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Contamination of Fresh Produce by Microbial Indicators on Farms and in Packing Facilities: Elucidation of Environmental Routes

Faith E. Bartz,[a]* Jacquelyn Sunshine Lickness,[a]* Norma Heredia,[b]
Anna Fabiszewski de Aceituno,[a] Kira L. Newman,[c] Domonique Watson Hodge,[a]
Lee-Ann Jaykus,[d] Santos García,[b] Juan S. Leon[a,c]

Hubert Department of Global Health, Rollins School of Public Health, Emory University, Atlanta, Georgia, USA[a];
Departamento de Microbiología e Inmunología, Facultad de Ciencias Biológicas, Universidad Autónoma de
Nuevo León, San Nicolás, Nuevo León, México[b]; Department of Epidemiology, Rollins School of Public Health,
Emory University, Atlanta, Georgia, USA[c]; Department of Food Science, North Carolina State University, Raleigh,
North Carolina, USA[d]

ABSTRACT To improve food safety on farms, it is critical to quantify the impact of environmental microbial contamination sources on fresh produce. However, studies are hampered by difficulties achieving study designs with powered sample sizes to elucidate relationships between environmental and produce contamination. Our goal was to quantify, in the agricultural production environment, the relationship between microbial contamination on hands, soil, and water and contamination on fresh produce. In 11 farms and packing facilities in northern Mexico, we applied a matched study design: composite samples ($n = 636$, equivalent to 11,046 units) of produce rinses were matched to water, soil, and worker hand rinses during two growing seasons. Microbial indicators (coliforms, *Escherichia coli*, *Enterococcus* spp., and somatic coliphage) were quantified from composite samples. Statistical measures of association and correlations were calculated through Spearman's correlation, linear regression, and logistic regression models. The concentrations of all microbial indicators were positively correlated between produce and hands (ρ range, 0.41 to 0.75; $P < 0.01$). When *E. coli* was present on hands, the handled produce was nine times more likely to contain *E. coli* ($P < 0.05$). Similarly, when coliphage was present on hands, the handled produce was eight times more likely to contain coliphage ($P < 0.05$). There were relatively low concentrations of indicators in soil and water samples, and a few sporadic significant associations were observed between contamination of soil and water and contamination of produce. This methodology provides a foundation for future field studies, and results highlight the need for interventions surrounding farmworker hygiene and sanitation to reduce microbial contamination of farmworkers' hands.

IMPORTANCE This study of the relationships between microbes on produce and in the farm environment can be used to support the design of targeted interventions to prevent or reduce microbial contamination of fresh produce with associated reductions in foodborne illness.

KEYWORDS environmental microbiology, food-borne pathogens, produce

Address correspondence to Juan S. Leon, juan.leon@emory.edu.

* Present address: Faith E. Bartz, U.S. Agency for International Development, Addis Ababa, Ethiopia; Jacquelyn Sunshine Lickness, Centers for Disease Control and Prevention, Atlanta, Georgia, USA.

F. E. Bartz and J. S. Lickness contributed equally to this work.

F resh produce-related foodborne illnesses and outbreaks have increased over the past few decades (1, 2). Although numerous fresh produce types may be contaminated with foodborne pathogens, certain produce types are more frequently impli-

TABLE 1 Percentages of produce farm samples for which evidence of microbial contamination could be found

Sample type	Coliforms		E. coli		Enterococcus		Coliphage	
	No. of samples	No. (%) positive	No. of samples	No. (%) positive	No. of samples	No. (%) positive	No. of samples	No. (%) positive
Produce	250	243 (97)	254	69 (27)	254	215 (85)	191	159 (83)
Hands	171	164 (96)	171	63 (37)	171	171 (100)	130	85 (65)
Soil	84	80 (95)	85	17 (20)	85	56 (66)	65	22 (34)
Source water	44	42 (95)	51	28 (55)	48	42 (88)	26	12 (46)
Irrigation water	73	68 (93)	76	31 (41)	75	63 (84)	47	21 (45)

cated in foodborne outbreaks, including leafy greens, jalapeños, tomatoes, and melons (3–5).

