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Ability of Hand Hygiene Interventions Using Alcohol-Based Hand Sanitizers and Soap To Reduce Microbial Load on Farmworker Hands Soiled during Harvest

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ABSTRACT

Effective hand hygiene is essential to prevent the spread of pathogens on produce farms and reduce foodborne illness. The U.S. Food and Drug Administration Food Safety Modernization Act Proposed Rule for Produce Safety recommends the use of soap and running water for hand hygiene of produce handlers. The use of alcohol-based hand sanitizer (ABHS) may be an effective alternative hygiene intervention where access to water is limited. There are no published data on the efficacy of either soap or ABHS-based interventions to reduce microbial contamination in agricultural settings. The goal of this study was to assess the ability of two soap-based (traditional or pumice) and two ABHS-based (label-use or two-step) hygiene interventions to reduce microbes (coliforms, *Escherichia coli,* and *Enterococcus* spp.) and soil (absorbance of hand rinsate at 600 nm [A_{600}]) on farmworker hands after harvesting produce, compared with the results for a no-hand-hygiene control. With no hand hygiene, farmworker hands were soiled (median A_{600}, 0.48) and had high concentrations of coliforms (geometric mean, 3.4 log CFU per hand) and *Enterococcus* spp. (geometric mean, 5.3 log CFU per hand) after 1 to 2 h of harvesting tomatoes. Differences in microbial loads in comparison to the loads in the control group varied by indicator organism and hygiene intervention (0 to 2.3 log CFU per hand). All interventions yielded lower concentrations of *Enterococcus* spp. and *E. coli* ($P < 0.05$), but not of coliforms, than were found in the control group. The two-step ABHS intervention led to significantly lower concentrations of coliforms and *Enterococcus* spp. than the pumice soap and label-use ABHS interventions ($P < 0.05$) and was the only intervention to yield significantly fewer samples with *E. coli* than were found in the control group ($P < 0.05$). All interventions removed soil from hands ($P < 0.05$), soap-based interventions more so than ABHS-based interventions ($P < 0.05$). ABHS-based interventions were equally as effective as hand washing with soap at reducing indicator organisms on farmworker hands. Based on these results, ABHS is an efficacious hand hygiene solution for produce handlers, even on soiled hands.

Increases in produce-associated outbreaks highlight the need for effective microbial risk management on produce farms and in packing sheds. In the United States, from 1999 to 2008, contaminated produce was responsible for at least 23% of all reported foodborne illnesses *(33)*. Produce contamination may occur at various points in the farm-to-fork continuum *(19, 31)*. Some produce-associated outbreaks have been thought to be caused by infected farmworker and, possibly, inadequate hand hygiene *(14, 16, 42)*.

Farmworker hands may be vehicles for microbial contamination of produce *(23, 29)*. Harvest and packing, often done by hand, have been associated with increases in microbial contamination *(2, 18, 22)*. A 2010 study found that of seven major fruit and vegetable crops, all were either exclusively or partially harvested by hand *(7)*. Because "workers often touch produce with their bare hands" the U.S. Food and Drug Administration Food Safety Modernization Act (FSMA) Proposed Rule for Produce Safety states that hand washing is a "key control measure in preventing contamination" of produce *(39)*.

Effective hand hygiene reduces microbial risks and disease in health care and community settings *(1, 6, 43)*, but there are few data on its efficacy in food handling settings *(4)*, and it has just begun to be studied in the agricultural environment. The FSMA Proposed Rule for Produce Safety defines hand hygiene as "washing hands thoroughly, including scrubbing with soap and running water … and drying hands thoroughly using single-service towels, clean cloth towels, sanitary towel service or other adequate hand drying devices" *(39)*. However, soil on farmworker hands may limit the ability of hand washing to remove or inactivate microbes. Thus, it is important to assess the hypothesis that hand washing with soap is the most efficacious hygiene intervention for the agricultural envi-

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ronment. In addition, hand washing with soap may be difficult to achieve on every occasion specified in the rule due to barriers such as limited access to potable water near all work areas. Alcohol-based hand sanitizers (ABHS) are a logical alternative because they do not require potable water, and a large body of evidence exists to show that their antimicrobial efficacy results in reduced spread of infection in health care environments *(6, 43)*. The FSMA Proposed Rule for Produce Safety prohibits the sole use of ABHS because "the effectiveness of hand sanitizers has been shown to be highly dependent upon the removal of organic material from the hands prior to their use" *(39)*. However, a large body of research suggests that the efficacy of ABHS is not impacted when hands are soiled *(10, 12, 25, 26, 28, 30, 35)*. One limitation of ABHS is that hands may still appear dirty, even if microbes have been inactivated. One method that may address this limitation is SaniTwice, a two-step technique where an excess of ABHS is applied to hands and removed with paper towels, followed by a second ABHS application *(11)*. This technique has been shown to reduce *Escherichia coli* on hands soiled with beef broth and raw hamburgers *(11)* and to reduce bacteria and soil on agricultural workers' hands *(13)*.

