

# Management of Risk of Microbial Cross-Contamination from Uncooked Frozen Hamburgers by Alcohol-Based Hand Sanitizer

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## ABSTRACT

This research was undertaken to determine the effectiveness of an alcohol-based hand sanitizer on hands contaminated with a nonpathogen surrogate for *Escherichia coli* O157:H7, where the source of the contamination was frozen hamburger patties. A nonpathogenic nalidixic acid-resistant food-grade strain of *Enterobacter aerogenes* was used to inoculate frozen hamburger patties composed of 76% lean beef and 24% fat. Thirty-two individuals participated to produce the data used in this study. Each participant handled nine patties at least three times, a sample for microbiological analysis was collected from the surface of one hand, the participant sanitized both hands, and a sample was collected from the other hand. Burger handling created perceptible and visible food debris on the hands of most participants. Computer simulations also were used to perform a variety of risk calculations. The average reduction in bacteria from the use of sanitizer on hands contaminated by frozen burgers containing *E. aerogenes* was  $2.6 \pm 0.7$  log CFU per hand. An experiment designed to simultaneously test the effect of sanitizer on *E. aerogenes* and *E. coli* O157:H7 also revealed no significant difference in sanitizer effectiveness against the two organisms. The results of the real-world risk estimation calculations (using the actual prevalence and concentration of *E. coli* O157:H7 in ground beef) predict that once in 1 million trials, a single pathogen cell will be transferred to a single lettuce piece. The effectiveness of this sanitizer intervention was similar to that for hand washing and glove use previously reported. The person-to-person microbial reduction variability from sanitizer use is similar to published data for glove use and was less variable than published data on hand washing effectiveness.

Microbial cross-contamination appears to play an important role in the transmission of foodborne disease, both in the home (9) and in food service settings (10, 12). Proper hand washing and the use of hand sanitizers has been recognized as an important means of controlling cross-contamination in those instances where hands may contact food (5).

The U.S. Food and Drug Administration (FDA) 2005 Food Code (15) states that employees shall clean their hands for at least 20 s with soap when switching between working with raw food and working with ready-to-eat food. The Food Code also stipulates that a hand sanitizer shall only be applied to hands that have already been washed with soap and water.

The Centers for Disease Control and Prevention (CDC) also has recently issued guidelines for hand hygiene in health care (not food service settings) (2), which suggest that alcohol-based hand sanitizers may offer a suitable alternative to hand washing for health care personnel. The CDC report included an analysis of more than 400 published articles, and the authors concluded that alcohol-based products are more effective than plain soap or antimicrobial soaps when used for antisepsis by health care workers. The CDC also noted that alcohol-based hand rubs require less time that washing with soap and water and irritate hands less often.

The FDA has commented on the CDC guidelines (16) and noted that they are not applicable in food service establishments for a variety of reasons: (i) hand sanitizers may not be effective against some pathogens that are transmitted in food service settings and (ii) the level and types of soils found on the hands of food service and health care employees are different.

This present study was undertaken to determine the effectiveness of an alcohol-based hand sanitizer on hands contaminated from handling frozen hamburger patties inoculated with a nonpathogenic surrogate for *Escherichia coli* O157:H7 and to model the effectiveness of this intervention relative to other possible interventions.

## MATERIALS AND METHODS

**Experimental methods.** The methods used in this study were based in part on techniques developed in our laboratory (3, 6) and elsewhere (17). These techniques involve use of a nonpathogenic food-grade strain of *Enterobacter aerogenes* that is resistant to nalidixic acid, which allows it to be quantified in the presence of other microorganisms in food and of resident bacteria on the hands of study participants. Frozen hamburger patties obtained from a commercial grinder and composed of 76% lean beef and 24% fat were used for all experiments. Patties were maintained at  $-7^{\circ}\text{C}$  (20°F), representing worst-case frozen storage conditions. Thirty-two Rutgers University staff members and students (12 men and 20 women) participated in this study.