Outbreak investigations on the farm and in packing facilities suggest that sources of produce contamination may include animal droppings, farmworkers' hands (subsequently simply referred to as "hands"), soil, agricultural water, tools, equipment, and other contact surfaces (6–12). Under laboratory conditions, such contamination events have been demonstrated (13–16). However, these studies have limited public health relevance because outbreak investigations generally lack temporality and the laboratory environment may not reflect natural conditions. Ultimately, there are no comprehensive prospective studies directly linking microbial contamination of fresh produce to that of hands, soil, water, or surfaces in the natural setting of the agricultural production environment. This may be due to logistical constraints in setting up a matched study design (6, 17), resource constraints in acquiring large sample sizes (18), application of the appropriate statistical analyses to account for multivariable factors affecting microbial contamination (6, 19–21), and data variability from diverse microbial distributions on produce and environmental samples (22–24). Because of the low rate of detection of pathogen contamination (25–28), populations of microbial-indicator organisms, including organisms that indicate filth (coliforms) and/or fecal contamination (*Escherichia coli*, *Enterococcus* spp., and somatic coliphage), can be monitored (reviewed in references 29 to 31).

Our previous studies (6, 27, 28) quantified microbial indicators and pathogen contamination among fresh produce and environmental samples during the production chain. However, neither these studies nor any others to date have quantitatively compared the direct impacts of microbial contamination of hands, soil, water, or surfaces on fresh produce in the agricultural environment. Thus, the goal of this study was to use indicator organisms to quantify the relationship between microbial contamination on hands, soil, and water and microbial contamination on fresh produce. This work provides a model for a matched design in longitudinal epidemiological field studies and compares the impacts of specific environmental routes on fresh produce microbial contamination.

RESULTS

The percentages of samples positive for indicators (Table 1) and the concentrations of indicators (Table 2) varied by sample type. The percentages of samples positive for coliforms were over 93% for all sample types and ranged from 66 to 100% for

TABLE 2 Geometric mean microbial indicator concentrations of samples from produce farms

Sample type	Sample unit	Geometric mean microbial indicator concn (95% confidence interval) for:			
		Coliforms (log$_{10}$ CFU)	E. coli (log$_{10}$ CFU)	Enterococcus (log$_{10}$ CFU)	Coliphage (log$_{10}$ MPN)
Produce	Fruit	5.22 (4.96, 5.49)	1.31 (1.06, 1.56)	5.06 (4.76, 5.36)	2.12 (1.85, 2.38)
Hands	Hand	5.72 (5.43, 6.00)	2.40 (2.12, 2.67)	6.53 (6.29, 6.76)	2.13 (1.84, 2.43)
Soil	g	2.52 (2.28, 2.76)	−0.03 (−0.27, 0.21)	1.38 (1.18, 1.59)	−0.39 (−0.62, −0.16)
Source water	100 ml	1.58 (1.28, 1.89)	0.13 (−0.15, 0.41)	0.51 (0.26, 0.76)	1.25 (0.51, 1.98)
Irrigation water	100 ml	1.65 (1.35, 1.95)	−0.09 (−0.29, 0.12)	0.52 (0.25, 0.80)	1.00 (0.54, 1.45)
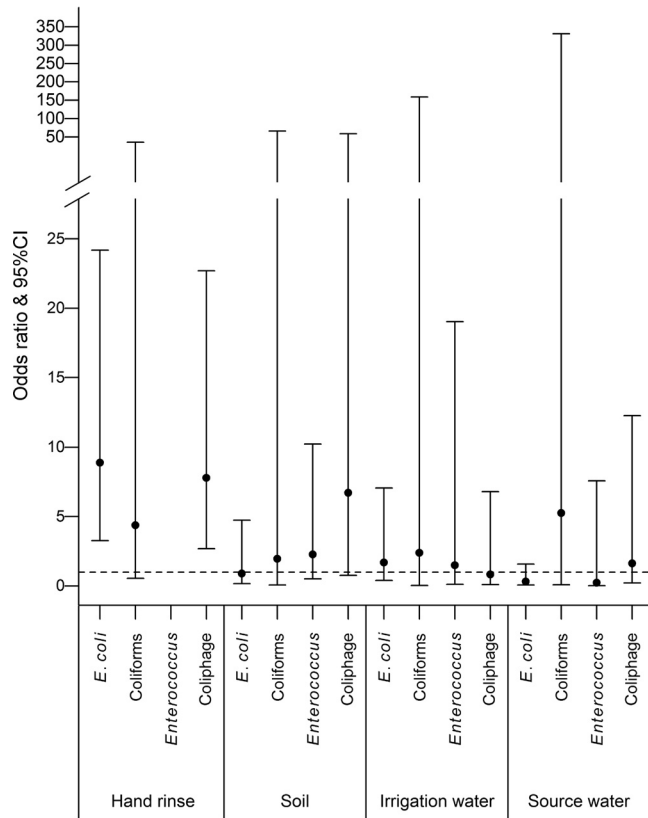
FIG 1 Logistic model odds ratio estimates (dots) and 95% confidence intervals (error bars) represent the odds of the presence of indicators on produce compared to the odds of the presence of the same indicator on matched environmental samples (hand rinse, soil, irrigation, and source water). Models were adjusted for crop type (cantaloupe, jalapeño, and tomato), step in the production process (before harvest, after harvest, distribution, and packing facility), and year of sample collection (2011 or 2012). The dashed line indicates an odds ratio of 1.0. When the 95% confidence intervals cross the dashed line, this indicates a lack of statistical significance (i.e., $P > 0.05$). When both the odds ratio estimate and the 95% confidence intervals are higher than the dashed line, this indicates a positive and significant ($P > 0.05$) relationship. A model was not constructed for *Enterococcus* spp. because this genus was found in 100% of the hand rinse samples.

