

Research Note

Efficacy of a Commercial Produce Wash on Bacterial Contamination of Lettuce in a Food Service Setting

SARAH SMITH, MILA DUNBAR, DIANA TUCKER, AND DONALD W. SCHAFFNER*

Food Risk Analysis Initiative, Rutgers—The State University of New Jersey, 65 Dudley Road, New Brunswick, New Jersey 08901-8520, USA

MS 03-117: Received 19 March 2003/Accepted 17 June 2003

ABSTRACT

Many microorganisms (including a number of important foodborne pathogens) can be present on raw fruits and vegetables. Since these products are frequently eaten raw, any pathogens present represent a potential risk to the consumer. The objective of this study was to compare the efficacy of a commercial produce wash with that of water for reducing the total bacterial population on lettuce when used by food service employees in university dining halls. Because this study was carried out in actual food service facilities during their daily operation, we used indigenous produce microflora instead of actual pathogens. Over the course of the study, more than 40 heads of lettuce were divided into thirds, and each section was analyzed for total plate count either before washing, after washing in water, or after washing in Victory produce wash. When initial contamination levels were ≥ 100 CFU/g ($n = 36$ samples), reductions obtained with Victory produce wash (1.8 log CFU/g) were significantly larger ($P = 0.0006$) than those obtained with water (0.8 log CFU/g). Our results indicate that Victory produce wash is effective in reducing indigenous flora on lettuce during food service preparation. Our results also show that care must be taken in the analysis of microbial reduction data: only a slight reduction in total plate count (ca. 0.1 log CFU/g) and no significant difference in reductions ($P = 0.84$) were observed when all samples (irrespective of initial contamination level) were compared.

Lettuce sold to consumers in an unprocessed or minimally processed form has in some cases resulted in food poisoning outbreaks. Outbreaks occurring between 1984 and 1998 and involving *Salmonella* spp., *Escherichia coli* O157:H7, and *Campylobacter jejuni* (10) have been linked to lettuce consumption. In 2002, lettuce was confirmed to be the vehicle for the *Shigella sonnei* that sickened five people after the consumption of a salad in a food service establishment (10). Many techniques have been used to reduce lettuce contamination (including irrigation, harvest, and postharvest processing interventions), yet contamination may sometimes be unavoidable. In addition to contamination attributable to farming and harvesting practices, further handling in the food service setting can add to levels of bacteria as well. Chen et al. (2) reported that even if proper hand-washing methods are followed, microorganisms may still be present and can be transferred from washed hands to lettuce during chopping.

Research on the efficacy of washing with water and that of washing with sanitizing solutions has yielded conflicting results. Li et al. (6) reported that the immersion of cut lettuce in water with or without chlorine reduced initial populations of aerobic bacteria by 1.73 to 1.96 log CFU/g. Singh et al. (8) reported that the washing of shredded lettuce with deionized water reduced populations of *E. coli* O157:H7 by only 0.22 log CFU/g but that multiple washings in deionized water resulted in a reduction of 0.76 log

CFU/g. The rinsing of lettuce with common household sanitizers, including distilled water, apple cider vinegar (5%), lemon juice (13%), bleach (4%), and white vinegar (35%), reduced aerobic bacterial populations by averages of 0.6, 1.2, 1.8, 1.9, and 2.3 log/g, respectively, without severely affecting sensory attributes (11). Kim et al. (4) reported the inactivation of 1.4 and 1.8 log CFU/g of mesophilic and psychrotrophic bacteria, respectively, after treatment with a 1 mM chlorine solution and the inactivation of 2.0 and 2.9 log CFU/g with a 2 mM chlorine solution. Washing cut lettuce in chlorinated water (100 mg of total chlorine per liter) reduced *E. coli* O157:H7 populations by 1 to 2 log CFU/g, depending on the temperature of the wash water (3). Yet, Li et al. (5) reported no significant decrease in *E. coli* O157:H7 levels on cut lettuce after treatment with chlorine at 20 ppm before or after inoculation at either 50 or 20°C. Inoculum levels of *Listeria monocytogenes* were reduced by 1 log CFU/g after washing with chlorinated water (100 of total chlorine mg per liter) at 4 or 47°C (3). Beuchat et al. (1) found that rinsing lettuce with chlorine for 1 to 10 min lowered the *Salmonella* concentration from 4.53 to 2.93 log CFU/g.

