

Quantitative Microbial Risk Assessment of Antibacterial Hand Hygiene Products on Risk of Shigellosis

DONALD W. SCHAFFNER,^{1*†} JAMES P. BOWMAN,² DONALD J. ENGLISH,³ GEORGE E. FISCHLER,⁴
JANICE L. FULS,⁵ JOHN F. KROWKA,⁶ AND FRANCIS H. KRUSZEWSKI⁷

¹Department of Food Science, Rutgers University, 65 Dudley Road, New Brunswick, New Jersey 08901; ²James P. Bowman & Associates LLC, 6409 Roth Ridge Drive, Loveland, Ohio 45140; ³Avon Products, Inc., One Avon Place, Suffern, New York 10901; ⁴Gefmey Consulting LLC, 4808 North 24th Street, Phoenix, Arizona 85016; ⁵Henkel Consumer Goods Inc., 19001 North Scottsdale Road, Scottsdale, Arizona 85255; ⁶Personal Care Products Council, 1101 17th Street N.W., Suite 300, Washington, D.C. 20036-4702; and ⁷American Cleaning Institute, 1331 L Street N.W., Suite 650, Washington, D.C. 20005, USA

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ABSTRACT

There are conflicting reports on whether antibacterial hand hygiene products are more effective than nonantibacterial products in reducing bacteria on hands and preventing disease. This research used new laboratory data, together with simulation techniques, to compare the ability of nonantibacterial and antibacterial products to reduce shigellosis risk. One hundred sixty-three subjects were used to compare five different hand treatments: two nonantibacterial products and three antibacterial products, i.e., 0.46% triclosan, 4% chlorhexidine gluconate, or 62% ethyl alcohol. Hands were inoculated with 5.5 to 6 log CFU *Shigella*; the simulated food handlers then washed their hands with one of the five products before handling melon balls. Each simulation scenario represented an event in which 100 people would be exposed to *Shigella* from melon balls that had been handled by food workers with *Shigella* on their hands. Analysis of experimental data showed that the two nonantibacterial treatments produced about a 2-log reduction on hands. The three antibacterial treatments showed log reductions greater than 3 but less than 4 on hands. All three antibacterial treatments resulted in statistically significantly lower concentration on the melon balls relative to the nonantibacterial treatments. A simulation that assumed 1 million *Shigella* bacteria on the hands and the use of a nonantibacterial treatment predicted that 50 to 60 cases of shigellosis would result (of 100 exposed). Each of the antibacterial treatments was predicted to result in an appreciable number of simulations for which the number of illness cases would be 0, with the most common number of illness cases being 5 (of 100 exposed). These effects maintained statistical significance from 10⁶ *Shigella* per hand down to as low as 100 *Shigella* per hand, with some evidence to support lower levels. This quantitative microbial risk assessment shows that antibacterial hand treatments can significantly reduce *Shigella* risk.

The spread of transient bacteria by hands plays a significant role in the direct and indirect transmission of disease (9). Proper hand washing with soap and water is accepted as one of the most effective ways to reduce the spread of disease in a variety of environments, including community, health care, and food-related contexts. Studies have compared the effectiveness of antibacterial and nonantibacterial soaps, using a variety of antibacterial formulations under a variety of conditions. Based on these studies, there has been little consensus on whether antibacterial soaps are more effective than nonantibacterial soaps in reducing bacteria and preventing disease (17). There are some population studies that show the benefit of hand washing as compared with no hand washing (12, 15, 18, 20, 21) and a reduction in the amount of enteric disease infection or a reduction in diarrhea when soap is provided for hand washing.

A U.S. Food and Drug Administration Nonprescription Drug Advisory Committee review of consumer antiseptic hand wash product studies concluded in 2005 that existing data failed to demonstrate any association between specific log reductions of bacteria achieved by antiseptic hand washing in surrogate testing and a reduction of infection (25). A consumer product industry-sponsored expert panel meeting held in 2007 reviewed new methods for assessing the efficacy of antibacterial hand washes. The panel reviewed a testing protocol for linking the effectiveness of antibacterial hand washing to infection reduction and made recommendations for conducting future studies designed to demonstrate the efficacy of antibacterial hand wash formulations (3). As it is potentially difficult to determine the dose of bacteria delivered directly from the hand to the mouth, the testing protocol focused on enteric infection and disease in which transient bacteria are transferred from the hand directly to the mouth or indirectly to the mouth via the hands that contaminate the food that is subsequently ingested.

It has been shown that hand contamination by bacteria during food preparation can be as high as 5 to 6 log CFU,

* Author for correspondence. Tel: 732-982-7475; Fax: 732-932-6776; E-mail: schaffner@aesop.rutgers.edu.

† With the exception of the first author, all other authors contributed equally to the manuscript and are listed alphabetically.

for instance, when contaminated meat is handled (5, 14). A review of the literature on the dose response of enteric organisms such as *Shigella*, *Escherichia coli* O157:H7, and *Campylobacter jejuni* indicates that reducing exposure to these organisms to less than 3 log could significantly reduce the infection rate (6, 11, 16, 22).