Enterococcus spp. Percentages of *E. coli*- and somatic-coliphage-positive samples varied from 20 to 83%. For further details on these results stratified by produce commodity and production step, please see reference 28.

The associations between the detection of contamination of hands, soil, and agricultural water with an indicator organism and the detection of produce with the same indicator organism were quantified (Fig. 1). Significant associations between indicators on hands and on produce were observed during the detection of two of the four indicators tested: *E. coli* and coliphage. If *E. coli* was detected on hands, produce harvested by those hands was nine times more likely to contain *E. coli*. Similarly, if coliphage was detected on hands, produce harvested by those hands was eight times more likely to be contaminated with coliphage. No significant relationships were observed between indicators detected in soil, irrigation water, or source water and indicators on produce ($P > 0.05$). In summary, the relative odds of the occurrence of any produce contamination with *E. coli* and coliphage was higher when these indicators were detected on hands, and this was the strongest association observed between produce and other environmental samples.

Similarly, the relationships between the concentrations of an indicator organism on hands, in soil, and in agricultural water and the concentrations of the same indicator organism on produce were quantified (Table 3). Overall, indicator concentrations on hands had a significant positive association with concentrations on produce (Spear-

TABLE 3 Spearman correlation coefficients between microbial indicator concentrations on produce rinses and matched environmental samples from farms producing various types of produce

