Effects of various hand hygiene regimes on removal and/or destruction of Escherichia coli on hands

Monique Courtenay,* Lina Ramirez,† Beth Cox,† Inyee Han,† Xiuping Jiang† and Paul Dawson†

*South Carolina Governors School of Science and Mathematics, Hartsville, SC, USA; †Department of Food Science and Human Nutrition, Clemson University, Clemson, SC, USA

Abstract

Various hand hygiene techniques have been recommended by sanitarians. In the USA, the National Restaurant Association (NRA) ServSafe® program guidelines include a recommended hand washing regime. The ServSafe regime was compared to rinsing with warm and cool water and no washing/rinsing for bare hands and gloves after exposure to ground beef (approximately 10⁶ cells/g) or liquid solution (approximately 10⁶ cells/mL) contaminated with an ampicillin-resistant Escherichia coli JM 109 strain. The efficacy of alcohol-based hand sanitizers to replace hand washing was also evaluated. ServSafe, warm water rinse and cool water rinse reduced E. coli cells on hands by 98.0, 64.4 and 42.8% log₁₀ cfu/mL, resulting in <1, 1.4, and 2 log₁₀ cfu/mL E. coli on hands, respectively, from 3.6 log₁₀ cfu/mL on unwashed hands. When vinyl food service gloves were worn during the hand washing treatments, gloves retained more bacteria than when only hands were rinsed or washed. From 2.9 to 3.4 log₁₀ cfu/mL remained on hands when ethanol-based sanitizers were used instead of hand washing. Of all hand washing treatments tested in these experiments, the US NRA recommended method was most effective (P < 0.05) in removing E. coli from hands and the levels remaining after this method were below the threshold of detection (<10 cfu/hand).

Introduction

Food service industry workers receive lessons in cleanliness and sanitation when handling food on the job as part of a comprehensive HACCP (hazard analysis critical control point) program. Many are instructed to wear gloves, wash their hands at certain time intervals, wash their hands after certain tasks and wash their hands in a specific manner. The reasons for these guidelines are to ensure the safety of consumers and workers as well as to maintain the quality of the food product. When meats are concerned, safety has to be ensured because disease causing bacteria may be present on the raw product and in the food preparation environment. Meats, fruits and vegetables can be contaminated with enteric bacteria from exposure to manure, contaminated equipment, tainted water and human handling (Pennington 2003). The fecal-oral route transmission pathway by food service workers to customers is another hazard where hand washing can be a preventative control point. Pathogenic Escherichia coli have been linked to food-borne illness along with other bacteria such as Salmonella spp., Staphylococcus spp., and Campylobacter spp. (Donnenberg 2002). Various researchers have used a surrogate bacterium to measure transmission. Zhao et al. 1998 reported that 10⁵ cfu Enterobacter aerogenes per cm² were transferred from chicken skin to hands then subsequently 10⁵–10⁶ cfu/cm² were transferred from hands to vegetables upon handling. In another surrogate study transfer rates of E. aerogenes between hands, food and kitchen utensils varied from 100% to less than 1% (Chen et al. 2001). One of the first reported E. coli-related outbreaks in the US occurred in 1982 in Oregon and Michigan from improperly prepared hamburgers at local fast food restaurants. While most of the E. coli O157:H7 deaths and illnesses are a result of contaminated food and water, numerous food-borne disease outbreaks have
been linked to hand contamination (Hansen & Knochel 2003). Hand sanitation practices were reported in a survey of 1448 participants in a food safety certification program using the US National Restaurant Association (NRA) ServSafe curriculum (McElroy & Cutter 2004). In this survey, nearly 80% of the respondents self-reported they either failed to sanitize between tasks or sanitize hands properly prior to the completion of the training program. The same group reported practicing these procedures 26% more frequently after completing the NRA ServSafe program. In a study of unsafe home practices, Califiano et al. (2000) reported that only 20% of those surveyed only ‘sometimes’ washed their hands before food preparation and Toshima et al. (2001) reported that hand washing techniques were inconsistent and judged unsatisfactory most of the time. Hygiene data based on self-reporting (i.e. McElroy & Cutter 2004) should be distinguished from data based on observation (i.e. self-reporting (i.e. McElroy & Cutter 2004) should be unsatisfactory most of the time. Hygiene data based on observation when determining food worker hygiene practices.