A simulation model that uses hand washing to reduce the bacterial load from a predetermined baseline and that determines the bacterial load transferred to a ready-to-eat food could provide significant insight into the potential effect of antiseptic hand washing on reduction of infection. Published infection rates derived from dose-response studies can be combined with data on the number of bacteria on hands, the effectiveness of antibacterial products, and hand-to-food transfer rates to predict likely clinical outcomes.

The work presented in this study confirms and builds on previously published *Shigella*-based data and methods (7, 8) and incorporates recommendations of the expert panel (3). The previous scope of work (7, 8) used *Shigella* as the test organism, one hand wash product containing triclosan as the active ingredient, and one nonantibacterial product. The current work also uses *Shigella* and triclosan and additional active ingredients (ethyl alcohol, chlorhexidine gluconate) as well as an additional nonantibacterial hand wash product. The number of subjects used in the present study is significantly higher than in previous work, allowing for a more powerful statistical analysis. Microbial risk modeling analyses that provide insight into the potential benefit of using antibacterial products for hand washing are also included in this study.

MATERIALS AND METHODS

Subject qualification, enrollment, and prestudy restrictions. All prospective test subjects signed an informed consent form; and subject qualification, enrollment, and prestudy restrictions were as per ASTM International protocols (1, 2). Briefly, test subjects were instructed to avoid contact with antimicrobial products, including antimicrobial-containing personal care products, and to avoid bathing in biocide-treated pools or spas, and they were screened for any conditions that would exclude participation in the study. Eligible subjects were given nonantibacterial personal care products and a copy of the study instructions. Test subjects followed the study restrictions for at least 7 days prior to the test day. Subjects still meeting the study criteria on the day of the test were randomly assigned to one of five groups of 32 subjects, one for each of the five treatments. Subjects (268) were enrolled in the pretest conditioning phase; of those, 163 (56 male and 107 female) met the study criteria, were enrolled in the test phase, and completed the study. The study was double blinded, so that neither test subjects nor laboratory personnel knew which treatment a subject was using on any given day. An Institutional Review Board reviewed the investigation for informed consent and to determine risk to any participants of physical or psychological harm. Approval by the Board was obtained prior to initiation of the study. Hill Top Research Corporation conducted the laboratory portion of this study according to their Good Clinical Practices and Standard Operating Procedures.

Marker organism and preparation. *Shigella flexneri*, an invasive pathogen causing shigellosis, was used as the marker

organism. The specific strain *S. flexneri* 2a ATCC 700930 has been used in numerous human oral-dosing studies to evaluate potential vaccines and has been recognized as safe for this use (13, 23). Preparation of the freeze-dried culture of *S. flexneri* ATCC 700930 and preparation of the inoculum was as indicated elsewhere (2, 7, 8), resulting in a suspension containing $>1 \times 10^6$ CFU/ml viable *S. flexneri* in 0.85% physiological saline (BD, Sparks, MD).

Preparation of melon balls. Risk reduction from hand washing includes not only the effect of the wash, but also of subsequent transfer to ready-to-eat foods. Freshly cut cantaloupe melon balls were used as the ready-to-eat food item, following a previously developed protocol (7). Briefly, prior to a test day, fresh whole cantaloupe was purchased at a local store. Four 2.2-cm melon balls per subject were prepared no more than 24 h in advance of experiments. The melon balls were refrigerated until testing and were brought to room temperature prior to handling.

Conditioning wash and hand contamination. All subjects underwent a conditioning wash prior to beginning the experimental treatment as per Fischler et al. (7), using a nonantibacterial soap (Johnson's Head-To-Toe baby wash, Johnson & Johnson Consumer Companies, New Brunswick, NJ). Hands were contaminated as per ASTM E2784 (2). Briefly, two paper towel pads were placed about 1 ft (~0.3 m) apart on top of the foil pouch used to hold them during autoclaving. Viable *S. flexneri* (30 ml, $\sim 1 \times 10^6$ CFU/ml) in suspension were added to each paper towel pad. Subjects pressed both hands onto pads for 5 ± 1 s and allowed their hands to air dry for 90 ± 5 s. Hands were then sampled as indicated in the section "Measuring transfer to food and effect of hand treatment" as the baseline measurement. Hands were rewashed with nonantibacterial soap after baseline sampling and were rinsed to remove residual sampling solution, dried with paper towels, and recontaminated as above.

Hand treatment. Five different hand treatments were investigated in this study: (i) Tone Foaming Hand Wash, Island Mist, a nonantibacterial product (The Dial Corporation, a Henkel Company, Scottsdale, AZ); (ii) Kiss My Face Self Foaming Liquid Soap, Lavender & Chamomile, a nonantibacterial product (Kiss My Face, LLC., Gardiner, NY); (iii) Dial Complete Antibacterial Foaming Hand Wash, an antibacterial hand wash containing 0.46% triclosan (The Dial Corporation); (iv) Hibiclens, an antibacterial hand wash containing 4% [wt/vol] chlorhexidine gluconate (Mölnlycke Health Care US, LLC, Norcross, GA); and (v) Purell Instant Hand Sanitizer, 62% ethyl alcohol (GOJO Industries, Inc., Akron, OH).