| Sample type	Produce type[a]	Coliforms		E. coli		Enterococcus		Coliphage	
		n[c]	ρ (P value)	n	ρ (P value)	n	ρ (P value)	n	ρ (P value)
Hands	All	192	0.75 (<.0001)[b]	194	0.59 (<.0001)[b]	194	0.66 (<.0001)[b]	143	0.41 (<.0001)[b]
	Cantaloupe	90	0.76 (<.0001)[b]	90	0.50 (<.0001)[b]	90	0.66 (<.0001)[b]	63	0.21 (0.1021)
	Jalapeño	41	0.64 (<.0001)[b]	43	0.51 (0.0005)[b]	43	0.57 (<.0001)[b]	31	0.25 (0.1770)
	Tomato	61	0.58 (<.0001)[b]	61	0.51 (<.0001)[b]	61	0.35 (0.0053)[b]	49	0.20 (0.1642)
Soil	All	82	−0.08 (0.4909)	84	−0.20 (0.0702)	84	0.06 (0.5775)	62	0.23 (0.0739)
	Cantaloupe	37	−0.36 (0.0283)[b]	37	0.25 (0.1429)	37	0.23 (0.1722)	29	0.05 (0.7799)
	Jalapeño	20	−0.28 (0.2342)	21	0.43 (0.0493)[b]	21	0.01 (0.9712)	14	−0.20 (0.5027)
	Tomato	25	0.28 (0.1801)	26	0.39 (0.0523)	26	0.22 (0.2884)	19	0.09 (0.7307)
Source water	All	75	0.14 (0.2360)	82	−0.22 (0.0526)	79	−0.34 (0.0022)[b]	40	−0.17 (0.2913)
	Cantaloupe	30	0.32 (0.0890)	30	−0.39 (0.0340)[b]	30	0.03 (0.8672)	16	−0.07 (0.7968)
	Jalapeño	21	0.13 (0.5889)	24	−0.02 (0.9161)	23	−0.09 (0.6759)	11	−0.06 (0.8540)
	Tomato	24	−0.17 (0.4419)	28	0.35 (0.0687)	26	−0.11 (0.6084)	13	0.25 (0.4101)
Irrigation water	All	72	0.01 (0.9398)	75	−0.17 (0.1359)	74	−0.14 (0.2284)	46	−0.25 (0.1004)
	Cantaloupe	37	−0.20 (0.2406)	37	−0.37 (0.0236)[b]	37	−0.21 (0.2040)	29	−0.09 (0.6434)
	Jalapeño	14	0.33 (0.2442)	15	0.50 (0.0570)	14	0.06 (0.8393)	7	0.15 (0.7419)
	Tomato	21	−0.23 (0.3076)	23	0.13 (0.5469)	23	−0.21 (0.3321)	10	−0.09 (0.8129)

[a]Indicator concentration units: $\log_{10}$ CFU (bacterial indicators) or most probable number (coliphage) per fruit (produce), hand (hands), gram (soil), or 100 ml (water).
[b]Significant correlation ($\alpha = 0.05$).
[c]n, number of samples.

man's correlation coefficient [ρ] = 0.41 to 0.75, $P < 0.0001$ for all overall comparisons). This significant association was also observed for coliforms, *E. coli*, and *Enterococcus* spp., after stratifying for produce type ($\rho = 0.35$ to 0.76, $P < 0.01$). There were some correlations between indicator concentrations in soil, irrigation water, and source water and indicator concentrations on produce. For example, among cantaloupes, we found significant negative associations for concentrations of *E. coli* organisms between produce and source water ($\rho = -0.39$, $P = 0.03$) and produce and irrigation water ($\rho = -0.37$, $P = 0.02$) and for concentrations of coliforms between produce and soil ($\rho = -0.36$, $P = 0.03$). Similarly, we found a significant negative association between concentrations of *Enterococcus* spp. on produce and in source water overall ($\rho = -0.34$, $P < 0.01$). Interestingly, the association between source water and produce for the concentration of *Enterococcus* spp. was lost when the analysis was stratified by produce type. In general, there were few significant negative correlations between concentrations of indicators in environmental samples (i.e., soil and water) and those on produce, though there was a significant positive correlation between microbial-indicator concentrations on hands and on produce of all types.