The role of hand washing on the presence and transfer of bacteria has been studied in a variety of settings including hospitals (Vollaard et al. 2004), food service facilities (Paulson et al. 1999; Paulson 2000; Taylor 2000; Lane 2001; Michaels 2002; Michaels et al. 2002; Kerr et al. 2003), food processing establishments (Lane 2001; Bennett 2003; Featherstone 2003), in the home (Califano et al. 2000; Kassa et al. 2001; Toshima et al. 2001; Kohl et al. 2002; Clayton et al. 2003; Schaffner 2003), fairs (Worsfold 2003), assisted living facilities (Sneed et al. 2004), elementary schools (In & Yeon 2004) and child care locations (Albrecht et al. 1992). Hand sanitation has also been studied as to the effects of gloves (Guzewich 1995; Fendler et al. 1998a,b; Paulson 2000; Montville et al. 2001), sanitizers/antibacterial soaps (Miller et al. 1994; Paulson 1994; Morita et al. 1999; Toshima et al. 2001; Michaels et al. 2003) and water temperature during washing (Michaels et al. 2001, 2002). Larson et al. (2003) found a lack of validity for two ATP bioluminescence-based hygiene testing kits on measuring microbial contamination of hands and kitchen surfaces. These researchers reported between 3.2 and 7.0 log<sub>10</sub> cfu/hand on washed hands. Sneed et al. 2004 reported that only two out of 40 assisted living facilities in Iowa met all surface sanitation standards and the researchers concluded that attention needs to be given to training and supervision to ensure proper hand washing and appropriate cleaning and sanitation procedures to reduce or eliminate cross-contamination. To encourage safe handling of foods in food service settings various agencies and industry groups have promoted various hand washing protocols for workers to follow. The US National Restaurant Association (NRA) has a food safety ServSafe (ServSafe 2004) training program that includes hand washing guidelines. The objective of this study was to compare the ServSafe hand washing procedure to warm water rinsing, cool water rinsing, no washing on bare hands and gloves for the ability to remove E. coli. In addition, several commercial alcohol-based sanitizers were tested for the ability to eliminate/kill E. coli from/ on hands.

Materials and methods

Preparation of inoculum

Escherichia coli ampicillin-resistant strain with a fluorescent gene was used for this study. A non-pathogenic E. coli strain JM109 was labeled with jellyfish green fluorescent protein according to the following protocol as described previously (Jiang et al. 2002). The competent bacterial cells were electroporated in a Gene Pulser II (Bio-Rad) with plasmid vector pGFPuv (ClonTech, Palo Alto, CA). Transformants were selected from isolated colonies grown on Luria-Bertani agar (LB) plates containing 100 µg of ampicillin/mL. The resulting ampicillin-resistant transformants emitted bright green fluorescence under a handheld UV light. The stability of GFP label in the E. coli strain was determined by streaking on Trypticase soy agar (TSA) plates containing 100 µg ampicillin/mL for several generations.

To prepare ground beef inoculums, 0.1 mL of E. coli was pipetted into 10 mL of Tryptic Soy Broth (TSB) under a Germfree® Bioflow Chamber. Ampicillin was added at 100 µg/mL to the TSB. The inoculum was placed in an agitator inside of an incubator at 37°C for 16–18 h. Afterwards, the inoculum was centrifuged at 2700 r.p.m. for 15 min, and the supernatant decanted. Next, 10 mL of peptone water (0.1%) was added to the cell pellet and the solution was mixed with a Fisherbrand® Vortex Genie 2 machine.