In all cases, subjects were directed to maintain their hands parallel to their elbows for treatments in order to prevent contamination of their clothing or upper forearms. In the case of the two nonantibacterial hand wash products and the product containing 0.46% triclosan, approximately 3 g of the product was dispensed into the dry cupped palm of one hand. The test subject then distributed the product over all surfaces of both hands. The subject was then directed to work the product vigorously over all surfaces of the hands and lower third of the forearm for a period of 30 ± 5 s. The hands and wrists were then rinsed under $40 \pm 2^\circ\text{C}$ running tap water for 30 ± 5 s and were allowed to air dry for 90 ± 5 s.

In the case of the alcohol-based hand sanitizer, 3.0 ml of the product was dispensed into the dry cupped palm of one hand and distributed over the surfaces of both hands and lower third of the forearm. The subjects were directed to rub their hands briskly until dry, or up to 2 min.

As per manufacturer's directions, Hibiclens (4% [wt/vol] chlorhexidine gluconate) was used in a slightly different way than the three other hand wash products described here. Immediately prior to treatment with the chlorhexidine gluconate-containing product, the subject's hands were wetted with a small amount of water. After wetting, 5.0 ml of the product was dispensed into the wet cupped palm of one hand and distributed over all surfaces of both hands. The subject was then directed to work the material vigorously over all surfaces of the hands and the lower third of the forearm for 15 s. The subject's hands were then rinsed under running tap water for 30 s and allowed to air dry for 90 s.

Measuring transfer to food and effect of hand treatment.

After hand treatment, the procedure of Fischler et al. (7) was used for bacterial transfer to melon balls. Briefly, the dominant hand was used to handle four melon balls, whereas the nondominant hand was sampled directly, as detailed below.

Four melon balls were dispensed into the cupped dominant hand of the test subject and rolled using the thumb and fingers for 15 ± 2 s. The melon balls were placed into a sterile Stomacher bag and weighed and mixed with 20 ml of sterilized sampling solution (KH_2PO_4 [0.4 g], Na_2HPO_4 [10.1 g], Tween 80 [10 g], lecithin [3 g], sodium thiosulfate [1 g], and Triton X-100 [1 g] in 1 liter of purified water adjusted to 7.8 ± 0.1). The melon balls were macerated for 1 min at 260 rpm using a Stomacher Laboratory Blender (Seward Laboratory Systems Inc., Port Saint Lucie, FL).

The treated nondominant hand was sampled by placing low bioburden plastic bags (29.2 by 31.8 cm; Glad Food Storage Bags, The Clorox Company, Oakland, CA) on the subject's hand and adding a 75-ml aliquot of sampling solution. The bag was massaged for 1 min in a uniform manner by a laboratory technician. A 3- to 5-ml aliquot of the sampling solution from the bagged hand was placed into tubes containing sterile dilution fluid (1.25 ml of phosphate buffer stock [34 g KH_2PO_4 /liter], Tween 80 [10.0 g], lecithin [3.0 g], and sodium thiosulfate per liter [1.0 g], adjusted to pH 7.2 ± 0.1).

Aliquots of the sampling solution or dilutions of the sampling solution were collected from subjects' hands or melon balls. These aliquots were spread plated in duplicate on Hektoen enteric agar (Remel, Lenexa, KS, or 3M, St. Paul, MN). Petri plates were incubated for 18 to 24 h at $35 \pm 2^\circ\text{C}$, and colonies were enumerated. The calculated detection limits of the test method were 75 CFU per hand and ~ 40 CFU per melon ball. When a sample had no detectable organisms (i.e., below the detection limit), it was reported to be at the detection limit of the method.

Disinfection of subject hands and follow-up. After the final sampling was completed, subject hands and wrists were disinfected as per ATSM E2784 (2). Hands were rinsed with water, washed with Hibiclens, treated with 70% isopropyl alcohol, and air dried. All test subjects were given a sheet containing instructions for examining hands for signs of skin infection and were instructed to call the clinical site immediately if they should have any intestinal illness symptoms. The subjects returned to the clinical test site within 8 to 14 days to have their hands examined for any signs of infection.

Statistical analyses: microbiology. The changes from baseline counts were obtained for each treatment. The data used in the statistical analysis were the differences between the averages of the right- and left-hand log baseline counts and the log posttreatment count from the treated hand not used for conducting the melon ball portion of the study. Within-treatment analysis used Student's *t* test for paired data to compare the posttreatment counts to the baseline counts. Between-treatment analysis was conducted

on the changes from baseline counts as well as on the melon ball counts using analysis of covariance with treatment as a factor and the average log baseline count as the covariate. Multiple comparison testing of all hand treatments, as well as all differences in counts on melon balls between treatments, was conducted using least-squares means. A comparison of difference in nondetectable count rates on hands and on melon balls was performed using Fisher's exact test. Statistical tests of these hypotheses employed a level of significance of 0.05 using SAS software (SAS Institute Inc., Cary, NC).