To control for potential confounding in the results of the preceding paragraph, multivariable linear regression models were used (see Materials and Methods and Fig. 2). The relationship between concentrations of all four microbial indicators on hands and concentrations of the same microbial indicators on produce remained significant and positive ($\beta = 0.17$ to 0.57, $P < 0.05$) (Fig. 2). Models estimated that for every 1-$\log_{10}$-CFU/hand increase of a given microbial indicator on hands, there was a corresponding increase in that indicator concentration on produce equal to 0.37 $\log_{10}$ CFU/fruit for *E. coli*, 0.55 $\log_{10}$ CFU/fruit for coliforms, 0.57 $\log_{10}$ CFU/fruit for *Enterococcus* spp., and 0.17 $\log_{10}$ CFU/fruit for somatic coliphage. In contrast to results obtained from a correlation analysis that showed a significant negative relationship between *E. coli* in soil and on hands, linear models that adjusted for crop type, year of data collection, and step in the production process showed a significant positive relationship between *E. coli* concentrations in soil and on produce ($\beta = 0.40$, $P < 0.05$). In support of the results of the correlation analysis, linear models found a significant negative association between *E. coli* concentrations on produce and in irrigation water ($\beta = -0.45$, $P < 0.05$). In summary, greater concentrations of microbial indicators on
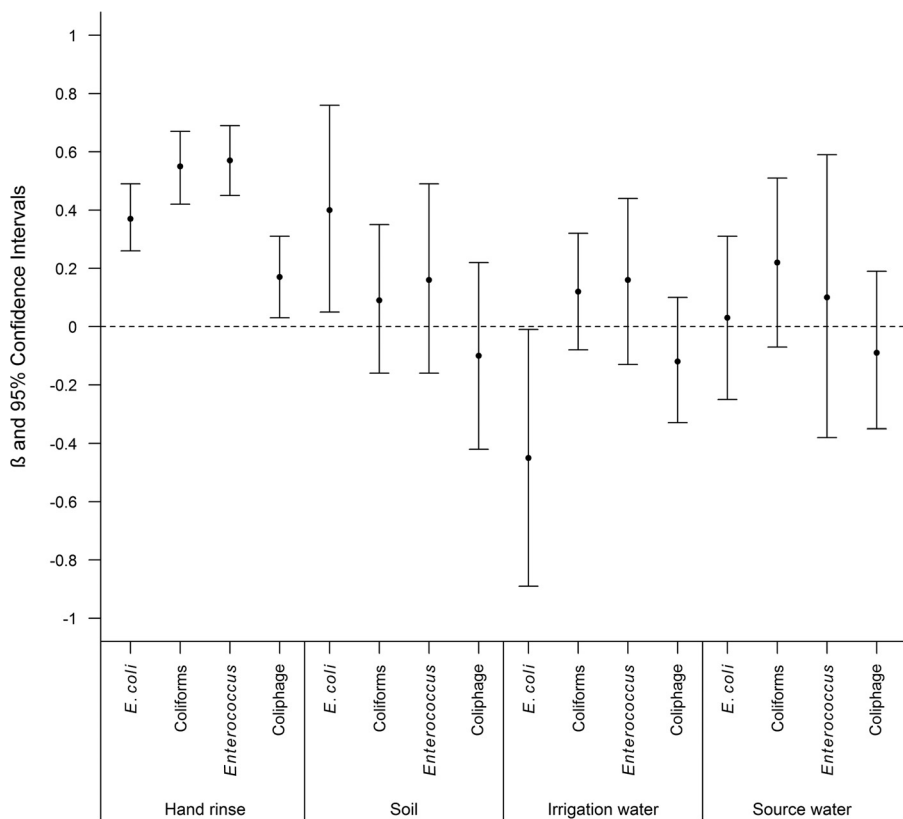
FIG 2 Linear model estimates (β, represented by dots) and 95% confidence intervals (error bars) represent the $\log_{10}$ change of the produce indicator concentration given a 1-$\log_{10}$ increase in the matched environmental indicator concentration (hand rinse, soil, irrigation water, and source water). Models were adjusted for crop type (cantaloupe, jalapeño, and tomato), step in the production process (before harvest, after harvest, distribution, and packing facility), and year of sample collection (2011 or 2012). The dashed line indicates a linear estimate of zero. When the 95% confidence intervals cross the dashed line, this indicates a lack of statistical significance (i.e., $P > 0.05$). When the β and 95% confidence intervals do not cross the dashed line, they indicate a significant and positive (both are higher than the dashed line) or negative (both are lower than the dashed line) relationship.

produce were associated with greater concentrations on hands (across all indicators examined), and high *E. coli* concentrations on produce were associated with high concentrations in soil and low concentrations in irrigation water.

Farm conditions and production practices. Interviewed farm managers reported the total size of each farm (13 to 325 ha), area of each produce type planted (tomato, 5 to 30 ha; jalapeño, 3.5 to 30 ha; cantaloupe, 15 to 65 ha), and information about workers employed (permanent, 6 to 60; seasonal, 10 to 250; literacy rate, 50 to 90%; non-Spanish speakers, up to 20%). Produce was distributed domestically (in northern Mexico) or exported to the United States (three tomato/jalapeño farms and one jalapeño farm were export certified; two cantaloupe farms were undergoing the export certification process).