Previous studies have evaluated the removal of natural microflora or inoculated pathogens in a laboratory setting. The present study was undertaken to investigate the efficacy of a produce wash in the removal of native microflora during daily food service tasks in a real-world setting. The lettuce was processed and washed by food service employees according to Rutgers Division of Dining Services stan-

* Author for correspondence. Tel: 732-932-9611, Ext 214; Fax: 732-932-6776; E-mail: schaffner@aesop.rutgers.edu.

TABLE 1. Methods used to test for coliforms and pathogenic bacteria

Microorganism(s)	Method
Coliforms	Most-probable-number (MPN) method with triplicate brilliant green bile (2%).
<i>Escherichia coli</i>	MPN method with EC broth (BAM ^a). <i>E. coli</i> was confirmed by subculturing the gas-positive tubes on eosin methylene blue agar at 37°C for 24 h. The positive colonies on the agar were tested with BBL Enterotube II (Becton Dickinson).
<i>Salmonella</i> spp.	Samples were enriched in lactose broth and then in tetrathionate broth for 4 and 24 h, respectively, at 37°C. The broth was streaked onto bismuth sulfite, xylose lysine desoxycholate, and Hektoen enteric agar and incubated for 24 h at 37°C. Suspect colonies were Gram stained, and enterotubes were used if morphological characteristics were correct.
<i>Staphylococcus aureus</i>	Homogenate was plated on Baird Parker agar supplemented with egg yolk tellurite and incubated for 48 h at 37°C. The coagulase test was performed on presumptive positive colonies for confirmation.
<i>Listeria monocytogenes</i>	Samples were enriched in <i>Listeria</i> enrichment broth for 48 h. The enrichment was streaked on PAL-CAM agar after 24 and 48 h. Suspect colonies were streaked onto tryptic soy agar supplemented with yeast extract and incubated for 24 h at 37°C. Gram staining and MicroID <i>Listeria</i> (Remel, Lenexa, Kans.) were used for confirmation.

^a U.S. Food and Drug Administration's *Bacteriological Analytical Manual*.

dard procedures. Because the study was completed in a functioning real-world establishment, aerobic mesophilic plate counts were used instead of pathogenic bacteria.

MATERIALS AND METHODS

Collection of lettuce samples. The collection of all samples took place at two Rutgers University dining halls over a period of 3 months. Heads of lettuce were collected from produce refrigerators, and the outermost two or three layers and the core of each head of lettuce were removed in accordance with standard Division of Dining Services practices. Each head was then divided into thirds. One third was immediately placed in a sterile 55-oz Whirl-Pak bag (Nasco, Fort Atkinson, Wis.) for transport. The second third was held and rotated under the faucet of a food service sink with cold water for ca. 20 s before it was placed in a Whirl-Pak bag. The remaining third was held and rotated for ca. 20 s under the faucet of a food service sink equipped to dispense Victory produce wash (Ecolab, St. Paul, Minn.). Faucets equipped to dispense Victory produce wash in this manner produce a stream of tap water containing hydrogen peroxide and peroxyacetic acid at a concentration of ca. 60 ppm. All preparation, cutting, and cleaning was completed by a food service employee at the establishment. The samples were transported to the laboratory within 1 h in an insulated bag containing ice packs. Lettuce samples were held at 4°C until microbial testing began (within 2 h). A total of 43 heads of lettuce were analyzed.

Microbial analysis of lettuce samples. Twenty-five grams of lettuce was weighed into a sterile filter bag (BA6141/STR, Seward Ltd., London, UK) and masticated (Lab-Blender 400, Cooke Laboratory Products, Alexandria, Va.) with 225 ml of sterile Butterfield's diluent for 2 min. The samples were serially diluted, surface plated on total plate count (TPC) agar, and incubated at 37°C for 24 h. The homogenate from the sample preparation was used to test for total coliforms, *E. coli*, *Salmonella* spp., *Staphylococcus aureus*, and *L. monocytogenes* according to the methods outlined in Table 1. Unless otherwise noted, all microbiological media were obtained from Difco (Becton Dickinson and Co., Sparks, Md.).

Statistical analysis. Data were compiled and plotted with Sigma Plot version 8.0 (SPSS Inc., Chicago, Ill.). Log-transformed data were compared for significant differences ($P = 0.05$) with SAS statistical software (SAS Institute Inc., Cary, N.C.).

RESULTS AND DISCUSSION

No pathogenic bacteria were detected in the first 24 lettuce samples tested. Total coliform levels for all samples were <100 log CFU/g. Subsequent lettuce samples were tested only for TPC. These results are not unusual and are consistent with those reported by the Public Health Laboratory Services (PHLS) in the United Kingdom. The PHLS tested 151 imported whole-lettuce samples and did not detect *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *E. coli* O157:H7, or *L. monocytogenes* (7).

