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Comparative efficacy of hand hygiene agents in the reduction of bacteria and viruses

Emily E. Sickbert-Bennett, MS, David J. Weber, MD, MPH, Maria F. Gergen-Teague, MT(ASCP), Mark D. Sobsey, PhD, Gregory P. Samsa, PhD, and William A. Rutala, PhD, MPH
Chapel Hill and Durham, North Carolina

Background: Health care-associated infections most commonly result from person-to-person transmission via the hands of health care workers.

Methods: We studied the efficacy of hand hygiene agents (n = 14) following 10-second applications to reduce the level of challenge organisms (Serratia marcescens and MS2 bacteriophage) from the hands of healthy volunteers using the ASTM-E-1174-94 test method.

Results: The highest log10 reductions of S marcescens were achieved with agents containing chlorhexidine gluconate (CHG), triclosan, benzethonium chloride, and the controls, tap water alone and nonantimicrobial soap and water (episode 1 of hand hygiene, 1.60-2.01; episode 10, 1.60-3.63). Handwipes but not alcohol-based handrubs were significantly inferior from these agents after a single episode of hand hygiene, but both groups were significantly inferior after 10 episodes. After a single episode of hand hygiene, alcohol/silver iodide, CHG, triclosan, and benzethonium chloride were similar to the controls in reduction of MS2, but, in general, handwipes and alcohol-based handrubs showed significantly lower efficacy. After 10 episodes, only benzethonium chloride (1.33) performed as well as the controls (1.59-1.89) in the reduction of MS2.

Conclusions: Antimicrobial handwashing agents were the most efficacious in bacterial removal, whereas waterless agents showed variable efficacy. Alcohol-based handrubs compared with other products demonstrated better efficacy after a single episode of hand hygiene than after 10 episodes. Effective hand hygiene for high levels of viral contamination with a nonenveloped virus was best achieved by physical removal with a nonantimicrobial soap or tap water alone. (Am J Infect Control 2005;33:67-77.)
efficacy studies have tested the efficacy of hand hygiene agents for unrealistically long contact times of at least 30 seconds, whereas health care professionals have been observed in 8 out of 14 studies to wash their hands for less than 10 seconds and never exceeding 24 seconds. No observational data were available on the duration of hand hygiene with alcohol-based handrubs. Thus, the largest comparative efficacy study to date using a standard methodology was undertaken to test 14 different hand hygiene agents for their efficacy in the reduction of both bacteria and viruses from the hands, using the realistic hand hygiene use time of 10 seconds. This study was conducted in conjunction with an analysis of the effects of test variables on the efficacy of hand hygiene agents. Based on our previously published work, this study employed those parameters that most clearly mimicked clinical use and enhanced the validity of a human challenge study. In addition, we observed hand hygiene among health care providers with an alcohol-based handrub to determine the duration of hand hygiene under actual use conditions.

Fig 1. Schematic of experimental design that defines "baseline," "episode," and "evaluation."
MATERIALS AND METHODS

Study population

Sixty-two healthy, adult, human volunteers were recruited for 70 hand hygiene efficacy evaluations for 14 different hand hygiene test agents (5 evaluations per test agent). The study was approved by the University of North Carolina School of Medicine Institutional Review Board, and written informed consent was obtained. Subjects were screened by questionnaire and physical examination for skin disorders and allergies prior to participation and excluded if they had eczema, psoriasis, any other chronic skin condition, nonintact skin, or an allergy to any active ingredients in the hand hygiene agents. For at least 7 days prior to their participation in the study, the subjects were instructed to avoid antimicrobial hand agents and were provided with a nonantimicrobial hand soap (Soft Soap Hand Soap; Colgate Palmolive Company, New York, NY) and a pair of reusable rubber gloves (Playtex Hand Saver Gloves, Platex Products, Inc., Dover, DE) to use while cleaning or washing dishes. Only a single subject was a health care worker who provided clinical care. Subjects were allowed to participate in no more than 3 evaluations during this study. An experimental evaluation is defined as a cleansing wash, followed by 10 episodes of hand contamination, hand hygiene, and efficacy evaluation following episodes 1, 3, 5, 7, and 10 (Fig 1). The period of time between participation, called the washout period, was at least 2 weeks. Five subjects were randomly assigned to each hand hygiene agent, and testing was done in a random manner.

Study methods for evaluating efficacy of hand hygiene agents

The Standard Test Method for Evaluation of the Effectiveness of Healthcare Personnel Handwash Formulations was used to measure the comparative efficacy of various health care hand hygiene agents. Modifications to the Standard Test Method included the following: (1) All hand hygiene was performed for 10 seconds, and (2) the efficacy of hand hygiene agents was evaluated against MS2 bacteriophage in addition to S. marcescens.