The goal of this study was to assess the ability of two soap-based and two ABHS-based hygiene interventions to reduce microbes and soil on farmworker hands after harvesting produce, compared with a no-hygiene control. Traditional (nonantibacterial and nonabrasive) soap was included as the current "gold standard" *(38)*. Pumice soap was chosen because it may be able to remove particles and organic compounds from hands that traditional soaps do not. ABHS interventions were included as waterless hygiene options as alternatives to traditional soap. The two-step ABHS intervention was included because of its previously demonstrated efficacy on soiled hands *(10)*.

MATERIALS AND METHODS

Setting and population. This study took place over a 4-week period in August and September 2014 on a farm that produces tomatoes in the state of Nuevo León, Mexico. The farm exported its produce to the United States and sold it to Mexican retailers and had established food safety protocols in place, as well as a dedicated food safety specialist on site. Approval for research on human subjects was conferred after ethics review by Emory University (institutional review board no. 00035460).

The study population consisted of 181 farmworkers who were employed by this farm to harvest tomatoes. Participants routinely used gloves for tomato harvest but removed them when participating in our study in order that the interventions be tested on the most highly soiled and microbially contaminated hands possible. During each of the five nonconsecutive days of the study prior to study enrollment, the farm food safety specialist introduced the study staff, who described the study and solicited volunteers. Inclusion criteria included that the participant was an employee of the farm assigned to harvest tomatoes and provided oral informed consent to participate in the study according to the institutional review board–approved protocol. There were no exclusion criteria. Oral consent was documented by study staff for each participant.

Farm activities and intervention groups. After consent was received, the farmworkers were randomly assigned to one of five groups (described below), and each was given a name tag to indicate his or her group and unique sample identifier. To standardize the microbial load on farmworker hands, all farmworkers were asked to wash their hands with traditional (nonantibacterial and nonabrasive) soap (~3.5 ml of Pearl Lotion Hand Soap; Noble Chemical, Inc., Lancaster, PA) and potable water at a nearby hand washing station stocked with paper towels for drying (Servitoalla double-ply, 28 by 22.8 cm; Pétalo, Kimberly-Clark, Mexico City, Mexico). All potable water used in the study was provided by the Universidad Autónoma de Nuevo León (UANL) laboratory and assured to have no coliforms, *E. coli,* or *Enterococcus* spp. in a 100-ml aliquot (see "Absorbance and microbial analyses" for general description of microbial assays). The farmworkers were then asked to harvest tomatoes for 1 to 2 h (collecting approximately 30 bins per person), using their standard procedure but without gloves. After harvesting, each farmworker completed activities described below based on their assigned group, following the instructions and demonstration of study staff (Fig. 1). A convenience sample of at least 10 participants per study group also had their hands photographed before and after the activities described below.

After harvesting, individuals in the control group did not perform any hand hygiene. Individuals in the label-use ABHS group used ABHS according to the product label instructions, with minor modifications. Individuals in this group received one pump of sanitizer gel (~3.5-ml of GOJO Purell Advanced Instant Hand Sanitizer, active ingredient 70% ethanol; GOJO Industries, Akron, OH) in the palm of one hand. They were then asked to rub their hands in the following manner used in all interventions: rub hands palm-to-palm, rub each palm on the dorsal surface of the opposite hand, and interlace fingers to distribute product over the fingers. They were asked to continue rubbing their hands until dry.

Individuals in the two-step ABHS group performed SaniTwice hand hygiene as described previously, with minor modifications *(11)*. Briefly, they received three pumps of sanitizer gel (~10.5 ml, enough to keep hands wet for 20 s) in the palm of one hand. They were then asked to rub their hands as described above for about 20 s. After ~20 s of rubbing, they were given a paper towel to remove all remaining sanitizer on their hands. They then followed the steps described above for the label-use ABHS group.