The aerobic plate counts for unwashed lettuce samples ranged from 0 to 5.14 log CFU/g, with 88% of the samples having microbial loads of >2 log CFU/g and 65% of the samples containing >3 log CFU/g. The distribution of total aerobic counts is shown in Figure 1. The aerobic plate counts for unwashed lettuce samples were in general agreement with results obtained by Vijayakumar and Wolf-Hall (11), who found 10³ to 10⁵ CFU of aerobic, mesophilic microorganisms per g of untreated lettuce. The values obtained in the present study and those obtained by Vijayakumar and Wolf-Hall were generally lower than those obtained by Soriano et al. (9), who reported values of 3.0 to 7.8 log CFU/g for untreated lettuce.

Minimal decreases were seen after lettuce ($n = 43$) was washed with tap water (resulting in decreases of 0.1 ± 1.42 log CFU/g) or Victory produce wash (resulting in decreases of 0.05 ± 1.76 log CFU/g), and no significant difference was detected between the two methods with respect to the reductions achieved ($P = 0.84$). Some studies have shown insignificant reductions (0.04 log CFU/g (9)) or only marginally significant reductions (0.6 log CFU/g (11)) in aerobic plate counts after washing in distilled water.

Further data analysis revealed that unwashed, water-washed, and Victory-washed lettuce samples had different final TPC distributions (Fig. 1a through 1c, respectively). Washing with water shifted the TPC distribution lower, and an even more pronounced shift was evident after lettuce was washed in Victory produce wash. The average TPC values decreased from 3.4 ± 1.2 to 3.0 ± 1.0 log CFU/g

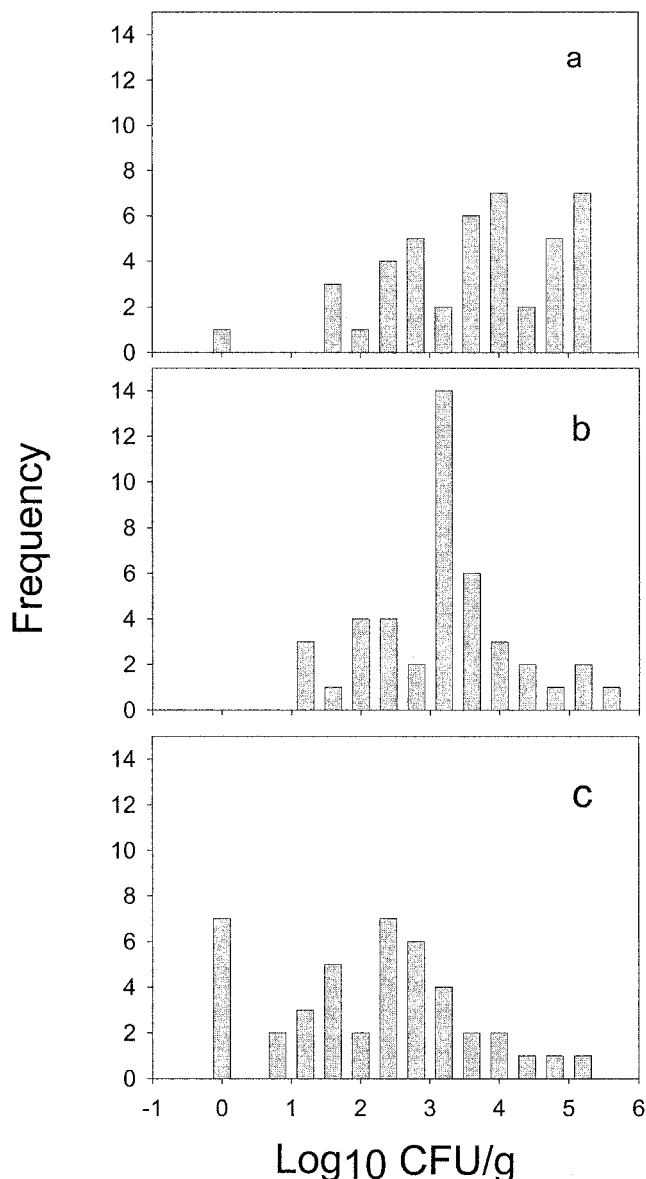


FIGURE 1. Frequency distribution of total aerobic plate counts on lettuce samples (a) before washing, (b) after washing in water, and (c) after washing in Victory produce wash, respectively, in a food service setting.

after lettuce was washed in water and to 2.0 ± 1.3 log CFU/g after lettuce was washed in Victory. The percentage of samples containing >2 log CFU/g decreased by 7% after lettuce was washed in water and by 32% after lettuce was washed in Victory. The percentage of samples containing >3 log CFU/g was reduced by 14% after lettuce was washed in water and by 42% after lettuce was washed in Victory. It should be noted that the presence of large bacterial populations in some samples washed with either water or Victory suggest that some bacteria in these samples are located in protected sites or are internalized within the lettuce tissue.