Study methodology is summarized as a flow diagram in Figure 1. Prior to beginning the experiment, subjects washed their hands for 10 seconds with a nonantimicrobial soap (Soft 'N Sure; Steris, St. Louis, MO) to cleanse the hands and to become familiar with the procedure. Prior to the baseline measurement and each hand hygiene, the hands were contaminated with a liquid suspension containing both MS2 and S. marcescens. The stock preparation of the bacterial test organism S. marcescens (ATCC 14756) was stored frozen in skim milk at −80°C. One liter of tryptic soy broth (Becton, Dickinson, and Company, Sparks, MD) was inoculated with a liquid suspension containing both MS2 and S. marcescens. The suspension containing the microorganisms was poured into the subjects’ cupped hands and was spread over their entire hands for 45 seconds. Next, the subjects allowed their hands to air-dry for 60 seconds. For the baseline measurement, the hands were sampled immediately following this contamination and air-dry period using the glove juice method described below. The baseline measurement was used to ensure that the organisms had adhered to the hands; our data showed that the method allowed the recovery of \(10^7\) CFU/mL for S. marcescens and \(10^8\) PFU/mL for MS2 on each hand. Immediately after the baseline measurement, the hands were recontaminated, air-dried for 60 seconds, cleaned with the selected hand hygiene agent for 10 seconds, rinsed for 10 seconds (if rinse was indicated per manufacturer’s instructions), and then immediately sampled according to the schedule displayed in Table 1.

The glove juice method, used to sample the hands for remaining organisms, consisted of placing each hand into a large-size, nonsterile latex glove filled with 75 mL of a sampling and neutralizing solution and massaging the gloved hand for 30 seconds. Nonsterile gloves were chosen because we had previously shown no difference in recovery or contamination with the use of nonsterile gloves as compared with a sterile glass flask. After the hand massage, 5 mL glove juice was aseptically retrieved from each glove, serially diluted, and assayed by the spread plate technique (S. marcescens) and by the double-agar layer technique (MS2) on tryptic soy agar plates (Remel, Lenexa, KS). At the end of the experiment, the subjects washed their hands extensively with 4% chlorhexidine gluconate (CHG) followed by 95% ethanol.

Test organisms

The stock preparation of the bacterial test organism S. marcescens was stored frozen in skim milk at −80°C. One liter of tryptic soy broth (Becton, Dickinson, and Company, Sparks, MD) was inoculated with a liquid suspension containing both MS2 and S. marcescens. The suspension containing the microorganisms was poured into the subjects’ cupped hands and was spread over their entire hands for 45 seconds. Next, the subjects allowed their hands to air-dry for 60 seconds. For the baseline measurement, the hands were sampled immediately following this contamination and air-dry period using the glove juice method described below. The baseline measurement was used to ensure that the organisms had adhered to the hands; our data showed that the method allowed the recovery of \(10^7\) CFU/mL for S. marcescens and \(10^8\) PFU/mL for MS2 on each hand. Immediately after the baseline measurement, the hands were recontaminated, air-dried for 60 seconds, cleaned with the selected hand hygiene agent for 10 seconds, rinsed for 10 seconds (if rinse was indicated per manufacturer’s instructions), and then immediately sampled according to the schedule displayed in Table 1.

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**Table 1. Schedule of contaminations and washes**

| Contamination | Hand hygiene | Recovery |
|---------------|--------------|----------|
| Nonantimicrobial cleansing wash | No | Yes | No |
| Baseline | Yes | No | Yes |
| Episode 1 | Yes | Yes | Yes |
| Episode 2 | Yes | Yes | No |
| Episode 3 | Yes | Yes | Yes |
| Episode 4 | Yes | Yes | No |
| Episode 5 | Yes | Yes | Yes |
| Episode 6 | Yes | Yes | No |
| Episode 7 | Yes | Yes | Yes |
| Episode 8 | Yes | Yes | No |
| Episode 9 | Yes | Yes | Yes |
| Episode 10 | Yes | Yes | Yes |
### Table 2. Hand hygiene test agents