Individuals in the traditional soap group received two pumps of potable water (approximately 220 ml) to wet their hands. They then received one pump (~3.5 ml) of the same traditional soap used by all participants prior to harvesting. They were asked to rub their hands as described above for about 20 s. After rubbing, they rinsed their hands with three pumps of the potable water provided (approximately 330 ml). A paper towel was provided, and they were asked to dry their hands as they normally would.

Individuals in the pumice soap group received two pumps of pumice soap (~6 ml of GOJO Natural Orange Pumice Hand Cleaner, a gel-based surfactant formula with pumice particles; GOJO Industries) in the palm of one hand. They were then asked to rub their hands as described above for about 20 s. During this rubbing, they also received a splash of potable water (approximately 2 ml). After rubbing, they rinsed their hands with three pumps of the potable water provided (approximately 330 ml). A paper towel was provided, and they were asked to dry their hands as they normally would.

Immediately after the activities described above were completed, the farmworkers were asked to provide a hand rinsate sample by inserting one hand in a Whirl-Pak bag (Nasco, Fort Atkinson, WI) containing 750 ml of sterile 0.1% peptone water (Thermo Fisher Scientific, Waltham, MA) while study staff massaged their fingers through the bag for 20 to 30 s. This process

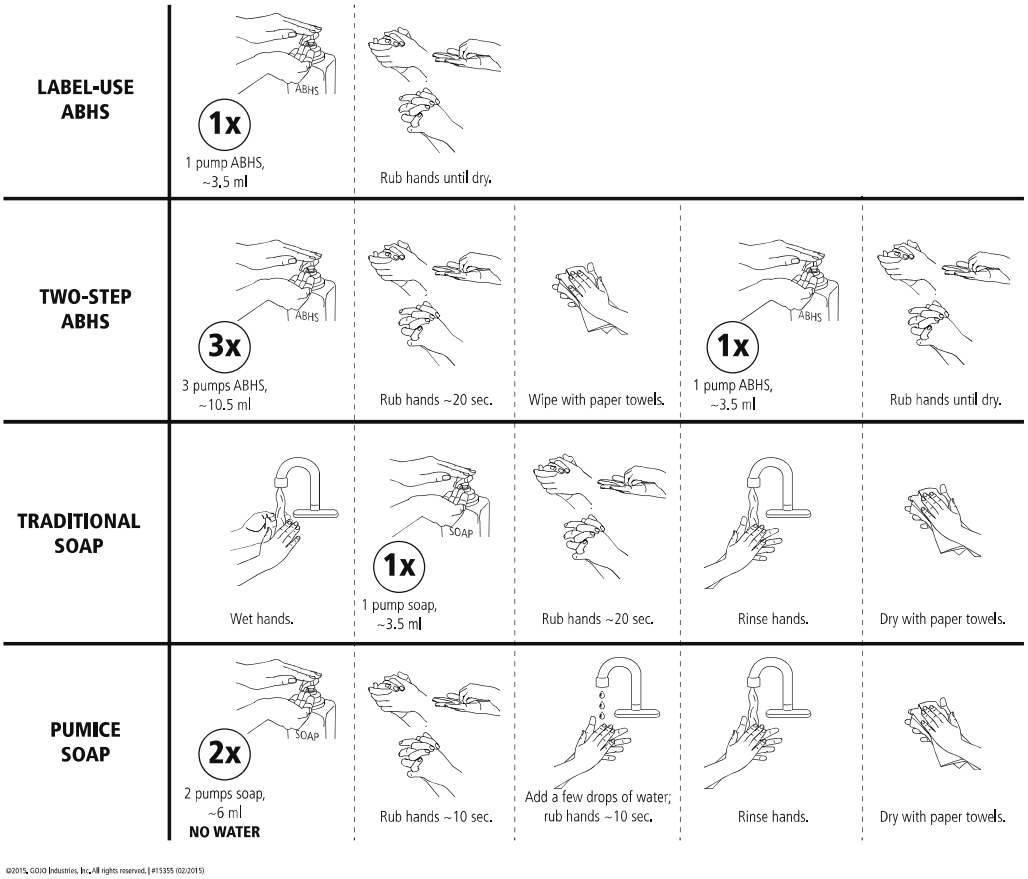

FIGURE 1. *Visual description of the two ABHS-based and two soap-based hand hygiene interventions. Illustrations in this figure are courtesy of GOJO Industries, Inc.*

was repeated for the second hand. The worker was provided a paper towel and small token of thanks for participation (e.g., bottled water, a cap, a bandana, or similar item). The labeled hand rinsate sample was stored on ice packs in a cooler. For each study staff member collecting samples, at the end of the day, an additional unopened Whirl-Pak bag containing 750 ml of peptone water was retained as a negative collection control. All samples were transported to the Laboratory of Microbial Biochemistry and Genetics at UANL, where they were stored at 4°C until analysis. Analysis was performed within 48 h of field collection. If the microbial analysis results were outside the quantifiable range and a repeat analysis was necessary, the repeat analysis was conducted within 72 h of field collection.