Meat preparation and bacterial enumeration

All of the ground beef used was obtained from a South Carolina, state-inspected university meats laboratory. Ground beef was approximately 15% fat, 15% protein and 70% water. Using 100 g of beef for each inoculum, 10 mL of the E. coli re-suspended pellet (from a 10<sup>5</sup> cells/mL culture) was poured evenly onto the meat surface and distributed throughout by hand-kneading while wearing sterilized gloves (resulting in approximately 10<sup>5</sup> cells per 100 g or approximately 10<sup>6</sup> cells/g). The ground beef was then measured and divided
into four 25 g lots. Each lot was used for 1 of 4 hand cleaning treatments in three separate experiments (hand washing, washing with gloves on, and alcohol-based sanitizers). Each of the following experiments were replicated using three separate meat lots with three different inoculation cultures on different days.

Enumeration of bacteria in the inoculated meat was determined by placing 11 g of meat into a stomacher bag with 99 mL of peptone water followed by stomaching (Stomacher® 400 Circulator machine) for 1 min at 230 r.p.m. Before starting the treatments, all subjects washed their hands following the recommended ‘ServSafe’ protocol. Subjects used a commercial food service hand cleanser (Purex Industrial Clean Hands lotion for dispensers) containing water, ammonium sulfate, coco diethanolamide and sodium alpha olefin sulfonate.

Experiment 1: hand washing hand hygiene treatment methods

A 100 g sample of inoculated ground beef was kneaded with both hands for 30 s by each of three subjects per washing treatment and hands were then air dried for 15 s. Each of four hand washing treatments were tested; no wash, cool rinse, warm rinse and ServSafe. For ‘cool rinse’ treatment, the subject rinsed their hands while rubbing their hands together with cool water (26°C) for 15 s. This same method was also used for ‘warm rinse’ treatment with the 40°C water. For the ‘ServSafe’ treatment, hands were rinsed for 10 s with the warm water, lathered with soap (Purex International Clean Hands lotion for dispensers) for 15 s, and rinsed again for 15 s. No rinsing was used for the ‘no wash’ treatment. After each treatment, subjects were given paper towels to gently pat their hands dry. To recover the remaining bacteria both hands were placed in a pre-labeled stomacher bag with 50 mL of peptone water and gently mixed. Each subject dipped their hands in the E. coli solution for 5 s then gently patted hands dry with a paper towel. Hands were then allowed to air dry for 30 s before receiving a dime size amount of sanitizer (one pump from the automatic dispenser = 1.2 mL). Hands were rubbed together for 30 s and then allowed to air dry again for 30 s. Afterwards, hands were rinsed in stomacher bags with 50 mL of peptone water for 30 s each. The four brands of ethanol-based sanitizers were used in the experiment (designated as brand B, C, P and S) each containing 62% ethanol and skin conditioners.

Experiment 2: glove washing

The glove washing experiment was conducted identically was experiment 1 except the subjects conducted hand washing treatments while wearing gloves. Food preparation gloves from a University food service facility were used (GlovePlus Latex Free Industrial Vinyl™ by Ammex).

Experiment 3: ethanol-based sanitizers

The sanitizer experiment was similar to experiment 1 except an E. coli solution was prepared rather than using meat. An E. coli pellet was added to 850 mL of sterile water and gently mixed. Each subject dipped their hands in the E. coli solution for 5 s then gently patted hands dry with a paper towel. Hands were then allowed to air dry for 30 s before receiving a dime size amount of sanitizer (one pump from the automatic dispenser = 1.2 mL). Hands were rubbed together for 30 s and then allowed to air dry again for 30 s. Afterwards, hands were rinsed in stomacher bags with 50 mL of peptone water for 30 s each. The four brands of ethanol-based sanitizers were used in the experiment (designated as brand B, C, P and S) each containing 62% ethanol and skin conditioners.