For 9 of 11 farms, the farm manager reported using drip irrigation with additives (all 9 used fungicides and insecticides; the majority used synthetic fertilizers), with water obtained from deep wells. All reported hand harvesting with no glove use and the majority used knives for cutting stalks, with 5/9 farms reporting field packing, 2/9 using a conveyor belt for truck loading, and 6/9 using on- or off-site packing facilities for further sorting. Of 9 of the 11 farms observed, 5 farms had 1 to 3 bathrooms on site (up to 1.9 miles from the workers). Only 3 of 9 farms always had handwashing stations near bathrooms. Study staff observed that some workers using the bathroom did not use handwashing facilities afterwards. Animals were not raised commercially on or adjacent to the farms, but wild and domestic animals, namely, birds (all farms) and cows, dogs,

and rabbits (one farm) were observed. Because of the limited sample sizes (5 farms/commodity) and similar production practices, an analysis of the effect of different production practices on relationships between produce and environmental samples could not be conducted.

DISCUSSION

The goal of this study was to quantify the relationship between microbial contamination, using indicator organisms, on hands, soil, and water and microbial contamination on fresh produce from farms and packing facilities in northern Mexico.

The most significant finding was the associations between the detection and concentrations of microbial indicators on hands and produce. A high proportion of hand samples were positive for all four microbial indicators. These two observations together suggest that hands are a reservoir of microorganisms and an important vehicle for their transfer to fresh produce during manual production, harvesting, and/or packing (32–34), common practices in produce production in this region (32). Though no other studies to date that we know of have quantified the relationships of microbes between hands and produce in the agricultural environment, this implication is consistent with those of other studies suggesting that hands are an important vehicle of contamination of produce (13, 33–36). We hypothesize that microorganisms measured in our study could have been transferred either from hands to produce or produce to hands, as has been demonstrated to occur (33, 37–39). These findings and our hypothesis led to testing the effectiveness of hand hygiene interventions (soap, hand sanitizer) to improve the microbial quality of hands and produce (33, 34). These intervention trials on farms showed that farm worker hands treated with hand hygiene products had significantly lower microbial levels immediately following hand hygiene treatment. However, 30 min after jalapeño harvest, microbial levels on these hands were no different than those in the no-hygiene control group. This microbial recontamination of hands suggests that contact with produce or environmental surfaces on farms rapidly transfers and recolonizes microbes on hands, despite hand hygiene. Little evidence of correlation was observed in indicator detection and concentration between soil and fresh produce. This is not unexpected, as common regional farming practices (e.g., no nearby animal production facilities, use of synthetic fertilizers rather than compost, and use of plastic mulch), along with the arid growing conditions, likely minimized microbial contamination of soil in this production region (40–42). Although bivariate correlation analysis (Table 3) suggested that there was a significant negative correlation between microbial-indicator concentrations in irrigation and source water and indicator concentrations on produce, this relationship did not remain after adjustment for any linear model, except that for *E. coli* on produce and irrigation water (Fig. 2). The reason for these inconsistent sporadic relationships was unclear but is likely associated with the use of groundwater and perforated hoses for irrigation, both of which reduce contamination risk (43, 44). Although microbial indicators were found in water, levels were below U.S. Food and Drug Administration (FDA) Food Safety Modernization Act (FSMA) Final Rule on Produce Safety standard limits (28, 31, 45); this is consistent with other studies demonstrating that groundwater carries a relatively low risk of pathogen detection, compared to that of surface water (9, 46). Regardless, our results do not support a positive relationship between indicator detection or concentration on produce and that in source (i.e., groundwater) and irrigation (drip) water.