| Active ingredient                  | Form     | Method of application | Brand name       | Manufacturer                                          |
|-----------------------------------|----------|-----------------------|------------------|-------------------------------------------------------|
| 60% Ethyl alcohol                 | Gel      | Waterless handrub     | Prevacare        | Johnson and Johnson, Arlington, TX                    |
| 61% Ethyl alcohol                 | Lotion   | Waterless handrub     | Avagard          | 3M Healthcare, St. Paul, MN                          |
| 61% Ethyl alcohol and 1% CHG      | Lotion   | Waterless handrub     | Avagard          | 3M Healthcare, St. Paul, MN                          |
| 62% Ethyl alcohol                 | Foam     | Waterless handrub     | Alcare           | Steris, St. Louis, MO                                |
| 70% Ethyl alcohol and 0.005% silver iodide | Wipe 256 cm² | Waterless handwash   | Surfacine        | Intelligent Biocides, Tyngsborough, MA                 |
| 0.5% Parachlorometaxylol and 40% SD alcohol | Wipe 296 cm² | Waterless handwipe   | Sanidex          | Professional Disposables, Inc, Orangeburg, NY          |
| 0.4% Benzalkonium chloride        | Wipe, 296 cm² | Waterless, handwipe  | Wash and Dri     | First Brands, Danbury, CT                             |
| 0.73% CHG                         | Liquid   | Handwash              | PrimaKare        | Steris, St. Louis, MO                                |
| 2% CHG                            | Liquid   | Handwash              | Bactoshield      | Steris, St. Louis, MO                                |
| 4% CHG                            | Liquid   | Handwash              | Bactoshield      | Steris, St. Louis, MO                                |
| 1% Triclosan                      | Liquid   | Handwash              | Prevacare        | Johnson and Johnson, Arlington, TX                    |
| 0.2% Benzethonium chloride        | Liquid   | Handwash              | Pure Cleanse     | Puresoft Solutions, Newfields, NH                     |
| Control: Nonantimicrobial soap    | Liquid   | Handwash              | Soft 'N Sure     | Steris, St. Louis, MO                                |
| Control: Tap water                | Liquid   | Handwash              | N/A              | N/A                                                   |

from a subculture of the frozen stock grown on tryptic soy agar and was incubated for 24 ± 4 hours at 25°C. The culture was stirred vigorously prior to removing 5-mL aliquots for inoculation onto the hands, and each aliquot was vortexed prior to application to the hands. Serial dilutions of the *S marcescens* suspension were assayed using the spread plate technique (100 µL per plate) at the beginning and end of each experiment. The suspension was used for no more than 4 hours.

As a surrogate for a nonenveloped human virus, MS2 (ATCC 15597-B1) was chosen for its stability and resemblance to clinically important families of viruses, including the picornaviridae and caliciviridae families. A stock preparation was made using plaque purification, propagation in host *Escherichia coli* C3000 bacterial cells (ATCC 15597), and chloroform extraction of infected cell lysate for final purification.28 Aliquots of MS2 were titered using the double-agar layer technique and then stored in phosphate buffer solution (pH 7.5) containing 20% (vol/vol) glycerol at −80°C. Fifty microliters of MS2 were added to the 5-mL aliquots of *S marcescens* and vortexed prior to inoculation. The host bacterial strain for the MS2 assay, *E coli* C3000, was stored at −80°C in tryptic soy broth containing 20% (vol/vol) glycerol. To prepare the *E coli* C3000 for use in the bacteriophage assay, 30 mL tryptic soy broth were inoculated with the stock preparation of *E coli* C3000 and incubated overnight at 37°C with constant rotary shaking at approximately 150 RPM.

### Solution preparation

Both the Butterfield’s phosphate buffer (KH₂PO₄ in water) diluent and the sampling solution were prepared according to the description in the ASTM E 1174-94. In addition, a neutralizing solution (Tween 80, lecithin, sodium oleate, sodium thiosulfate, proteose peptone, tryptone)29 was added to the sampling solution to quench the antimicrobial action of the hand hygiene agent applied to the hands; this neutralizing solution was validated using ASTM E 1054-91.30

### Hand hygiene test agents

The test agents displayed in Table 2 were used for the 10-second hand hygiene applications. Triclosan was not tested against MS2 because triclosan damaged the confluent layer of *E coli* host cells. All hand hygiene agents were purchased from commercial sources with the exception of Surfacine, which was provided by the manufacturer (Intelligent Biocides, Tyngsborough, MA). All hand hygiene agents were used at room temperature, and the temperature of the tap water used for hand hygiene and rinsing was adjusted to 40°C ± 3°C. The lotion, gel, and liquid soaps were dispensed into 3-mL aliquots using sterile syringes; the foam was dispensed by weight as 3-g aliquots, and 1 wipe was used per hand hygiene application. Controls consisted of a nonantimicrobial soap with water and tap water alone. Three-milliliter aliquots of each control agent were dispensed onto the hands, and the subjects rubbed their hands in a similar fashion as with a hand hygiene agent. Rinsing with tap water was only performed with handwashing products and with controls.