Enumeration of bacteria

Enumeration of bacteria for all three experiments was accomplished by spiral plating with an Autoplate® 4000 Spiral Biotech machine and Petri dishes previously filled with TSB and ampicillin. All plates were inverted and incubated at 36–38°C for 24 h. Plates were counted after incubation using a Leica® Quebec Darkfield Colony Counter. Bacterial numbers were obtained by counting each visible colony that was fluorescent under UV light (Fig. 1). The results for the duplicate plates were recorded and were converted to log10 of colony forming units per mL of rinse solution (log10 cfu/mL) and log10 of cfu/hand. The log10 of cfu/hand were determined by converting the total number of bacteria recovered from both hands in the rinse solution, dividing that number in half to represent one hand and converting this to a log10 value. In addition, the percentage of cfu reduction (% reduction) and percentage of log10 reduction on a mL rinse (% log10 reduction) and per hand (log10 cfu/mL or per hand) and total cfu per hand were calculated using the following:

\[
\frac{- \text{wash treatment} \times (\text{cfu/ml})}{\text{no wash (cfu/ml)}} \times 100 \%
\]

\[
\frac{- \text{wash treatment} \times (\text{log10 cfu/ml})}{\text{no wash (log10 cfu/ml)}} \times 100 \%
\]

\[
\frac{- \text{wash treatment} \times (\text{log10 cfu/hand})}{\text{no wash (log10 cfu/hand)}} \times 100 \%
\]
The asterisk * indicates that wash treatment is either cool rinse, warm rinse, ServSafe or a sanitizer.

Experimental design and statistical analysis

Each experiment was replicated three times and each experiment was analyzed separately using a statistical analysis program (SAS 2001). Experiment 1 and 2 had four hand or glove washing treatments and each of these treatments were subjected to an analysis of variance to determine if washing treatments had a significant effect on bacterial numbers. Experiment 3 was analyzed similarly except there were five cleansing treatments (no wash and four sanitizers). Since cleansing treatment effects were significant \( P < 0.05 \) for each experiment, individual treatment means were separated using the pdiff command of SAS.

Results and discussion

Experiment 1: hand washing experiment

Each of the hand washing treatments resulted in different \( E. \ coli \) levels recovered from hands \( P < 0.05 \) (Fig. 2). In the present study, transfer of bacteria from inoculated meat \( \log_{10} 6.3 \text{ cfu/g} \) to hands \( 3.65 \text{ cfu/mL rinse} \) was similar to rates reported by Hansen & Knochel (2003). Hansen & Knochel (2003) tested palm surface imprinting with image analysis as a rapid method to assess hand hygiene. Palm surface area for their subjects ranged from 110 to 190 cm\(^2\) with median of about 120 and 170 cm\(^2\) for women and men respectively. Hansen & Knochel (2003) inoculated hands with from 0.5 to 6.5 \( \log_{10} \text{ cfu/cm}^2 \) of \( E. \ coli \) and recovered from 0.5 to 3.5 \( \log_{10} \text{ cfu/cm}^2 \) from hands using the image analysis/palm imprint method. Over this inoculation range, these researchers found that a two-phase linear relationship between the applied bacterial concentrations and the number of cells recovered from hands held for both \( Listeria \) and \( E. \ coli \). De Wit & Kampelmacher (1981) reported that most food industry workers had between 2 and 3 and 5.4–7.3 \( \log_{10} \text{ cfu/hand} \) of \( Enterobacteriaceae \) and mesophilic bacteria respectively. Hansen & Knochel (2003) reported an 85% reduction in bacterial levels for hands contaminated with raw chicken then washed with soap. In the current study, ‘ServSafe’ was the most effective hand-washing method followed by ‘Warm Rinse’, ‘Cool Rinse’, and ‘No Wash’ in that order (Fig. 2). Michaels et al. (2002) reported a fraction of a log \( 10 \) reduction improvement when higher water temperatures were used to wash and rinse hands and a greater variation at lower water temperatures. Unwashed hands carried 3.6 \( \log_{10} \text{ cfu/mL} \) rinse and 5.0 \( \log_{10} \text{ cfu/hand} \) while hands washed using the ServSafe method retained <1 \( \log_{10} \text{ cfu/mL} \) rinse and <1 \( \log_{10} \text{ cfu/hand} \). The transfers or removal rate of bacteria from surfaces to food and vice versa has been calculated in a variety of ways and Moore et al. (2003) contrasted several of these methods while reporting on transfer rates between lettuce and food preparation surfaces. In the current study, three methods to calculate the percentage of cells removed during hand cleansing
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treatments are presented; % cfu/mL of hand rinse, % 
log_{10} cfu/mL of hand rinse and % log_{10} cfu/hand 
(Tables 1, 2). Bacterial reduction due to antimicrobial 
treatments are often measured in log_{10} values since 
percentages can be misleading. The confusing nature 
of using education percentages is illustrated in this study 
where per cent reduction due to cool water rinsing and 
ServSafe hand washing was 94.96 and 99.98% cfu/mL; 
42.8 and 98.0% log_{10} cfu/mL; and 28.6 and 82.0% 
log_{10} cfu/hand, respectively. Depending on the units 
used to calculate the percentage reduction, very differ-
ent inferences can be drawn. With a starting population 
of 3–5 log_{10} cfu/hand, as was found for food industry 
workers (De Wit & Kampelmacher 1981), a 2–3 log_{10} 
population remains on cool water rinsed hands with a 
approximately 95% reduction in cells. Thus for com-
paring hand washing treatments, the percentage of the 
log_{10} reduction may be the best unit comparison of 
cleansing treatments.