Further statistical analysis revealed that when samples containing initial contamination levels of ≥ 2 log CFU/g were analyzed separately ($n = 36$), the reduction achieved with the Victory produce wash (1.8 log CFU/g) was significantly larger

($P = 0.0006$) than that achieved with tap water (0.8 log CFU/g). No correlation between the contamination level of unwashed lettuce and the log reduction in microflora was observed for either washing method (data not shown).

Victory sanitizing wash was more effective in reducing indigenous microflora on lettuce in a food service setting than water was. This is an important finding, since most other published studies on the removal of aerobic bacteria have been carried out in a laboratory setting with distilled or deionized water.

The results of the present research also show the importance of thorough data analysis. Care must be taken when analyzing the effectiveness of treatments designed to produce reductions in microbial populations. Our analysis shows that the initial microbial population may influence the apparent effectiveness of a given treatment. The inclusion of a number of samples with low initial contamination levels was able to confound a cursory statistical analysis and mask the effectiveness of a produce sanitizer compared with that of water.

ACKNOWLEDGMENT

We thank the Rutgers University Division of Dining Services for their time, for their cooperation, and for funding this study.

REFERENCES

1. Beuchat, L. R., B. V. Nail, B. B. Adler, and M. R. S. Clavero. 1998. Efficacy of spray application of chlorinated water in killing pathogenic bacteria on raw apples, tomatoes, and lettuce. *J. Food Prot.* 61:1305–1311.
2. Chen, Y., K. M. Jackson, F. P. Chea, and D. W. Schaffner. 2001. Quantification and variability analysis of bacterial cross contamination rates in common foodservice tasks. *J. Food Prot.* 64:72–80.
3. Delaquis, P., S. Stewart, S. Cazaux, and P. Toivonen. 2002. Survival and growth of *Listeria monocytogenes* and *Escherichia coli* O157:H7 in ready-to-eat iceberg lettuce washed in warm chlorinated water. *J. Food Prot.* 65:459–464.
4. Kim, J. G., A. E. Yousef, and G. W. Chism. 1999. Use of ozone to inactivate microorganisms on lettuce. *J. Food Saf.* 19:17–34.
5. Li, Y., R. E. Brackett, J. Chen, and L. R. Beuchat. 2001. Survival and growth of *Escherichia coli* O157:H7 inoculated onto cut lettuce before or after heating in chlorinated water, followed by storage at 5 or 15°C. *J. Food Prot.* 64:305–309.
6. Li, Y., R. E. Brackett, R. L. Shewfelt, and L. R. Beuchat. 2001. Changes in appearance and natural microflora on iceberg lettuce treated in warm, chlorinated water and then stored at refrigeration temperature. *Food Microbiol.* 18:299–308.
7. Little, C., D. Roberts, E. Youngs, and J. deLouvois. 1999. Microbiological quality of retail imported unprepared whole lettuces: a PHLS food working group study. *J. Food Prot.* 62:325–328.
8. Singh, N., R. K. Singh, A. K. Bhunia, and R. L. Stroschine. 2002. Effect of inoculation and washing methods on the efficacy of different sanitizers against *Escherichia coli* O157:H7 on lettuce. *Food Microbiol.* 19:183–193.
9. Soriano, J. M., H. Rico, J. C. Molto, and J. Manes. 2000. Assessment of the microbiological quality and wash treatments of lettuce served in university restaurants. *Int. J. Food Microbiol.* 58:123–128.
10. U.S. Food and Drug Administration and Institute of Food Technologists. 2001. Analysis and evaluation of preventive control measures for the control and reduction/elimination of microbial hazards on fresh and fresh-cut produce. Available at: <http://www.cfsan.fda.gov/~comm/ift3-toc.html>. Accessed 30 September 2001.
11. Vijayakumar, C., and C. E. Wolf-Hall. 2002. Evaluation of household sanitizers for reducing levels of *Escherichia coli* on iceberg lettuce. *J. Food Prot.* 65:1646–1650.