### Observation of health care personnel for determining duration of hand hygiene with an alcohol-based handrub

Direct observations of hand hygiene, as practiced by health care professionals in our institution, were undertaken by trained infection control professionals. Subjects were unaware that they were being observed. No demographic information or subject identifiers were obtained. The type of hand hygiene (chlorhexidine wash or alcohol-based handrub) and duration of hand hygiene were recorded on a data abstraction form.
Table 3. Log reductions of Serratia marcescens

| Agent                          | Episode 1 | Episode 3 | Episode 5 | Episode 7 | Episode 10 |
|-------------------------------|-----------|-----------|-----------|-----------|------------|
| 60% Ethyl alcohol              | 1.15 (0.75, 1.55) | 1.14 (0.68, 1.59) | 1.02 (0.40, 1.63) | 0.78 (0.21, 1.35) | 0.42 (0.01, 0.83) |
| 61% Ethyl alcohol              | 1.55 (0.89, 2.20) | 1.54 (0.81, 2.27) | 1.54 (1.00, 2.08) | 1.39 (0.62, 2.15) | 1.35 (0.66, 2.03) |
| 62% Ethyl alcohol              | 1.51 (1.19, 1.83) | 1.15 (0.78, 1.53) | 0.92 (0.26, 1.57) | 0.82 (0.28, 1.37) | 0.67 (0.23, 1.12) |
| 61% Ethyl alcohol/1% CHG       | 1.74 (1.39, 2.09) | 1.58 (1.27, 1.89) | 1.46 (1.10, 1.83) | 1.37 (0.86, 1.88) | 1.08 (0.55, 1.61) |
| 70% Ethyl alcohol/0.005% silver iodide | 1.78 (1.25, 2.31) | 1.52 (0.90, 2.15) | 1.40 (0.82, 1.98) | 1.38 (0.71, 2.05) | 1.07 (0.52, 1.62) |
| 0.5% Parachlorometaxylenol/40% SD alcohol | 0.57 (0.35, 0.80) | 0.68 (0.43, 0.94) | 0.64 (0.39, 0.90) | 0.62 (0.31, 0.93) | 0.84 (0.52, 1.17) |
| 0.4% Benzalkonium chloride    | 0.25 (0.13, 0.36) | 0.07 (0.00, 0.23) | 0.04 (0.00, 0.14) | 0.01 (0.00, 0.18) | 0.01 (0.00, 0.20) |
| 0.75% Chlorhexidine gluconate | 1.98 (1.68, 2.27) | 2.63 (2.46, 2.81) | 2.78 (2.48, 3.08) | 2.66 (2.52, 3.08) | 3.04 (2.75, 3.33) |
| 2% Chlorhexidine Gluconate     | 2.01 (1.91, 2.10) | 2.63 (2.43, 2.83) | 2.78 (2.44, 3.11) | 2.81 (2.37, 3.35) | 3.63 (3.08, 4.18) |
| 4% Chlorhexidine gluconate     | 1.89 (1.63, 2.16) | 2.72 (2.47, 2.98) | 2.41 (1.88, 2.94) | 2.75 (2.40, 3.09) | 3.14 (2.40, 3.89) |
| 1% Triclosan                   | 1.90 (1.50, 2.29) | 2.24 (1.85, 2.62) | 2.13 (1.73, 2.53) | 2.19 (1.88, 2.49) | 2.49 (1.77, 3.21) |
| 0.2% Benzethonium chloride     | 1.60 (1.40, 1.79) | 1.88 (1.56, 2.20) | 1.91 (1.66, 2.16) | 1.92 (1.58, 2.25) | 1.98 (1.77, 2.19) |
| Control: Nonantimicrobial soap | 1.87 (1.55, 2.19) | 1.73 (1.38, 2.08) | 1.66 (1.29, 2.02) | 1.56 (1.17, 1.96) | 1.60 (1.26, 1.95) |
| Control: Tap water             | 2.00 (1.80, 2.19) | 1.78 (1.66, 1.91) | 1.69 (1.55, 1.83) | 1.71 (1.55, 1.86) | 1.68 (1.55, 1.81) |

*p95% Confidence intervals are shown in parentheses.

Data analysis

After 24 hours of incubation at 37°C, MS2 plaques in the confluent E coli layer were enumerated and used to calculate the number of MS2 plaque forming units per milliliter (PFU/mL). After 48 hours of incubation at 25°C, red-pigmented colonies of S marcescens were enumerated and used to calculate the number of S marcescens colony forming units per milliliter (CFU/mL). Log reduction was determined by calculating the difference between the log10 of the baseline measurement (PFU/mL or CFU/mL) and the log10 of the measurement following each hand hygiene episode (PFU/mL or CFU/mL).