One of the major strengths of this study was its large sample size and diversity of fresh produce items and environmental samples taken from working farms in a relevant production region. The ability to match each produce sample to a corresponding, matched hand sample allowed us to provide compelling evidence supporting a direct relationship between hand and produce indicator organism contamination even across indicator organism types and when measuring the indicator detected or its concentration. The methodology employed here is unique and can be a resource for studies that address similar questions of produce contamination source or that test targeted interventions to prevent or reduce microbial contamination of fresh produce.

The inclusion of data for multiple microbial indicators enabled a robust study despite the varied efficacies of indicators at predicting pathogen risks (47). However, as noted in our previous studies (27, 28), the distributions of these four microbial indicators differed, and these had implications for the statistical analysis. The low rate of detection of *E. coli* was useful for logistic regression analyses. In contrast, the high rate of detection of *Enterococcus* spp. and coliforms hampered logistic models. For example, logistic regression analyses of *Enterococcus* spp. on hands could not be conducted because 100% of hand samples were positive for *Enterococcus* spp. However, the low concentrations and nonnormal distributions of *E. coli* bacteria did not permit construction of informative linear regression models. In this study, microbial-indicator concentration values were imputed for assays with plate counts that fell below the assay limit of detection (LOD) or above the limit of quantitation (LOQ) (48, 49). While this practice maximizes sample size, it assumes a normal distribution. Use of similar imputations in studies with smaller sample sizes would bias the regression estimates (50). The generalizability of these findings is limited as a function of the study region; the findings have greatest applicability to similar agroecologies. Also, as noted in Results, the study farms had similar agricultural practices that did not allow for the analysis of the effect of agricultural practices on the relationships between produce and environmental samples. Finally, correlation does not equate to causality, but a study to determine causality would require incorporation of a temporal component that would be logistically challenging and expensive. In the absence of a causal study, our approach of extensive sampling and rigorous statistical analysis to identify correlations between environmental and produce samples provides a basis for making causal inferences.

Overall, the results of this study showed a strong, significant, and positive association between the detection and concentrations of microbial indicators on the hands of produce harvesters and packers and on the matched produce samples. This suggests a need for hygiene interventions specifically designed for produce farm workers. For example, hygiene behavior can be affected by employer labor policies and food safety training. Employees of study farms were paid by the number of pieces of produce harvested (20% of U.S. farmworkers are paid by the piece), a practice that may discourage workers from taking time away from their activities to use sanitary facilities, especially if distant (51). Furthermore, the majority of workers on study farms were temporary employees, and a transitory workforce can present unique challenges to employers in ensuring that all employees receive adequate food safety training. Lastly, as mentioned, hand hygiene interventions in the agricultural environment (33, 34) result in only a transitory (<30-min) reduction in microbial load. Combined, these challenges require effective hand hygiene interventions to address the unique needs of the agricultural workforce: reduction in microbial load and persistence, accessibility of sanitary facilities, training, and facilitative labor policies to encourage hygiene practices among produce workers.

MATERIALS AND METHODS

Sample collection. The study was conducted from May to December in 2011 and 2012 in the Mexican states of Nuevo León and Coahuila. Eleven farms, three with packing facilities on site, participated; five produced jalapeño peppers (*Capsicum annuum*), five produced tomatoes (*Solanum lycopersicum*) (four of which also produced jalapeños), and five produced cantaloupes (*Cucumis melo* var. *cantalupensis*) (see references 27 and 28 for the study design). Production practices were characterized through farm manager interviews (5/6 tomato or jalapeño farms, 4/5 cantaloupe farms) and structured observations (4/6 tomato or jalapeño farms, 5/5 cantaloupe farms). Institutional review board approval was granted by Emory University (approval no. IRB00035460) and Universidad Autónoma de Nuevo León.

Samples (n = 636) of produce rinses (52), matched hand rinses, soil, and water were collected before harvest, during harvest, during distribution, and at the packing shed (when present), as described previously (28). Each sample corresponded to a specific farm, date, and field or packing facility and consisted of triplicate subsamples that were then composited into a single sample. Composite produce samples represented rinses of 54 tomatoes, 42 jalapeños, or 6 cantaloupes in 1,500 ml of 0.1% peptone water (PW). Composite matched hand rinses were collected after produce handling and represented hand rinses from three workers, each in 750 ml PW. Composite matched soil samples were collected immediately before harvest (21 15-g soil samples) and taken from near the sampled plants. Composite matched water (triplicate 1.5-liter samples) was sampled from the source well pumps (source water) and from irrigation hoses in the field (irrigation water) by disinfecting the point of sample collection with 200-ppm hypochlorite and allowing the water to run for 30 s before collection.