Quantitative microbial risk assessment. Relevant data on the behavior of *Shigella* from the experiments outlined above and from the peer-reviewed literature were used to construct a quantitative microbial risk assessment. Table 1 provides an overview of the simulation variables and distributions that were used in the risk assessment model; these will be discussed more fully in "Results." The dose-response model is based on that developed by Crockett et al. (4) for *Shigella*, using data for *S. dysenteriae* and *S. flexneri* from three different dose-response studies. Data, models, and user inputs were entered into an Excel (Microsoft, Redmond, WA) spreadsheet as described in Table 1. Modeling @RISK software (Palisade Corporation, Ithaca, NY) was used to perform Monte Carlo simulations of 10,000 iterations for each scenario evaluated. One iteration of the simulation represents 100 exposures to *Shigella* from melon balls with a given concentration of *Shigella*, originating from simulated food service workers who had washed their hands with either a nonantibacterial hand wash or an antibacterial hand wash product prior to handling melon balls. Quantitative microbial risk assessment simulation results were analyzed for statistically significant differences between use of a nonantibacterial hand wash or an antibacterial hand wash using the Mann-Whitney U, a nonparametric statistical hypothesis test for assessing whether one of two samples of independent observations tends to have larger values than the other. Simulation results were also analyzed for statistically significant differences using a two-sample *t* test, assuming unequal variances using Excel (Microsoft).

RESULTS

The effect of the five different treatments on the *Shigella* concentration found on contaminated hands is shown in Figure 1. The two nonantibacterial treatments each show about a 2-log reduction and were found not to be statistically significantly different from one another. The other three treatments show average log reductions greater than 3 but less than 4. The chlorhexidine gluconate treatment shows the least log reduction of the three but is not significantly different than that of the triclosan treatment. The ethyl alcohol treatment shows the greatest average log reduction, but this is not significantly different than that of the triclosan treatment.

The effect of the treatments on the eventual concentration of *Shigella* ending up on the melon balls is shown in Figure 2. As with the data shown in Figure 1, the two nonantibacterial treatments were found not to be statistically significantly different from one another. Both nonantibacterial treatments show close to 3 log CFU per melon ball. All three antibacterial treatments show a significantly different concentration on the melon balls relative to the nonantibacterial treatments. None of the three antibacterial

TABLE 1. Simulation model parameters and equations for the effect of hand washing treatments on risk of shigellosis^a

Variable name	Cell	Nonantibacterial hand wash	Cell	Triclosan hand wash	Data source
Initial contamination					
Starting log dose on hands	B4	6	D4	= B4 ^b	User input
Starting arithmetic dose on hands	B5	= 10^B4	D5	= 10^D4	Calculated
Treatment					
Treatment, mean log reduction	B8	2.1965	D8	3.4849	This study
Treatment, SD log reduction	B9	0.5039	D9	0.5226	This study
Actual log reduction on hands	B10	= RiskNormal(B8,B9) ^c	D10	= RiskNormal(D8,D9)	Calculated
Posttreatment on hands	B11	= B4-B10	D11	= D4-D10	Calculated
Reduction to melon					
Reduction to melon, mean	B14	0.9597	D14	= B14	This study
Reduction to melon, SD	B15	0.3699	D15	= B15	This study
Actual log reduction to melon	B16	= RiskNormal(B14,B15)	D16	= RiskNormal(D14,D15)	Calculated
Concn on melon, log CFU	B17	= B11-B16	D17	= D11-D16	Calculated
Concn on melon, CFU	B18	= 10^B17	D18	= 10^D17	Calculated
Dose response					
α parameter	B21	0.2099	D21	= B21	Crockett et al., 1996 (4)
N50 parameter	B22	1120	D22	= B22	Crockett et al., 1996 (4)
Probability of illness, given dose	B23	= 1-(1 + B18*(2^(1/B\$21)-1)/B\$22)^-B\$21	D23	= 1-(1 + D18*(2^(1/D\$21)-1)/D\$22)^-D\$21	Calculated
Exposures to consider	B24	100	D24	100	User input
Illnesses, given 100 exposures	B25	= RiskBinomial(B24,B23)	D25	= RiskBinomial(D24,D23)	Calculated

^a Food handlers' hands are contaminated with *Shigella* prior to hand washing, after which melon balls are handled.

^b The equations are shown in the center two columns of the table in Microsoft Excel format, and follow spreadsheet cell formatting such that “=B4” references cell B4, which in this case is to the left (i.e., “6”).

^c RiskNormal and RiskBinomial refer to functions in @Risk.