Absorbance and microbial analyses. Absorbance readings of hand rinsate at 600 nm (A_{600}) were taken to objectively measure the matter removed from hands during sampling, used as a proxy for "dirtiness of hands," referred to as "soil" herein. Absorbance reading is an objective approach to assessing dirt on hands that is comparable to assessing the turbidity of hand rinse samples *(27)* and may be preferable to other, subjective methods, such as visual inspection of hands *(25)*. Rinsate samples were inverted several times to resuspend any particulate matter, and then an aliquot was taken for measurement of absorbance at 600 nm (A_{600}) using a spectrophotometer (Sequoia Turner, Mountain View, CA).

Samples were analyzed in random order (without regard to study group) to detect and enumerate coliforms, *E. coli,* and *Enterococcus* spp., three common, nonpathogenic types of bacteria used to indicate microbial load, hereinafter called indicator bacteria. Serial volumes of each hand rinse sample (100 μl, 1 ml, and 10 ml) were filtered through separate 0.45-μm-pore-size cellulose filters (EMD Millipore Corporation, Billerica, MA) using a vacuum manifold filtration system (Pall Corporation, Port Washington, NY). When filtering volumes of less than 10 ml, the funnel (with the vacuum closed) was prefilled with 10 ml of peptone water before the sample was added to allow even sample dispersion across the membrane prior to opening the vacuum. Following filtration through duplicate membranes for each serial volume of rinsate, each membrane was placed on a separate petri dish containing solidified agar for bacterial enumeration. To enumerate *E. coli* and coliform bacteria, membranes were placed on chromogenic Bio-Rad Rapid'*E. coli* 2 agar (Bio-Rad, Hercules, CA) and incubated at 44°C for 24 h for enumeration of typical colonies (pink to purple for *E. coli* and both blue to green and pink to purple for coliforms). To enumerate *Enterococcus* bacteria, membranes were placed on Kenner Fecal *Streptococcus* agar (BD, Franklin Lake, NJ) plates and incubated at 37°C for 48 h before enumeration of red-centered colonies. For all three organisms, the limit of detection was 37 CFU per hand and the upper limit of quantification was 8.3 log CFU per hand.

The remaining sample rinsate was stored at 4°C for no more than 72 h postcollection and reprocessed, as described above, for cases in which colony counts were inconsistent or larger than assay detection limits (e.g., more than 250 colonies per plate). For each day of sample collection, study staff processed a negative sample collection control (described above), a negative water control (sampled from the municipal water used for hand rinsing in the field), and a positive control (mixture of *Enterococcus faecalis* [ATCC 19433], *Salmonella enterica* serovar Typhimurium [ATCC 19428] as a surrogate for coliforms *(15),* and *E. coli* [ATCC

TABLE 1. *Proportions of hand rinsate samples positive for indicator bacteria from the control group and four intervention groups of workers harvesting tomatoes on a farm in Mexico*

Group[a]	No. of positive samples/total no. of samples (%) tested for[b]:		
	Coliforms	*Enterococcus* spp.	*E. coli*
Control	30/42 (71)	41/42 (98)	10/42 (24)
Label-use ABHS	28/34 (82)	31/34 (91)	2/34 (6)
Two-step ABHS	21/35 (60)[c]	28/35 (80)	0/35 (0)[d]
Traditional soap	28/35 (80)	31/35 (89)	2/35 (6)
Pumice soap	35/35 (100)[d]	35/35 (100)	1/35 (3)

[a] The control group samples were collected after farmworkers harvested tomatoes for 1 to 2 h. Hand rinsate samples were collected from the four intervention groups immediately after performing hand hygiene.

[b] Values are for hand rinsate samples tested for the given indicator bacteria within each study group.

[c] Result is significantly different from the result for the pumice soap group ($\alpha = 0.05$)

[d] Result is significantly different from the result for the control group ($\alpha = 0.05$)

25922]; American Type Culture Collection, Manassas, VA). The positive control was created by growing each strain overnight on tryptic soy broth (Difco, BD) and then seeding 1 ml of each strain into 11 ml of sterile 0.85% NaCl (Sigma Aldrich, St. Louis, MO), pH 7.0.