*E. aerogenes* cells were grown overnight (18 to 24 h) at  $37^{\circ}\text{C}$  with shaking in tryptose phosphate broth (Becton Dickinson, Sparks, Md.) containing 50  $\mu\text{g/ml}$  nalidixic acid. *E. coli* O157:

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TABLE 1. Summary of information, assumptions, and data sources used in Monte Carlo simulations

Variable	Data or parameter values	Calculation or distribution	Source or assumption
Fraction of contaminated burgers	253 of 56,934	Beta distribution	Totals from 1997 to 2004 (14)
Concentration if contaminated	Minimum, 0.3 CFU/g; mode, 1.5 CFU/g; maximum, 15 CFU/g	Triangular	(13)
Fraction of cells on surface	Volume, 39 cm <sup>3</sup> ; surface area, 172 cm <sup>2</sup>	1 of 2,267 cells on surface	Assume one cell is 1 μm <sup>3</sup>
Transfer from burgers to bare hands	Mean (SD) transfer rate, -1.83 (0.07) log CFU	Normal	This research
Reduction from sanitizer	Mean (SD) reduction, -2.57 (0.63) log CFU	Normal	This research
Reduction from hand washing	Mean (DS) reduction, -2.2 (1.42) log CFU	Normal	(3)
Reduction due to gloves	Mean (SD) reduction, -4.56 (0.85) log CFU	Normal	(6)
Transfer from hands to lettuce	Mean (SD) transfer rate, -2.21 (1.07) log CFU	Normal	(3)

H7 ATCC 43895 cells were grown overnight (18 to 24 h) at 37°C with shaking in tryptic soy broth (Becton Dickinson).

Ten beef patties were removed from the freezer and placed on a wire rack. Each side (up to and including the edge) of each patty was inoculated with 0.5 ml of the *E. aerogenes* broth culture (~10<sup>9</sup> CFU/ml) and returned to the freezer until the participant arrived. Inoculation of frozen patties represents a worst-case situation, because inoculation of fresh beef that was then frozen might reduce the potential for cross-contamination. One patty was used to determine the *E. aerogenes* concentration before each handling experiment. The concentration on inoculated patties was 6.4 ± 0.4 log CFU/g (or ~2 × 10<sup>5</sup> CFU/cm<sup>2</sup>). The remaining nine patties were spread out on a clean plastic high-density virgin polyethylene cutting board (Dexas International, Inc., Dallas, Tex.) in a three-by-three arrangement.

Two or three participants usually were tested on a particular day; the same set of nine hamburger patties was reused and returned to the freezer between experiments. No significant differences were detected in transfer rates from the same set of burgers used up to three times. At the end of each day, the patties were discarded.

The participant was instructed to pick up the patties in whatever manner that felt natural to them, stack them (one on top of another) to one side, then spread them out into the original three-by-three arrangement to simulate placement on a grill for cooking. This stacking and respreading process was performed three times. In some experiments, participants also handled up to 10 sets of uninoculated patties before handling inoculated patties to increase potential levels of food debris on the hands.

In every experiment, participants rated the perceived level of debris on their hands on a 5-point scale (ranging from “very clean” to “very dirty”) while the investigators noted whether the hands appeared to contain visible debris from the burgers.

The investigator then immediately tested the participant’s dominant or nondominant hand via the glove juice method (8). The fingers of a sterile surgical glove (Allegiance Health care Corporation, McGraw Park, Ill.) were filled with phosphate-buffered saline (20 ml), and the glove was then fitted onto the participant’s hand. The hand was rubbed for 1 min by an investigator, and the resulting fluid was transferred into a sterile centrifuge tube.

The participant dispensed ~1 ml of alcohol-based sanitizer onto one hand and rubbed the sanitizer over both hands until he

or she determined that the process was complete (generally less than 30 s). The sanitizer used in this study contained 60% ethanol plus inactive ingredients (listed as water, triethanolamine, acrylic acid, propylene glycol, and tetradecanol) and meets Food Code hand sanitizer requirements with ingredients that are safe for use by employees with direct food contact. The investigator then immediately obtained samples from both of the participant’s hands via the glove juice method. To enumerate the collected samples, appropriate 10-fold dilutions of the glove fluid were made as needed, and 0.1 ml of that fluid was spread plated and 1.0 ml was pour plated on MacConkey agar containing 50 μg/ml nalidixic acid. Log transfer rates and sanitizer log reductions were assumed to be normally distributed and were calculated as described elsewhere (3).

For a direct comparison of the behavior of *E. coli* O157:H7 versus that of the surrogate *E. aerogenes*, 0.25 ml of each organism was inoculated onto each side of nine hamburger patties, and the experiment proceeded as described above with a single participant. Because variability between replicates was large, coinoculation was necessary to insure a valid comparison. Control experiments included recovery of both organisms from inoculated burgers and from both sanitized and unsanitized hands. Approximately 24 h later, the experiment was repeated to determine whether the organisms persisted on the participant’s hands; neither organism was detected.