This was a repeated measures design, with subjects nested within product (ie, 5 subjects per product), 10 hand hygiene episodes per subject, 2 hands per episode, and 2 measurements (ie, duplicate plate counts) per hand. S marcescens and MS2 were analyzed separately. One record per hand hygiene episode was created by averaging the 2 measures per hand and then by averaging the results of the left and right hand. Using the data at episodes 1 and 10, we performed a 1-way analysis of variance on each product. Here, contrasts were used (1) to assess whether products within the same category (ie, method of application) produced similar results and (2) to assess whether product categories differed. Using the data from all available episodes, we disaggregated the data by product and then used analysis of covariance methodology to implement a repeated measures analysis for each product. The predictors were subject (ie, to account for the repeated measures component of the design) and episodes (ie, operationalized as a continuous variable). The resulting slope coefficient for episodes estimates the change in the log10 number of organisms associated with each subsequent hand hygiene episode. Because each of the comparisons presented here was of independent interest, no formal adjustment was made for multiple comparisons. However, exact P values are provided for readers desiring to make such adjustments. The 95% confidence intervals provided in the Figures were calculated using Excel (Excel 97; Microsoft, Bellevue, WA).

RESULTS

Efficacy of hand hygiene agents against S marcescens

The relative efficacy of various hand hygiene agents is displayed in Tables 3 and 4. After episode 1, the hand hygiene agents that were most efficacious were agents containing chlorhexidine (H-J) and triclosan (K) with approximately a 2 log10 reduction of S marcescens (Fig 2). Both the nonantimicrobial control and the tap water control showed similar log10 reductions of S marcescens. Alcohol-based handrubs alone (A-C) or combined with CHG (D) or silver iodide (E), and benzethonium chloride (L), demonstrated somewhat lower log10 reductions of 1.5 to 1.78. Hand hygiene wipes (F,G) achieved a log10 reduction <0.6. There was no statistical difference within groups for alcohol-based handrubs (A-E), hand hygiene wipes (F,G), handwashing agents (H-L), and controls (M,N). Alcohol-based handrubs were inferior to handwashing agents (P < .01) and controls (P < .01) but were superior to hand hygiene wipes (P < .0001).

After episode 10 (Fig 3), the hand hygiene agents that were the most efficacious (1.98-3.63 log10 reductions) in the reduction of S marcescens on the hands were the handwashing agents (ie, liquids containing CHG, triclosan, or benzethonium chloride) (Table 3). By the tenth episode, these agents were all significantly more efficacious than the alcohol-based handrubs (P < .0001).
and waterless handwipes ($P < .0001$). Within the waterless handrub agents, the maximum difference in log reduction was 0.93 ($P = .0702$), and, within the waterless handwipe agents, the maximum difference in log reduction was 0.84 ($P = .0179$). Among the handwashing agents, the CHG agents were more efficacious than the agents containing triclosan ($P = .0070$) or benzethonium chloride ($P = .0001$). The triclosan agent was more efficacious ($P = .0060$) than either the nonantimicrobial soap or the tap water alone. Over the 10 episodes, improved efficacy in bacterial reductions was demonstrated by the CHG handwashing agents (slope coefficients, 0.096 to 0.158; $P = .0001$), triclosan (slope coefficient, 0.052; $P = .0056$), and benzethonium chloride (slope coefficient, 0.035; $P = .0005$). Decreased efficacy trends in reduction of $S$ marcescens on the hands across multiple episodes was observed with the 0.4% benzalkonium chloride waterless handwipe (slope coefficient, $-0.024; P = .0002$) and all of the waterless handrubs (slope coefficient $-0.088$ to $-0.070; P = .0001$ to $P = .0005$), except the 61% ethyl alcohol.

## Efficacy of hand hygiene agents against MS2

After a single episode, all handwashing agents and controls demonstrated a log$_{10}$ reduction of 1.35 to 2.56 (Fig 4). The alcohol-based handrub containing ethyl alcohol and silver iodide resulted in a statistically significant reduction of approximately 1 log$_{10}$. All other agents except PCMX, which included alcohol-based handrubs and wipes failed to demonstrate a statistically significant reduction of MS2. The controls demonstrated statistically improved reduction of MS2 compared with alcohol-based handrubs ($P < .0001$), hand hygiene wipes ($P < .0001$), and handwashing agents ($P < .01$). Handwashing agents were superior to both alcohol-based handrubs ($P < .0001$) and hand hygiene wipes ($P < .0001$).

