas all comparators yielded less than 1.0 log_{10} reductions in MS2. Only VR 7.8% led to a greater than 1.0 log_{10} reduction in FCV in 24 h dried residue testing. All VR dilutions led to greater reductions in FCV than did comparators in the 24 h dried residue testing.

In the testing of VR 4.68% and the comparators against MS2 following 30 s wet exposure, VR yielded significantly higher reductions in MS2 than did all tested comparator disinfectants. Although the log_{10} reduction differences were smaller in magnitude after 5 min wet contact (Table 3), VR 4.68% remained significantly more efficacious than all tested comparators. Similar trends were obtained for VR 7.8%. VR 4.68% also yielded significantly higher reductions in both MS2 and FCV than did all tested comparators when applied as a 24 h dried residue (Table 3). Log_{10} reductions in FCV viral load for VR 4.68% were significantly better than those of comparator disinfectants A–D and G after 30 s wet contact. After 5 min wet contact, VR 4.68% yielded significantly higher log_{10} reductions of FCV than did comparator disinfectants A and C (Table 3).

3.2. Bacterial Pathogens

Table 4 presents the comparative log_{10} reductions in bacterial pathogens for VR and the comparators applied at different exposure conditions. The results are presented as log_{10} reductions in microbes relative to a positive control of 2.8×10^6 CFU mL^{-1} for *E. coli*, 2.3×10^5 CFU mL^{-1} for *P. aeruginosa*, and 4.3×10^5 CFU mL^{-1} for MRSA.

VR 4.68% and VR 7.8% completely eradicated *E. coli* after 30 s wet exposure, and all VR dilutions completely eradicated *E. coli* after 5 min wet exposure. None of the comparators completely eradicated *E. coli* after 30 s wet exposure, and none of the comparators produced

a 3.0 $\log_{10}$ or higher reduction in *E. coli* in 24 h dried residue testing. In contrast, VR 4.68% and VR 7.8% produced greater than 3.0 $\log_{10}$ reductions in *E. coli* in 24 h dried residue testing. VR 2.34% produced a greater $\log_{10}$ reduction in *E. coli* than did all other comparators after only 30 s of wet exposure. VR 4.68% yielded statistically superior $\log_{10}$ reductions in *E. coli* compared with all comparators after 30 s wet exposure and in 24 h dried residue testing (Table 5).

Regarding *P. aeruginosa,* disinfectant B fully eradicated *P. aeruginosa* after 30 s wet contact. The only other disinfectant that yielded a greater than 3.0 $\log_{10}$ reduction in *P. aeruginosa* after 30 s wet contact was VR 7.8%. After 5 min wet contact, all disinfectants produced a greater than 3.0 $\log_{10}$ reduction in *P. aeruginosa*, except disinfectants A and C. Only VR 4.68% and VR 7.8% produced greater than 2.0 $\log_{10}$ reductions in *P. aeruginosa* as dried residues (Table 4). VR 4.68% yielded significantly higher reductions in *P. aeruginosa* than did disinfectants A–C and G in 30 s wet exposure (Table 5) and significantly higher reductions in *P. aeruginosa* than those for disinfectant A in 5 min wet exposure and 24 h dried residue testing (Table 5).

All VR dilutions yielded greater than 3.0 $\log_{10}$ reductions in MRSA after 30 s wet exposure. VR 7.8% and disinfectant B completely eradicated MRSA after 30 s wet exposure. After 5 min wet exposure, only disinfectant C yielded less than a 3.0 $\log_{10}$ reduction in MRSA and only disinfectant E did not fully eradicate MRSA. In 24 h dry residue testing, VR 4.68% and VR 7.8% yielded greater than 3.0 $\log_{10}$ reductions in MRSA. Of the remaining disinfectants tested, only VR 2.34% yielded greater than a 2.0 $\log_{10}$ reduction in MRSA; all comparator disinfectants yielded a 1.0 $\log_{10}$ reduction or less. VR 4.68% yielded significantly higher $\log_{10}$ reductions in MRSA than did disinfectant C in 30 s and 5 min wet exposure testing and compared with all comparator disinfectants in 24 h dried residue testing (Table 5).

3.3. Fungal Pathogens

Table 6 presents comparative $\log_{10}$ reductions in *C. auris* at different exposure conditions. Results are presented as $\log_{10}$ reductions in *C. auris* relative to a positive control of 3.8×10^5 CFU mL^{-1}. After 30 s wet exposure, only VR 4.68% and VR 7.8% yielded greater than 3.0 $\log_{10}$ reductions in *C. auris*, and VR 2.34%, and disinfectants E and F, yielded greater than 2.0 $\log_{10}$ reductions in *C. auris*. After 5 min wet exposure, VR at all dilutions, and disinfectants E and F, yielded greater than 3.0 $\log_{10}$ reductions in *C. auris*, and only VR 7.8% produced complete eradication. As a 24 h dried residue, only VR 7.8% yielded a greater-than-3.0 $\log_{10}$ reduction in *C. auris*, and all other disinfectants produced less than a 1.0 $\log_{10}$ reduction (Table 6). In 30 s wet contact testing, VR 4.68% yielded significantly higher $\log_{10}$ reductions in *C. auris* than did all comparator disinfectants (Table 7). In 5 min wet contact testing, VR 4.68% yielded significantly higher reductions in *C. auris* than did disinfectants A, B, and C (Table 7). In 24 h dried residue testing, VR 4.68% yielded significantly higher reductions in *C. auris* than did all tested comparators except C and E (Table 7).