Data entry and statistical analyses. All data were entered independently by two trained individuals into separate Microsoft Excel databases (Microsoft, Redmond, WA), compared, and reconciled by review of the original laboratory forms. An additional check showed no discrepancies when 5% of the original laboratory forms were randomly selected and compared against the final database. Statistical analyses were performed using Stata 10 (STATA Corp., College Station, TX), JMP Pro 10, and SAS 9.3 (SAS Institute Inc., Cary, NC). The Shapiro-Wilk test *(32)* indicated that all data (e.g., absorbance values of hand rinsates and log-transformed indicator organism concentrations) were not normally distributed (data not shown). Therefore, all statistical tests used were nonparametric. When calculating the concentrations of indicator bacteria, any sample without detectable bacteria was assigned a value of 18.5 CFU per hand, half the limit of detection *(37)*. Geometric means and standard deviations are used to describe bacterial concentrations as a convenience to the reader *(40)*, and medians and standard deviations are used to describe absorbance data. To compare differences in percentages of samples positive for microbial indicators across study groups, a Pearson χ^2 test *(9)* and Bonferroni correction *(17)* were used. To compare A_{600} and microbial concentration values across study groups, the Kruskal-Wallis test *(20)* followed by the Steel-Dwass multiple comparison procedure *(8)* were used.

RESULTS

In general, farmworkers' hands became contaminated with indicator bacteria (Table 1 and Fig. 2, control) and soiled while they harvested produce, prior to hand hygiene (Fig. 3, control). The percentages of samples positive for coliforms (71%) and *Enterococcus* bacteria (98%) in the control group were high (Table 1) relative to the percentage of samples positive for *E. coli* (24%) (Table 1). The concentrations of bacteria on control group hands ranged widely: coliform concentrations in positive samples ranged from the lower limit of detection to the upper limit of quantification (37 CFU per hand to 8.3 log CFU per hand) (Fig. 2), *Enterococcus* concentrations in positive samples ranged from 93 CFU per hand to the upper limit of quantification (8.3 log CFU per hand) (Fig. 2), and *E. coli* concentrations in positive samples ranged from the lower limit of detection (37 CFU per hand) to 3.3 log CFU per hand. The geometric mean concentrations of coliforms (3.4 log CFU per hand) and *Enterococcus* bacteria (5.3 log CFU per hand) in control group samples were relatively high (Fig. 2) compared with the geometric mean concentration of *E. coli* bacteria (1.7 log or 50 CFU per hand) (Fig. 2). For microbial assays, all negative and positive controls consistently yielded the expected results. The median absorbance of control hand rinsate samples was 0.48, and the values varied greatly across the control group, ranging from A_{600} 0.05 to 1.36. The visual appearance of hands postharvest and preintervention is shown in the "before intervention" photographs of hands in Figure 4. It appears that in just a few hours of harvesting produce, the farmworkers' hands accumulated high concentrations of some indicator bacteria and soil.

While hygiene interventions did not completely eliminate indicator bacteria from hands, in general, all hand hygiene interventions effectively reduced the concentrations of some bacteria. However, there were differences in the performance of the four interventions tested.

Compared with the results for the control group, none of the hand hygiene interventions yielded a significantly lower coliform concentration or percentage of samples positive for coliforms (Table 1 and Fig. 2). However, the two-step ABHS group had lower concentrations of coliforms than the label-use ABHS and pumice soap groups ($P < 0.05$) (Fig. 2). Compared with the control group, all four intervention groups had lower concentrations of *Enterococcus* spp. ($P < 0.05$) (Fig. 2), although similar to the result for coliforms, none of the hand hygiene interventions yielded significantly lower percentages of samples positive for *Enterococcus* than in the control group (Table 1). The two-step ABHS group had lower concentrations of *Enterococcus* than the label-use ABHS and pumice soap groups ($P < 0.05$) (Fig. 2). For *E. coli*, all four hand hygiene interventions yielded significantly lower concentrations on hands than were found in the control group ($P < 0.05$, Fig. 2). However, two-step ABHS was the only intervention to have significantly fewer samples with detectable *E. coli* than the control group, and this group had no samples positive for *E. coli* ($P < 0.05$) (Table 1). The other three interventions had only 1 or 2 samples positive for *E. coli* (3 to 6%), compared with 10 samples positive for *E. coli* (24%) in the control group (Table 1), but these differences did not reach statistical significance.