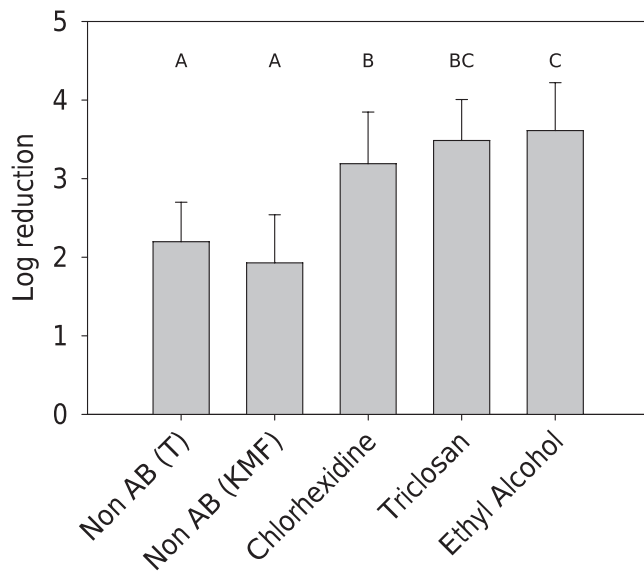


FIGURE 1. The effect of two nonantibacterial hand wash treatments, two antibacterial hand wash treatments, and ethyl alcohol hand sanitizer on *Shigella* concentration on inoculated hands. Bars with the same letter are not statistically significantly different ($P > 0.05$).

treatments (chlorhexidine gluconate, triclosan, or ethyl alcohol) are significantly different from one another.

The frequency of nondetection events on hands and melons is shown in Figure 3. A nondetection event occurs when the concentration of bacteria found on a hand or melon ball is less than the detection limit specified in the methods above. No nondetections of *Shigella* occurred on hands or melons when the first nonantibacterial hand wash product was used on contaminated hands, and they occurred only a small number of times for melons when

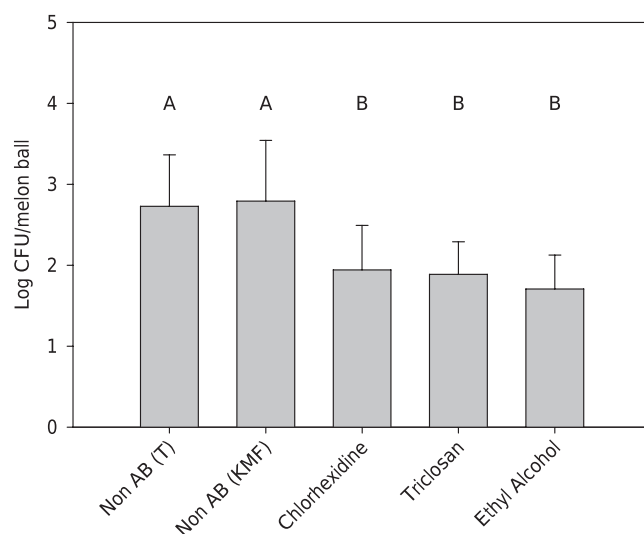


FIGURE 2. The effect of two nonantibacterial hand wash treatments, two antibacterial hand wash treatments, and ethyl alcohol hand sanitizer on the eventual concentration of *Shigella* transferred from hands to melon balls. Bars with the same letter are not statistically significantly different ($P > 0.05$).

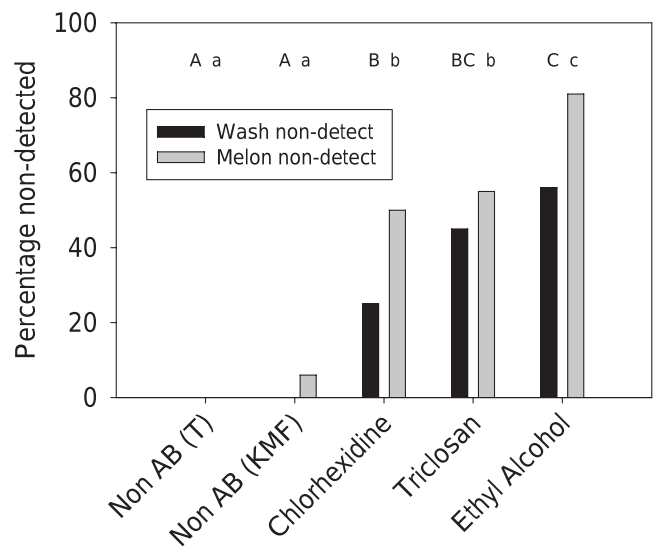


FIGURE 3. The frequency of nondetection events on hands and melons when hands are contaminated with *Shigella* and then subjected to two nonantibacterial hand wash treatments, two antibacterial hand wash treatments, or ethyl alcohol hand sanitizer. Nondetection events on hands are shown with a black bar, and nondetection events on melon balls are shown with a gray bar. Bars with the same letter are not statistically significantly different ($P > 0.05$); capital letters refer to hands, lowercase letters to melon balls.

the second nonantibacterial treatment was used on contaminated hands. The nondetection frequencies were not significantly different for the two nonantibacterial treatments. All three antibacterial treatments had significantly more nondetections than the nonantibacterial treatments, on both hands and melons. There were significant differences among the antibacterial treatments in some cases, as indicated by the letters shown in Figure 3.