**Simulation.** Computer simulations conducted with @Risk (Palisade Corporation, Ithaca, N.Y.), an add-in for Excel (Microsoft, Redmond, Wash.), also were used to perform some of the calculations. Table 1 lists the simulation variables, the data or parameter values, the numerical calculation of statistical distribution assumed, and the source or assumption used in the calculation. The spreadsheet containing the model is available for download (<http://foodsci.rutgers.edu/schaffner/files.htm>).

The “real-world” risk estimation calculations included the actual prevalence (14) and concentration of *E. coli* O157:H7 in ground beef (13). The fraction of *E. coli* O157:H7 on the surface of a hamburger was calculated based on a typical burger volume (39 cm<sup>3</sup>) and surface area (4,900 cm<sup>2</sup>) and the assumption that a single cell has a volume of approximately 1 μm<sup>3</sup>. It was assumed that an individual would handle 27 hamburger patties and then handle a single piece of lettuce. Transfer rates were derived from the research described here or elsewhere (3) as appropriate.

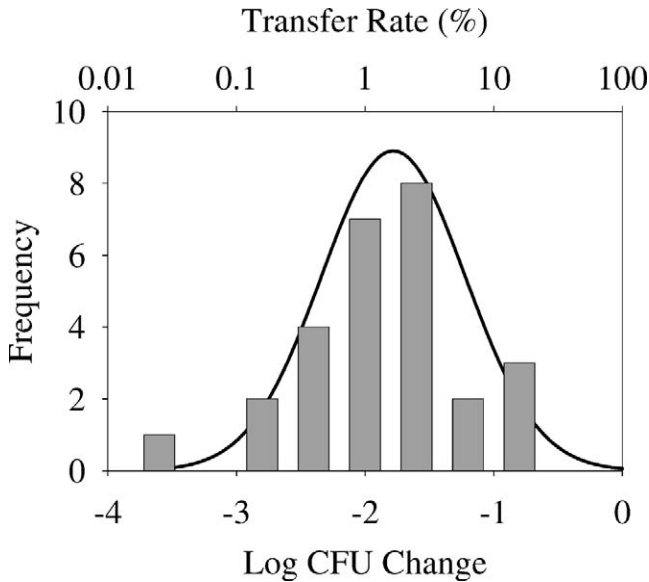


FIGURE 1. *E. aerogenes* transfer from frozen hamburgers to hands after nine burgers were stacked and unstacked three times. The experimental data are shown as shaded bars, and the normal distribution (mean  $\pm$  SD, 1.83  $\pm$  0.70 log CFU) that best fits the data is shown as the solid line.

Worst-case simulations also were carried out with the assumptions as above except that each burger was assumed to contain 100,000 CFU of *E. coli* O157:H7, all located on the burger surface. Simulated effectiveness of the sanitizer intervention was determined based on the research presented here. Simulated effectiveness of hand washing and glove use was determined based on data presented elsewhere (3, 6).

## RESULTS AND DISCUSSION

**Experiment.** Because preliminary data indicated that handling up to 10 sets of uninoculated patties prior to handling inoculated patties did not result in significant differences in bacterial transfer, results from both portions of the experiment were combined. Figure 1 is a summary of the transfer of *E. aerogenes* from frozen hamburgers to hands after stacking and unstacking nine burgers at least three times. For example, when seven participants handled the burgers, approximately 1% of the *E. aerogenes* on the burgers was transferred to their hands (i.e., 2-log reduction); rates for other participants are summarized in Figure 1. The average transfer rate was calculated as 1.48%, which corresponds to a 1.83-log reduction. This average corresponds to the peak of the normal distribution (black curve) shown in Figure 1.