After episode 10 (Fig 5), the greatest reductions (1.53-1.89 log$_{10}$ reductions) of MS2 on the hands were achieved with a handwashing agent containing benzethonium chloride, the nonantimicrobial control soap, and the tap water alone (Table 4). CHG was statistically less efficacious than benzethonium chloride ($P = .0002$) or the soap and the tap water controls ($P = .0001$). Furthermore, CHG showed a significantly decreased efficacy over the 10 episodes (slope coefficient, $-0.118$ to $-0.098; P = .0001$ to $P = .0103$). Over the ten episodes, every waterless handrub (slope coefficient, $-0.086$ to $-0.045; P = .0001$ to $P = .0025$) and waterless handwipe agent (slope coefficient, $-0.075$ to $-0.046; P = .0001$) showed a negative trend in log$_{10}$ reductions in the reduction of MS2 from the hands. These results indicate a progressive accumulation of test microbes on the hands (ie, decreased efficacy of hand hygiene agents).

### Table 4. Log reductions of MS2 bacteriophage* 

| Agent                                      | Episode 1 | Episode 3 | Episode 5 | Episode 7 | Episode 10 |
|--------------------------------------------|-----------|-----------|-----------|-----------|------------|
| 60% Ethyl alcohol                          | $-0.15$   | $-0.39$   | $-0.44$   | $-0.65$   | $-0.67$    |
| 61% Ethyl alcohol                          | $-0.08$   | $-0.24$   | $-0.31$   | $-0.52$   | $-0.59$    |
| 62% Ethyl alcohol                          | $-0.26$   | $-0.48$   | $-0.61$   | $-0.67$   | $-0.71$    |
| 61% Ethyl alcohol/1% CHG                   | $-0.03$   | $-0.34$   | $-0.61$   | $-0.76$   | $-0.87$    |
| 70% Ethyl alcohol/0.005% silver iodide     | 0.96      | 0.51      | 0.53      | 0.42      | 0.18       |
| 0.2% Benzethonium chloride                 | 0.21      | 0.00      | $-0.13$   | $-0.16$   | $-0.23$    |
| 4% Benzalkonium chloride                   | 0.23      | $-0.07$   | $-0.17$   | $-0.38$   | $-0.46$    |
| 0.75% Chlorhexidine gluconate              | 2.10      | 0.91      | 0.79      | 0.81      | 0.77       |
| 2% Chlorhexidine gluconate                 | 1.38      | 0.64      | 0.60      | 0.59      | 0.30       |
| 0.2% Benzethonium chloride                 | 1.92      | 1.61      | 1.53      | 1.48      | 1.33       |
| Control: Nonantimicrobial soap             | 1.85      | 1.77      | 2.03      | 1.54      | 1.59       |
| Control: Tap water                         | 2.56      | 2.24      | 2.25      | 2.06      | 1.89       |

*95% Confidence intervals are shown in parentheses.

### Efficacy of hand hygiene agents against MS2

After a single episode, all handwashing agents and controls demonstrated a log$_{10}$ reduction of 1.35 to 2.56 (Fig 4). The alcohol-based handrub containing ethyl alcohol and silver iodide resulted in a statistically significant reduction of approximately 1 log$_{10}$. All other agents except PCMX, which included alcohol-based handrubs and wipes failed to demonstrate a statistically significant reduction of MS2. The controls demonstrated statistically improved reduction of MS2 compared with alcohol-based handrubs ($P < .0001$), hand hygiene wipes ($P < .0001$), and handwashing agents ($P < .01$). Handwashing agents were superior to both alcohol-based handrubs ($P < .0001$) and hand hygiene wipes ($P < .0001$).

After episode 10 (Fig 5), the greatest reductions (1.53-1.89 log$_{10}$ reductions) of MS2 on the hands were achieved with a handwashing agent containing benzethonium chloride, the nonantimicrobial control soap, and the tap water alone (Table 4). CHG was statistically less efficacious than benzethonium chloride ($P = .0002$) or the soap and the tap water controls ($P = .0001$). Furthermore, CHG showed a significantly decreased efficacy over the 10 episodes (slope coefficient, $-0.118$ to $-0.098; P = .0001$ to $P = .0103$). Over the ten episodes, every waterless handrub (slope coefficient, $-0.086$ to $-0.045; P = .0001$ to $P = .0025$) and waterless handwipe agent (slope coefficient, $-0.075$ to $-0.046; P = .0001$) showed a negative trend in log$_{10}$ reductions in the reduction of MS2 from the hands. These results indicate a progressive accumulation of test microbes on the hands (ie, decreased efficacy of hand hygiene agents).

### Observation of health care personnel for determining duration of hand hygiene with an alcohol-based handrub

Fifty episodes of hand hygiene with an alcohol-based handrub were observed for health care personnel working in an intensive care unit. The mean time of application (rubbing product onto hands) was 11.6 seconds (SD $= 2$-45 seconds).