Each composite sample was assayed to measure three bacterial indicators (coliforms, generic *E. coli*, and *Enterococcus*) and one viral indicator (somatic coliphage). Bacterial indicators were assayed by enumerative methods on selective media, and coliphages were detected by using the Fast Phage most probable number (MPN) Quanti-Tray method (Charm Sciences, Lawrence, MA) (27, 28). The concentration of each bacterial indicator per sample was based on the combined replicate plate counts of CFU and effective sample volumes according to a U.S. Food and Drug Administration (FDA) methodology (53). A sample was considered positive for a given bacterial indicator if growth was observed on any plate. The MPN of coliphage was calculated using an IDEXX Quanti-Tray/2000 MPN table (IDEXX Laboratories, Westbrook, ME). A sample was considered positive for coliphage if fluorescence was observed (using an UV light).

The theoretical lower limit of detection (LOD) of the indicator assays was 1 CFU (bacteria) or MPN (coliphage) for the largest effective volume tested. The upper limit of quantification (LOQ) was 250 CFU (bacteria) or 2,420 (MPN for coliphage) for the smallest effective volume tested. The LODs and LOQs for each indicator assay varied by produce and sample type because of sample volume variability. Across all samples, limits ranged from (lower to upper, number of CFU or MPN per ml) 0.004 to 2,500 (coliforms and *E. coli*), 0.004 to 25,000 (*Enterococcus*), and 0.01 to 242 (coliphage). To avoid over- and underrepresentation of sample counts (48, 49), sample concentrations below the LOD were recorded as half the lower LOD, and sample concentrations above the LOQ were recorded as two times the upper LOQ. All microbial-indicator concentration data were adjusted to the number of CFU or MPN per fruit for produce samples, the number of CFU or MPN per hand for hand rinse samples, the number of CFU or MPN per gram for soil samples, and the number of CFU or MPN per 100 ml for water samples. For comparison to studies measuring surface area, rinses sampled a fruit surface area equivalent to approximately 4,500 cm^2/composite sample or 3 cm^2 of fruit surface area/ml PW.

Statistical analyses. Data were analyzed using SAS 9.3 or JMP Pro10 (SAS Institute, Cary, NC). Figures were generated using R v3.1.0 (R Foundation for Statistical Computing, Vienna, Austria). Because indicator concentration data were not normally distributed, they were transformed (log_{10}) for analysis. *P* values of 0.05 or less were considered significant.

The outputs of multivariable logistic regression models (PROC LOGISTIC) were odds ratios and associated 95% confidence intervals (54) that estimated the odds that an indicator was detected on produce when the same indicator was detected on matched environmental samples (e.g., hands, soil, or water). Models were adjusted for potential confounders: produce type, step in the production process, and year of collection. In models where variables had a very low or high rate of detection, the calculation of an odds ratio was not possible; therefore, Firth corrections were used to estimate an odds ratio (55). An odds ratio for the occurrence of *Enterococcus* spp. on hands could not be calculated because 100% of hand rinse samples were positive for *Enterococcus* spp.

Both Spearman's correlation (bivariate) and linear regression models (multivariable, PROC REG) were used to determine the association between the concentrations of an indicator on produce and the concentration of the same indicator in hand rinses, soil, and water. The outputs of Spearman's correlation analyses (56) were correlation coefficients (ρ), which indicated the magnitudes of correlation between 1 (perfect positive correlation), 0 (no correlation), and −1 (perfect negative correlation). The outputs of linear regression models (57) were β coefficients, which indicated the log_{10} change in indicator concentrations on produce given a log_{10} increase of 1 in indicator concentrations in the matched hand, water, or soil sample, adjusted for potential confounders. Linear regression models were adjusted for produce type, step in the production process, and year of collection by including these variables in the models.

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