When the data in Figure 1 are compared with similar data collected in our laboratory for *E. aerogenes* transfer from refrigerated (not frozen) chicken (3), the average transfer rates from refrigerated chicken are almost an order of magnitude higher (9.0 versus 1.5%), although the variability in the rates is similar ( $\pm 0.7$  log CFU). This higher transfer rate was not unexpected because moisture is a well-known facilitator of microbial transfer (4, 11) and the moisture in frozen beef was in the form of ice and thus not able to facilitate transfer. This finding highlights an important limitation of our study; if the frozen burgers were allowed

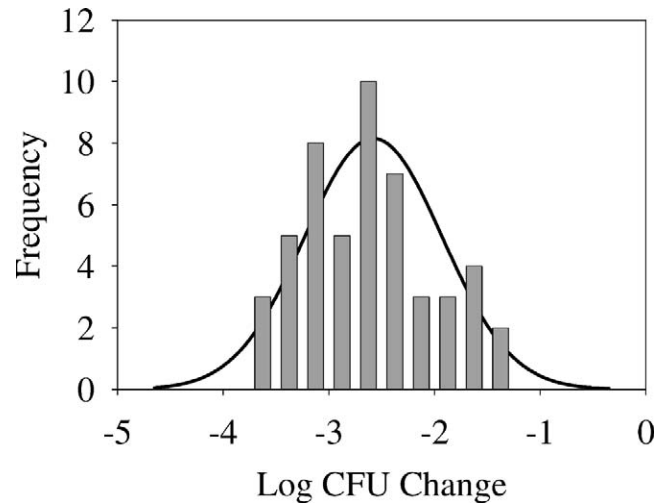


FIGURE 2. Effect of  $\sim 1$  ml of alcohol-based sanitizer on *E. aerogenes* deposited on hands after stacking and unstacking frozen hamburgers. Experimental data are shown as shaded bars, and the normal distribution (mean  $\pm$  SD, 2.58  $\pm$  0.65 log CFU) that best fits the data is shown as the solid line.

to thaw (even only at the surface), transfer rates (and risk) might be expected to rise by an order of magnitude.

Although the burgers were frozen, some food debris did transfer to the participants' hands. More than half of the participants (66%, 21 of 32) indicated that their hands felt either much dirtier (3 participants) or dirtier (18 participants) than normal after handling the frozen burgers. The investigators also noted that most (56%, 18 of 32) of the participants had visible debris on their hands after handling the frozen burgers.

Figure 2 summarizes the effect of  $\sim 1$  ml of alcohol-based sanitizer on *E. aerogenes* deposited on hands after stacking and unstacking inoculated frozen burgers. For example, a slightly greater than 2.5-log reduction in *E. aerogenes* CFU after using the sanitizer was found for 10 participants; rates for the other participants are summarized in Figure 1. The average (standard deviation [SD]) reduction was calculated as 2.58 (0.65) log CFU per hand, which correspond with the normal distribution (black curve) shown in Figure 2. The effectiveness of the alcohol-based sanitizer used in this study corresponds well with that reported in the literature (7). Data for participants who handled up to 10 sets of uninoculated burgers are included in Figure 2 and are not significantly different from the data collected in experiments in which uninoculated burgers were handled.

An experiment designed to test the effect of sanitizer simultaneously on *E. aerogenes* and *E. coli* O157:H7 also revealed no significant differences in sanitizer effectiveness against the two organisms. Transfer rates in the coinoculation experiment were within the normal experimental variation observed for the other experiments in this study.

**Simulation.** The results of the real-world risk estimation calculations for 1 million iterations (using the actual prevalence and concentration of *E. coli* O157:H7 in ground beef) are shown in Figure 3, which is a plot of the relative

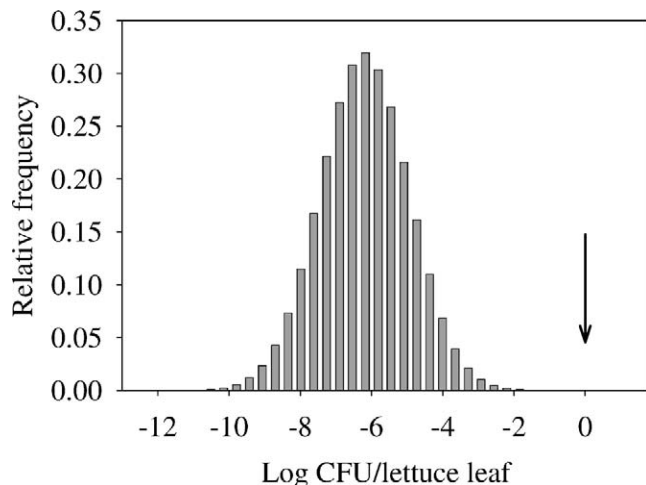


FIGURE 3. Risk estimation for transfer of *E. coli* O157:H7 to a single piece of lettuce after handling 27 raw hamburgers, based on the actual prevalence and concentration of *E. coli* O157:H7 in ground beef. The downward arrow indicates the single iteration (out of 1 million) when a single cell was transferred to lettuce.

frequency versus the predicted log CFU per lettuce leaf. The mode (most common) simulation result is  $10^{-6}$  CFU per lettuce leaf, or 1 one-millionth of a bacterial cell on one lettuce leaf. The simulation predicts that once (downward pointing arrow, Fig. 3) in 1 million trials a single cell will be transferred to a single lettuce piece. This event is so infrequent that the bar representing its frequency is not visible.