Experiment 2: glove washing

For the glove experiment, the ‘Warm Rinse’ and ‘ServSafe’ methods did not differ in removal of bacteria.

### Table 1

<table>
<thead>
<tr>
<th>Washing treatment</th>
<th>Hand washing (% reduction)</th>
<th>Glove washing (% reduction)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cfu*/hand</td>
<td>Log_{10} cfu/mL rinse*</td>
</tr>
<tr>
<td>Cool rinse</td>
<td>94.96*</td>
<td>42.8c</td>
</tr>
<tr>
<td>Warm rinse</td>
<td>99.78a</td>
<td>64.4b</td>
</tr>
<tr>
<td>ServSafe</td>
<td>99.96a</td>
<td>98.0a</td>
</tr>
<tr>
<td>SEM</td>
<td>2.0</td>
<td>5.7</td>
</tr>
</tbody>
</table>

% cfu reduction = \frac{\text{no wash (cfu/ml)} - \text{wash treatment* (cfu/ml)}}{\text{no wash (cfu/ml)}}

% log_{10} reduction ml = \frac{\text{no wash (log}_{10}\text{ cfu/ml)} - \text{wash treatment* (log}_{10}\text{ cfu/ml)}}{\text{no wash (log}_{10}\text{ cfu/ml)}}

% log_{10} reduction hand = \frac{\text{no wash (log}_{10}\text{ cfu/hand)} - \text{wash treatment* (log}_{10}\text{ cfu/hand)}}{\text{no wash (log}_{10}\text{ cfu/hand)}}

The asterisk * indicates that wash treatment is either cool rinse, warm rinse, or ServSafe respectively.

### Table 2

<table>
<thead>
<tr>
<th>Sanitizer</th>
<th>cfu*</th>
<th>Log_{10} cfu/mL rinse*</th>
<th>Log_{10} cfu/hand*</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>96.44</td>
<td>45.72a</td>
<td>37.77a</td>
</tr>
<tr>
<td>C</td>
<td>96.33</td>
<td>59.80a</td>
<td>52.62a</td>
</tr>
<tr>
<td>P</td>
<td>96.07</td>
<td>43.60a</td>
<td>36.14a</td>
</tr>
<tr>
<td>S</td>
<td>90.40</td>
<td>24.15b</td>
<td>17.49b</td>
</tr>
<tr>
<td>SEM</td>
<td>1.94</td>
<td>14.76</td>
<td>12.80</td>
</tr>
</tbody>
</table>