The basic structure of the quantitative microbial risk assessment is shown in Table 1. The first column shows the variable names. The second column shows the parameter values or equations representing one of the nonantibacterial treatments (Tone foaming hand wash). The third column shows the same information for one of the antibacterial treatments (triclosan). The last column shows the source of the values (user input, calculated, determined in this study, or from peer-reviewed literature). The equations are shown in Microsoft Excel format and reference spreadsheet cells; thus, for example, the equation “=10^B4” references cell B4, which in this case is immediately above that location (i.e., “6”). RiskNormal and RiskBinomial refer to functions in @Risk.

Figure 4 shows the results from simulation modeling that assumed a starting concentration of 1 million *Shigella* bacteria on the hands, and each of the five panels shows the simulated number of infection cases arising from 10,000 iterations in which the hands of food handlers are exposed to each of the five treatments. The first row of panels (A and B) shows the simulation results from the two nonantibacterial soaps (Tone and Kiss My Face, respectively). The most commonly simulated number of illness

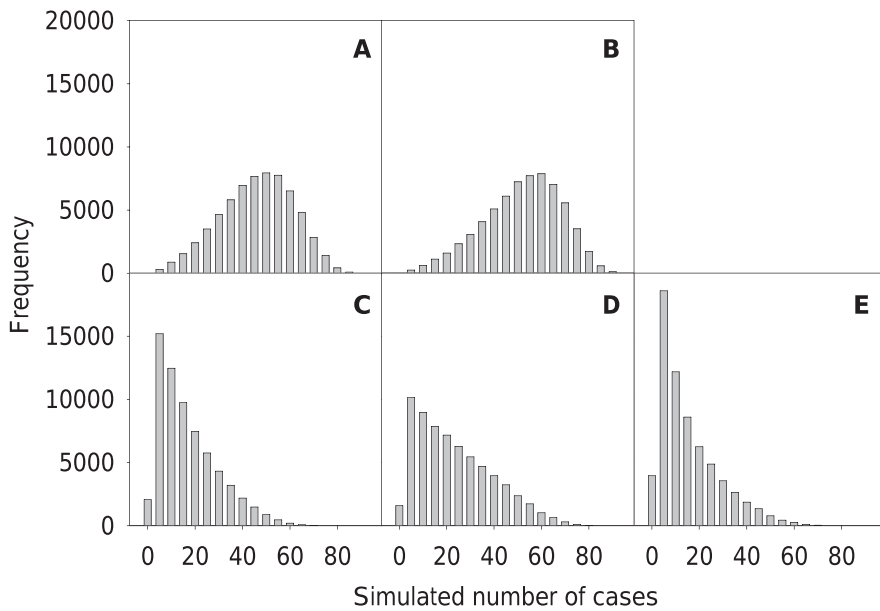


FIGURE 4. Simulation modeling results, assuming starting concentration of 1 million *Shigella* bacteria on the hands, and number of cases arising from 10,000 iterations in which the food service workers' hands are exposed to each of the five treatments: (A) Tone foaming hand wash, (B) Kiss My Face hand wash, (C) triclosan-containing hand wash, (D) chlorhexidine gluconate-containing hand wash, (E) ethyl alcohol hand sanitizer.

cases is 50 to 60 (of 100 exposed), with no interactions resulting in 0 simulated cases and with very few resulting in more than 90 simulated illness cases. The results with the three other treatments are markedly different, as shown

in panels C, D, and E (triclosan, chlorhexidine gluconate, and ethyl alcohol, respectively). Each of these antibacterial treatments resulted in an appreciable number of iterations in which the number of illness cases is 0. The most common number of illness cases is 5 (of 100 exposed), and

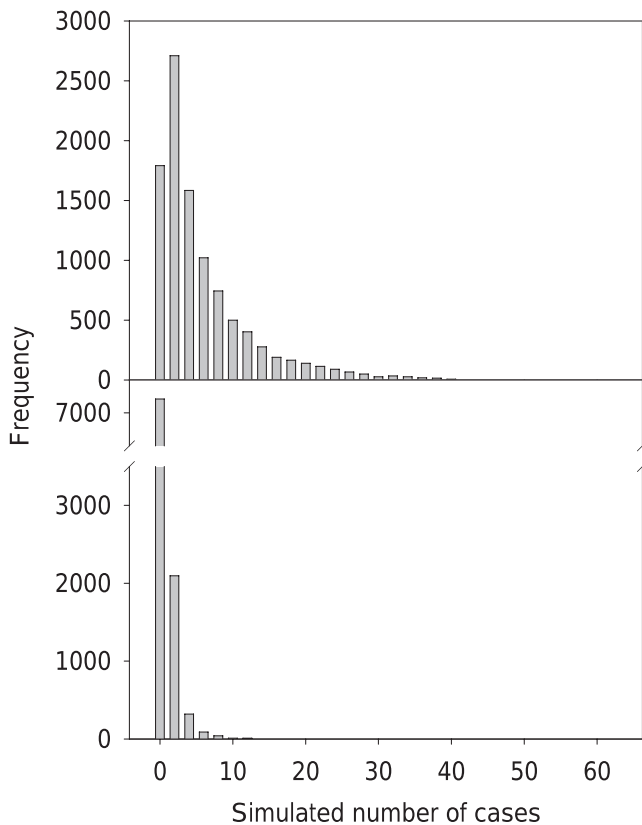


FIGURE 5. Simulation modeling results, assuming starting concentration of 10,000 *Shigella* bacteria on the hands, and number of cases arising from 10,000 iterations in which the food service workers' hands are exposed to (top) nonantibacterial hand wash and (bottom) triclosan-containing hand wash. The bottom panel contains a y axis break to allow better visualization of distribution tail.