The results of the intervention comparisons are shown in Figure 4. None of the interventions (hand washing, gloves, and sanitizer) were completely effective because there was some very small probability that a lettuce leaf would be contaminated with a single cell. All interventions were more effective than no intervention at all; the mean reduction achieved by hand washing and the use of gloves or sanitizer was about 3 log (1,000 times) greater than the result for no intervention at all. The three interventions appear to have similar effectiveness, with an average simulated *E. coli* O157:H7 concentration of  $10^{-2}$  CFU per lettuce leaf. Because the person-to-person variability of the hand washing process is greater than that for use of either gloves or sanitizer, the lowest and highest observed concentrations are lower and higher, respectively, than the lowest and highest values for either gloves or sanitizers. The minimum reduction using gloves or sanitizer was about 2 log (100 times) greater than that for either no intervention or hand washing.

These results lead to two important conclusions: (i) the risk of contamination of ready-to-eat food through transfer of *E. coli* O157:H7 from frozen hamburgers is very, very low, even when no intervention is used; and (ii) use of an alcohol-based hand sanitizing gel is an effective intervention for hands that have been contaminated with *E. coli* O157:H7 from frozen hamburgers. This research is the first to evaluate the effectiveness of a hand sanitizer on bacteria on hands in the presence of visible food debris. The results suggest that because the variability in the effectiveness of

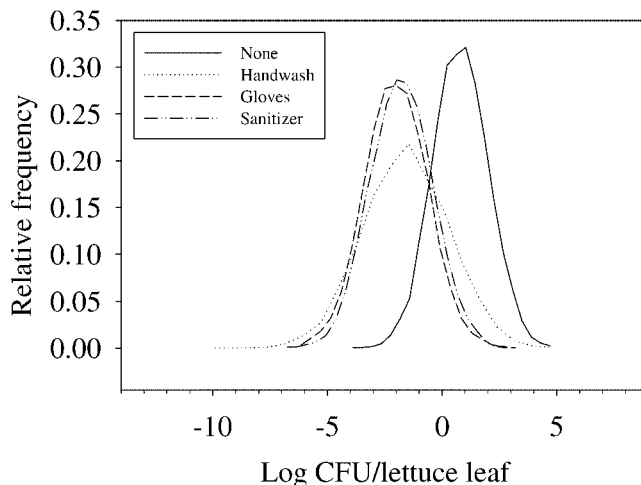


FIGURE 4. Risk estimation for transfer of *E. coli* O157:H7 to a single piece of lettuce after handling 27 raw hamburgers, assuming each burger contains 100,000 CFU of *E. coli* O157:H7, all located on the burger surface. Solid line represents no intervention. Simulated effectiveness of hand washing (dotted line) and glove use (dashed line) interventions was determined based on data of Chen et al. (3) and Montville et al. (6), respectively. Simulated effectiveness of the sanitizer intervention (dot-dash line) was determined based on the research presented here.

the hand sanitizer is less than the variability of the hand washing process, the minimally effective application of hand sanitizer is more effective than minimally effective hand washing.

The data presented here address the two FDA concerns. Hand sanitizers were effective against an enteric pathogen surrogate; side-by-side tests with *E. coli* O157:H7 and the surrogate revealed no significant difference in sanitizer effectiveness. This effectiveness was demonstrated in an experimental set-up mimicking a situation in which workers handled frozen hamburgers. The hand sanitizers were as or more effective than hand washing for reducing bacterial populations on hands, based on one specific level and type of contamination found on the hands of food service workers. The hand sanitizer was effective even when participants felt their hands were perceptibly dirty and investigators noted the presence of visible debris. The similarity in the effectiveness of sanitizer against *E. aerogenes* and *E. coli* O157:H7 also suggests that these results could be helpful for managing the risk of transfer of other enteric pathogens such as *Salmonella*, *Campylobacter*, and some viruses (1).

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