Using absorbance measurements of hand rinsate samples as a proxy for soil, all four interventions yielded significantly less soil on hands than in the control group (range, A_{600} 0.05 to 1.36); soap-based interventions (range, A_{600} 0.00 to 0.15) yielded significantly less soil remaining

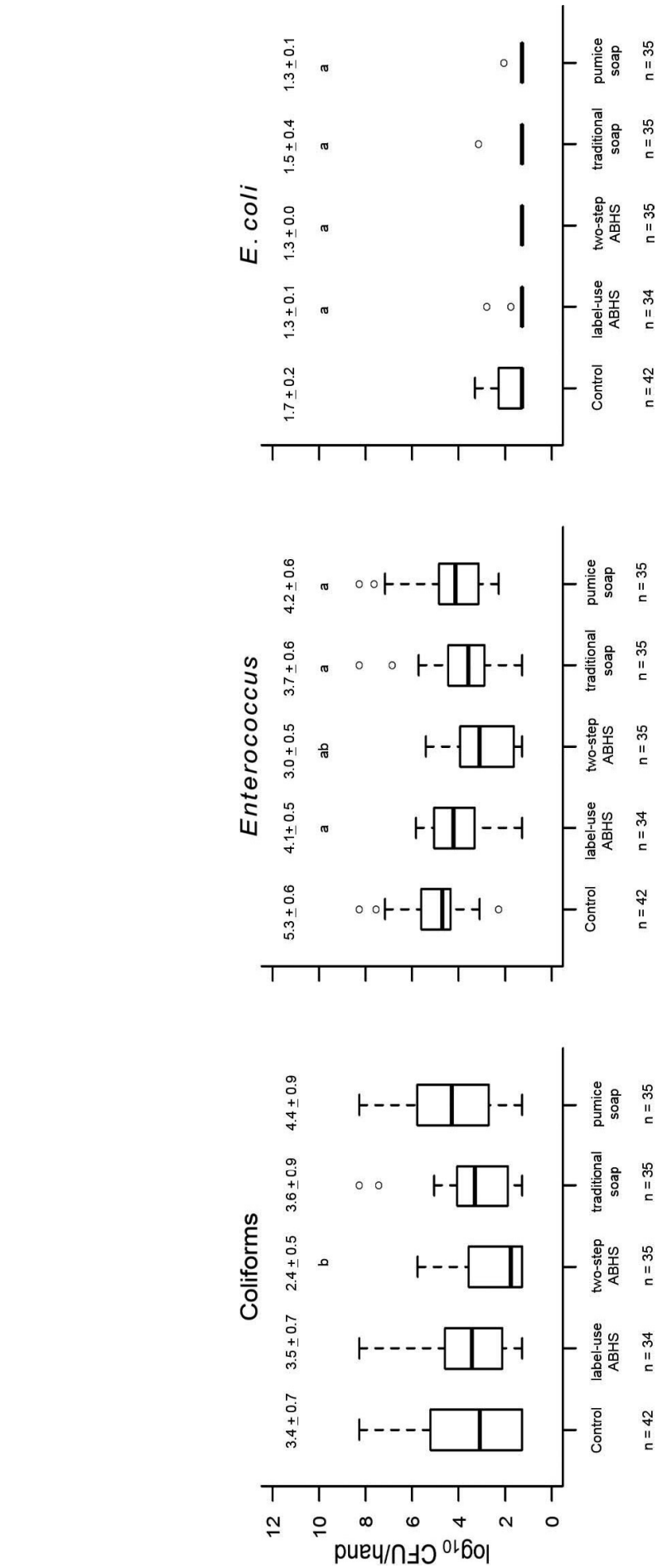

FIGURE 2. *Concentrations of coliform,* Enterococcus, *and* E. coli *bacteria in hand rinsate samples from the control group and four hand hygiene intervention groups of workers harvesting tomatoes. For each study group, the boxes display the quartiles (25th, 50th, and 75th) and whiskers extend to 1.5 times the interquartile range. Any data points outside the whiskers are displayed individually as dots. The values above each study group box plot indicate the geometric mean bacterial concentration and standard deviation (log CFU per hand). The control group samples were collected after farmworkers harvested tomatoes for 1 to 2 h. The four intervention groups had hand rinsates collected immediately after performing hand hygiene. a, significantly different from the control group ($\alpha = 0.05$); b, significantly different from the label-use ABHS and pumice soap groups ($\alpha = 0.05$)*