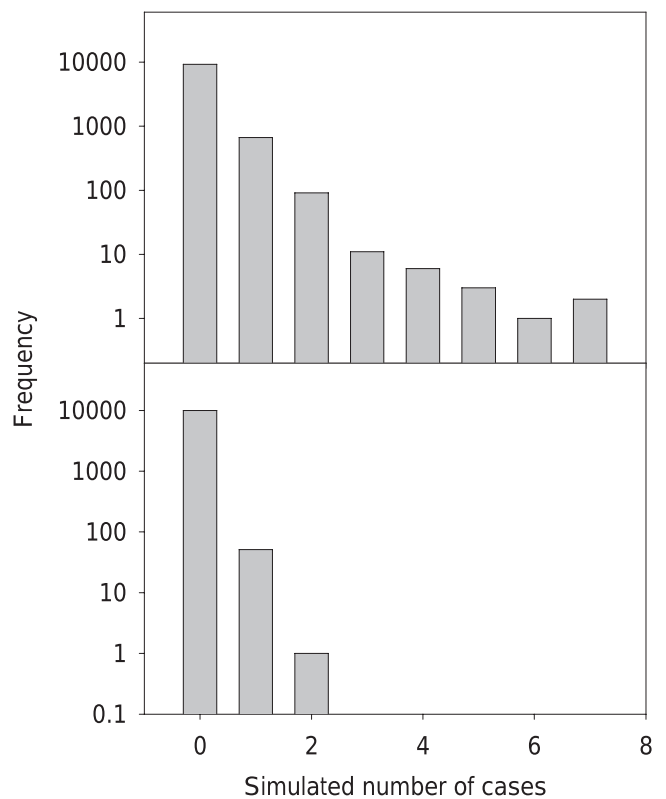


FIGURE 6. Simulation modeling results, assuming starting concentration of 100 *Shigella* bacteria on the hands, and number of cases arising from 10,000 iterations in which the food service workers' hands are exposed to (top) nonantibacterial hand wash and (bottom) triclosan-containing hand wash.

the maximum number of simulated illnesses cases is never more than about 70.

Figure 5 shows the results for the simulation in which the starting concentration of *Shigella* was lowered by two orders of magnitude from 10^6 (as shown in Fig. 4) to 10^4 . Figure 5 is also simplified to include only two treatments, a nonantibacterial hand wash (top panel) and an antibacterial hand wash using triclosan (bottom panel). In the case of the simulated nonantibacterial hand wash, the greatest number of illness cases predicted is approximately 40, with 0 cases simulated $\sim 18\%$ of the time, and with the most frequent number of simulated cases approximately 5. When the triclosan-containing hand wash is simulated, the maximum number of illnesses is predicted to be approximately 12, and the most frequently simulated number of illnesses is 0, which occurred in more than 70% of the interactions. Note the axis break for the y axis in the bottom panel. The Mann-Whitney U nonparametric statistical hypothesis test indicated that the difference between the two simulated treatments is highly significant ($P < 0.0015$), as did the two-sample *t* test, assuming unequal variances.

Figure 6 shows the simulation results for the same two treatments (top panel nonantibacterial hand wash, bottom panel antibacterial hand wash with triclosan) with an additional two orders of magnitude drop in the starting concentration, i.e., a starting concentration of 100 *Shigella* on the hands. As before, a marked contrast is seen. Note that the y axis in the plot has been transformed to a logarithmic scale to better visualize the results. The most frequent result from both simulations is zero illnesses, but note that when the nonantibacterial hand wash is used, more than 600 iterations (6.64% of iterations) resulted in one illness, whereas when the antibacterial hand wash is simulated, about 50 iterations (0.51% of all iterations) resulted in a single illness. In two iterations in which nonantibacterial hand wash was used (top panel), seven illnesses were simulated. The greatest number of illnesses simulated in the case of using an antibacterial hand wash with triclosan was only two, which occurred in only 1 of the 10,000 iterations (0.01% of all iterations). The Mann-Whitney U nonparametric statistical hypothesis test indicated that the difference between the two simulated treatments is highly significant ($P < 0.0015$), as did the two-sample *t* test, assuming unequal variances.