% cfu reduction = \frac{\text{no wash (cfu/ml)} - \text{sanitizer* (cfu/ml)}}{\text{no wash (cfu/ml)}}

% log_{10} reduction ml = \frac{\text{no wash (log}_{10}\text{ cfu/ml)} - \text{sanitizer* (log}_{10}\text{ cfu/ml)}}{\text{no wash (log}_{10}\text{ cfu/ml)}}

% log_{10} reduction hand = \frac{\text{no wash (log}_{10}\text{ cfu/hand)} - \text{sanitizer* (log}_{10}\text{ cfu/hand)}}{\text{no wash (log}_{10}\text{ cfu/hand)}}

The asterisk * indicates that sanitizer is one of the coded brands B, C, P or S respectively.

SEM, standard error of the mean.
and both were superior to ‘Cool Rinse’ and ‘No Wash’ (Fig. 2). Glove washing differed from bare hand washing where a stepwise difference in cells remaining after washing was seen (ServSafe < Warm Rinse < Cool Rinse < No Wash). During the experiment, the meat particles tended to cling to the vinyl surface of the glove and were more difficult to remove during washing. This may explain why although meat inoculum levels were identical for experiments 1 and 2, unwashed gloves retained an average of approximately 5 and 6.5 log_{10} cfu/mL and glove while unwashed hands retained an average of approximately 3.6 and 5.1 log_{10} cfu/mL and hand respectively. Therefore, based on the recovery methods used in this study, the transfer of *E. coli* from meat to gloves was greater than that from meat to hands. The greater retention of meat on gloves compared to hands after kneading make direct comparisons between experiment 1 and 2 questionable. However, since treatments within experiments were handled the same, comparisons between washing techniques are valid. The percentage reduction for gloves using cool rinsing and ServSafe techniques were similar to that for hands, while the warm rinse had a relatively higher percentage reduction for gloves than for hands (Table 1). Thus these results support those of previous researchers such as Fendler et al. 1998a, b) that gloves are more effectively cleaned than for hands (Table 1). The limited effectiveness of alcohol sanitizers was previously reported by several researchers including Hansen & Knochel (2003) who used a 70% alcohol solution after a non-medicated soap wash and found no further reduction in total aerobic and mesophilic bacteria. Two other studies reported an increase in bacterial hand flora after application of alcohol sanitizers (Miller et al. 1994; Charbonneau et al. 2000). Michaels et al. (2003) reported minimal increase in removal/killing of bacteria on hands when alcohol-based sanitizers were used after hand washing and only when larger quantities of 3 and 6 mL were used was there any advantage.

**Conclusions**

Food-borne diseases can be greatly reduced with proper food preparation and personal worker hygiene. Results obtained from this experiment supports the effectiveness of established food service hand-washing protocols. For all three experiments some form of hygiene was more effective in removing bacteria when compared to ‘No Wash’. In terms of sanitizers, although faster and easier, this method for cleaning was not as effective as washing. Finally, gloves are costly and are sometimes thought to be reusable if cleaned. Nevertheless, this study indicated that food handlers should not wash gloves when handling different meats or food products but rather should always change gloves as recommended by the ServSafe and other food worker hygiene programs. ServSafe protocol recommends changing gloves when soiled or torn, before beginning a different task, at least every four hours of continual use, after handling raw meat and before handling ready-to-eat foods. There are various hand washing
procedures that are effective in reducing hand contamination. Thus, this study is not an endorsement of the ServSafe method but rather supports the belief that hand rinsing and no washing is not as effective as a formal hand washing protocol that includes the basic elements adopted by the US NRA ServSafe program. To minimize the chance that food handling will result in food-borne illness, proper and timely hand washing is likely to be the best preventative measure available.

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