4. Discussion

Our results indicate that at appropriate dilutions, the novel VR disinfectant was more efficacious than seven other commercially available comparator disinfectants in both wet and dry residue states (up to 24 h) against surrogate viruses and various resistant bacterial and *C. auris* pathogens that are widely transmissible and can cause serious infections in healthcare and community settings.

A number of biocides (e.g., quaternary ammonium compounds, biguanides, phenolics) have been shown to have virucidal activity against enveloped viruses, but there is a general lack of efficacy against certain nonenveloped viruses [21,22]. Empirically, the explanation for this difference in activity has been based on both the interaction of these agents with the envelope of these viruses, and the agents' lack of activity against viral capsid proteins [23]. However, the new VR disinfectant with its components of 7% PHMB,

1.5% tetrasodium EDTA, 5% Dowanaol, 3% betaine, and 10% Glucopon showed high $\log_{10}$ reduction in nonenveloped viruses (the MS2 bacteriophage and FCV). FCV belongs to the Caliciviridae family, and is a surrogate of norovirus, which is the most prominent member of the Caliciviridae family, along with the Norwalk virus. The VR also yielded a high $\log_{10}$ reduction in resistant bacterial and fungal isolates.

Our results are consistent with those of Wang et al. (2021) [24], who found that PHMB-treated spandex fabric had strong antiviral behavior against feline coronavirus after 2 h of contact, with up to 99% viral inactivation, as well as strong antibacterial behavior, with 100% inhibitory action against both *S. aureus* and *Klebsiella pneumoniae* [24]. PHMB molecules can disrupt the microbial membrane and selectively condense the chromosomes, causing microbial death [25].

VR is hypothesized to be synergistically biocidal through the combined effects of its components. PHMB disrupts phospholipid membranes and binds to nucleic acids [26], and the other components destabilize membranes and other key microbial proteins through their synergistic surfactant effects [27]. The antibacterial mechanism of PHMB is highly dependent on the cationic biguanide moieties that can interact with the negatively charged phosphate head groups of the bacterial cell membrane and ultimately cause cell death [25,28]. PHMB also interacts with the viral capsid, to lead to virus death [29]. Tetrasodium EDTA reduces antimicrobial properties in vitro and disrupts ex vivo–generated biofilms in vivo [6,30–32]. Tetrasodium EDTA destabilizes biofilm and metalloproteases through its chelation activity [33]. Betaine, also known as trimethyl glycine, is a stable, nontoxic natural substance that is present in animals, plants, and microorganisms. Many microorganisms utilize betaine and have evolved different metabolic pathways for its biosynthesis and catabolism [34]. The main advantages of alkylpolyglucosides, known as Glucopon surfactants, are their ability to undergo a biodegradation process, and the fact that they can be obtained from natural and renewable sources such as corn, potatoes, wheat, or coconut oil [35].

The ability of VR to form a dry surface film that retains antiviral, antibacterial, and antifungal properties for 24 h is unique. In this study, VR demonstrated statistically superior efficacy and more prolonged activity compared with all tested disinfectants following 24 h dry residue exposure of viral pathogens, resistant *E. coli* and *S. aureus* bacteria, and the *C. auris* fungal pathogen. This unique feature of VR is of paramount importance because all tested pathogens and most transmissible infectious organisms can persist on various inanimate surfaces for several days and weeks, which enhance their transmission [1]. Viruses that belong to the Caliciviridae family can survive on plastics, cloth, and stainless steel for up to 168 days. Likewise, bacterial organisms (such as *S. aureus*, *E. coli*, and *P. aeruginosa*) and *C. auris* can survive on similar surfaces for up 70 days. Hence VR, unlike disinfectants with short-acting activity on surfaces, does not have to be used as frequently every day to prevent this prolonged and extended contamination.

A limitation of our study was the fact that we tested against only a few representative severe pathogens, and against one strain of each pathogen. In addition, testing was performed only on a single type of initially clean surface, with dilutions ranging from 3 to 9 ounces per gallon, and for either fairly brief wet, or very extended dry, durations.

Nevertheless, this promising combination merits further study in high-risk settings where frequent application of wet disinfectants cannot be consistently sustained.

5. Summary and Conclusions

The novel VR disinfectant at a concentration of 4.68% or above was the most effective among the comparator disinfectants when tested in both wet and dry residue states against a range of viral, bacterial, and fungal pathogens. Further study of VR is warranted, for extended eradication of highly infectious viruses on surfaces, given the potency of VR for the reduction in surrogate viruses and bacterial and fungal pathogens.

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