### DISCUSSION

Health care-associated infections rank in the top 5 causes of death, with an estimated 90,000 deaths each year in the United States. Cross transmission has been estimated to cause 40% of nosocomial infections.
most common pathogens involved in these health care-associated infections are aerobic gram-negative bacteria (E coli, Pseudomonas) and aerobic gram-positive bacteria (S aureus, coagulase-negative Staphylococcus). Although the impact of viral infections remains incompletely defined,33 epidemics because of influenza,34 respiratory syncytial virus (RSV),35 rotavirus,36 adenovirus,37 noroviruses,38 hepatitis A virus,39 and coronaviruses40 are well described in the health care setting.

Hand hygiene has been repeatedly shown to reduce the level of transient microorganisms on the hands.41,42 More recently, hand hygiene has been demonstrated to reduce the incidence of health care-associated infections.2,3,43-44 For these reasons, the Centers for Disease Control and Prevention (CDC) and professional organizations recommend hand antisepsis as a key measure for reducing the incidence and impact of health care-associated infections.24 However, many studies have demonstrated that, on average, only 40% of health care workers’ contacts with patients result in appropriate hand hygiene.24 Barriers to appropriate hand hygiene have been reported to be (1) inaccessibility of hand hygiene supplies, (2) skin irritation from hand hygiene agents, (3) an inadequate amount of time for hand hygiene, (4) interference with patient care, (5) lack of knowledge of the guidelines, and (6) lack of information on the importance of hand hygiene. Therefore, recent guidelines promote the use of alcohol-based handrubs that are easily accessible.

A variety of hand hygiene agents are now available, with different active ingredients and application methods. Common active ingredients include CHG, triclosan, and alcohols. Typical ways of using these agents include detergents (nonantimicrobial soap) used with water, antimicrobial soap used with water, waterless alcohol-based handrubs, and waterless handwipes, which are disposable papers impregnated with antimicrobial agents. The FDA’s standard method used to evaluate the efficacy of these agents is similar to the ASTM-E 1174-94, which involves inoculation of hands with a standardized suspension of S marcescens and hand hygiene with a specified volume of a test agent. Studies using at least a 30-second exposure time have shown high levels of reduction of transient microorganisms with many hand hygiene agents, including both antimicrobial handwashing agents and alcohol-based handrubs.9,10,45,46 A recent summary of the literature noted that of 22 studies that assessed the efficacy of hand hygiene agents in reducing the counts...
of viable bacteria on the hands, only 3 used an exposure time as short as 15 seconds. However, in 14 observational studies of health care workers, hand hygiene has averaged approximately 12.6 seconds. Our observations of hand hygiene with an alcohol-based handrub demonstrated a similar duration of use (mean, 11.6 seconds; median, 10 seconds) as previously demonstrated for handwashing agents. We have previously shown that the efficacy of hand hygiene with one alcohol rub (62% ethyl alcohol) was similar when comparing rubbing for 10 seconds with rubbing until dry (>2 minutes) for episodes 5 to 7; for episode 1, rubbing for 10 seconds was more efficacious, and, for episode 10, rubbing until dry was more efficacious. Overall, no consistent benefit was demonstrated for rubbing until dry. Based on the duration of hand hygiene with handwashing agents reported in the literature and hand hygiene with an alcohol product demonstrated in our observations, we undertook a comprehensive comparative trial of plain soap and tap water vs antimicrobial-based soaps and handrubs at a realistic exposure time of 10 seconds. We have previously evaluated the methodologic factors that may alter the efficacy of hand hygiene. Our data showed that, for the reduction of gram-negative bacteria from the hands after a single episode, CHG and triclosan handwashing agents achieved the highest reductions of *S marcescens*. However, benzthonium chloride handwashing agent and alcohol-based handrubs, although achieving slightly lower log reductions, were not statistically different from the levels achieved by CHG and handwashing agents. Handwipes that contained benzalkonium chloride or PCMX/alcohol were significantly inferior to all other agents tested. No agents were significantly superior to nonantimicrobial or tap water controls.

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After multiple (10) hand hygiene episodes, the CHG handwashing agent was the most efficacious, followed by triclosan, benzthonium chloride, nonantimicrobial soap, and tap water alone. Although the alcohol-based handrubs were as efficacious as the handwashing agents after the first episode, these handrubs were significantly less efficacious over repeated hand hygiene episodes and when compared with the handwashing agents. A recent publication reported that alcohol-based hand hygiene agents applied for 15 to 30 seconds achieved a 4- to 7-log reduction in test bacteria using a different methodology (EN15000) that
employs the fingertip method. In addition, these investigators used products containing much higher concentrations of ethanol (≥80%) than were present in our test products. The lowered efficacy of alcohol-based products in our study may be related to the shorter exposure time and specific composition of the alcohol hand hygiene agents tested (eg, concentration of alcohol, type of alcohol, other active ingredients, inert ingredients, and emollients). The decreasing efficacy of alcohol demonstrated in our study after episode 10 is most likely due to the lack of persistent antimicrobial effect of alcohols and the progressive accumulation of organisms on the hands following repeated episodes of contamination.