Simulations with intermediate *Shigella* concentrations (100,000 CFU and 1,000 CFU on the hands) were also carried out (data not shown). The Mann-Whitney U nonparametric statistical hypothesis test also indicated that the difference between the two simulated treatments was highly significant ($P < 0.0015$) in each case, as did the two-sample *t* test, assuming unequal variances. A simulation assuming only 10 *Shigella* bacteria on the hands to start was also conducted (data not shown). When a nonantibacterial treatment was simulated, 119 instances of a single case resulted. For triclosan treatment, only six instances (of 10,000 iterations) resulted in a single case. These differences were not statistically significantly different according to the Mann-Whitney U nonparametric statistical hypothesis test ($P = 0.1449$), but they were

significant ($P < 0.05$) for a two-sample *t* test, assuming unequal variances.

DISCUSSION

There has been little consensus on whether antibacterial soaps are more effective than nonantibacterial soaps in reducing bacteria on hands and preventing disease. A review of consumer antiseptic hand wash product studies in 2005 concluded that existing data failed to demonstrate any association between specific log reductions of bacteria achieved by antiseptic hand washing in surrogate testing and a reduction of infection (25). An expert panel report (3), commissioned in part in response to the Nonprescription Drug Advisory Committee review, noted that linking any nonspecific infection reduction directly and solely to a hand washing intervention would be a challenging goal. Linking the effectiveness of any antibacterial hand washing intervention to the reduction of disease in a population study would likely require hundreds of thousands of subjects representing the general population and millions of hand washing opportunities (3). The report did hold out hope that well-designed studies similar to those presented in the report itself could be used in the context of a quantitative microbial risk assessment to evaluate the magnitude of the likely beneficial effect (3).

The work presented in this study represents just such a quantitative microbial risk assessment. We also confirm and build on previously published research (7, 8) that used one antibacterial product with a single active ingredient, triclosan, and one nonantibacterial product formulation, as well as widely accepted ASTM protocols (1, 2). The current study compares triclosan and additional active ingredients (ethyl alcohol, chlorhexidine gluconate) and two nonantibacterial hand wash products. The number of subjects in the present study is also significantly higher than in previous work, allowing for a more complete statistical analysis of the data.

The conclusion supported by Figures 1 to 3 is that the use of an antibacterial hand wash is more efficacious than that of similar nonantibacterial products. This finding is in agreement with previously published work (7, 8, 10, 17). Fischler et al. (7) used a very similar protocol and found similar differences in antibacterial versus nonantibacterial hand wash efficacy for a 15-s hand wash on *E. coli* (1.15 to 1.85 greater log reductions for antibacterial products) and for a 30-s hand wash for *Shigella* (1.45 to 1.7 greater log reductions for antibacterial products). Fuls et al. (8) likewise found similar differences for a 15-s hand wash for *Shigella* (1.18 greater log reductions for antibacterial products) and for a 30-s hand wash for *Shigella* (1.66 greater log reductions for antibacterial products). The magnitude of the difference in antibacterial versus nonantibacterial hand washes reported in the meta-analysis by Montville and Schaffner (17) is less (~ 0.5 log reduction difference). But, as these authors note, methodological factors (e.g., the use of inoculated transient organisms and a sufficiently high inoculum level) as well as product

formulation (24) appear to play a significant role. The effect of ethyl alcohol is similar to that reported by Schaffner and Schaffner (19), who reported a mean log reduction of 2.58 of *Enterobacter aerogenes* on the hands of volunteers in a study that used a thawed hamburger matrix and treatment with 1 ml of 60% ethanol hand sanitizer.

The results shown here are also similar to those reported by Haas et al. (10). These researchers showed via quantitative microbial risk assessment that antibacterial soaps provided quantifiable benefit in the form of reduced risk of infection and illness using pathogenic *E. coli* in a ground beef simulation. In contrast to our findings, they reported that triclosan-containing products were less effective than those in which alcohols or chlorhexidine were active ingredients (10). It must be noted, however, that Haas et al. (10) used antibacterial efficacy data from the published literature and not that expressly designed for quantitative microbial risk assessment, as in our study. As indicated above, methodological factors (17) as well as product formulation (24) may play a role in reported antibacterial efficacy and subsequent risk reduction.

As Figures 4 to 6 show, a quantitative microbial risk assessment consisting of 100 servings, simulated 10,000 times (i.e., 1 million servings), did show statistically significant differences in predicted illness rates from melon balls that had been handled by simulated food handlers with *Shigella*-contaminated hands washed with either nonantibacterial or antibacterial soaps. These differences maintained statistically significant differences when the starting level of *Shigella* on the hands was as high as 1 million organisms, down to as low as 100 *Shigella* on the hands, or even lower.

The research presented above linked new experimental data with quantitative microbial risk assessment simulation techniques to compare the ability of nonantibacterial and antibacterial products to reduce shigellosis risk. This research provides strong evidence that antibacterial hand treatments are significantly more effective than nonantibacterial treatments in reducing *Shigella* on the hands and its subsequent transfer to ready-to-eat foods. When coupled with quantitative microbial risk assessment simulation techniques, these data show that antibacterial hand treatments can significantly reduce *Shigella* risk. Note that the reduction in risk shown by use of this quantitative microbial risk assessment is not limited to *Shigella* (3). The technique would likely give similar results for organisms like *Salmonella* or enteropathogenic *E. coli*, which have similar antibacterial susceptibility and dose-response relationships.

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