Improved compliance with hand hygiene using alcohol-based handrubs has been demonstrated in many studies. It would be difficult, if not impossible, to make handwashing sinks as readily accessible as waterless handrub agents. Therefore, the use of alcohol-based handrubs will continue to be an important addition to our existing infection control armamentarium to improve hand hygiene compliance and at those locations at which sinks are not available. Furthermore, the exact reduction of transient microorganisms required to prevent cross transmission is unknown, and it is likely that even a 90% reduction achieved by alcohol-based handrubs along with improved compliance will decrease the incidence of health care-associated infections. However, given the trend of a reduced efficacy of alcohol-based handrubs with multiple episodes, it is prudent to recommend traditional hand hygiene with an antiseptic agent or a nonantimicrobial soap periodically throughout the day.

The morphology of the MS2 bacteriophage closely resembles nonenveloped, hydrophilic viruses that are relatively resistant to disinfectants and antiseptics. Nosocomial outbreaks have been reported because of nonenveloped viruses, including noroviruses, hepatitis A, and others. Similarly, day care center outbreaks have been reported because of noroviruses and hepatitis A. By evaluating the efficacy of hand hygiene agents with a surrogate (MS2) of clinically important nonenveloped viruses, hand hygiene agents might be selected that are efficacious in the reduction of both bacteria and viruses. The bacteriophage MS2 has been demonstrated to have similar susceptibility to alcohols, organic acids, and alkalis as poliovirus. MS2 has previously been used in hand decontamination studies because it is an excellent surrogate for human enteroviruses, such as polio, which are known to be transmitted by hand contact.

In our study, after a single episode, products containing alcohol and silver, CHG, triclosan, and benzethonium chloride all showed significant reductions of MS2; however, none was superior to nonantimicrobial or tap water controls. Alcohol-based handrubs alone or combined with CHG did not demonstrate any significant reduction in MS2. After 10 episodes, every waterless agent showed low efficacy (<0.18 log10 reduction of MS2) and significantly decreasing reductions over the 10 episodes. These results are consistent with the literature, which reports that hydrophilic viruses are more resistant than lipophilic viruses to inactivation by alcohols. In addition, other studies conducted in vivo, which assessed viral reduction using MS2 as a surrogate, showed a log10 reduction of 2.1 with a 110-second contact time with 50% ethanol (pH 11.5), an ≈0.5 log10 reduction with a 60-second contact time with 70% ethanol, an ≈0.3 log10 reduction with a 60-second contact time with 0.5% CHG/70% isopropanol, an ≈0 log10 reduction with a 60-second contact time with 4% CHG, and a 2.29 log10 reduction with a 30-second contact time with plain soap. These studies are comparable with our results when accounting for methodologic differences in contact time, volume of agent used, and concentration of agents. Of all the hand hygiene agents, the most efficacious at reducing MS2 was the handwashing with tap water alone, followed by the nonantimicrobial soap handwash, and the 0.2% benzethonium chloride handwash. Our data support the proposition that reduction of a nonenveloped virus was achieved by physical removal rather than inactivation. Our data do not provide an explanation why chlorhexidine performed less well than soap and water in removing MS2. The most likely explanation is that chlorhexidine enhanced viral adherence to human skin leading to a lower reduction of the virus.

In conclusion, our study shows that, at a short exposure time of 10 seconds, all agents with the exception of handwipes and a 60% ethyl alcohol handrub performed similar to nonantimicrobial and tap water controls with reductions of 1.15 to 2.01 log10 of *Serratia marcescens*. After 10 episodes, which evaluates the efficacy of agents following multiple episodes of contamination, handwashing agents with 0.75% CHG, 2% CHG, 4% CHG, 1% triclosan, 0.2% benzethonium chloride, nonantimicrobial soap handwash, and tap water alone were efficacious (>1.5 log10) in reduction of bacteria. Our data demonstrate that short contact times are effective in reducing transient hand flora, and, therefore, the future focus of hand hygiene can be on improving the compliance rather than increasing the duration of hand hygiene. Because use of a shorter duration of hand hygiene is likely to improve compliance, greater compliance should then lead to a reduction in health care-associated infections. Alcohol-based handrubs and wipes were generally ineffective in demonstrating significant virus reduction.
from the hands either after a single episode or multiple episodes of hand hygiene. Although viruses are a less common cause of health-care-associated infections than are bacteria, in situations in which infection with viruses is likely (eg, gastroenteritis because of norovirus or hepatitis A infections), the use of soap and water washes should be considered.

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