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Efficacy of Two Hand Hygiene Interventions at Reducing Hand Contamination Among Produce Farmworkers in Northern Mexico

By

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B.A., University of Pittsburgh, 2016

Thesis Committee Chair: Juan S. Leon, PhD, MPH

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ABSTRACT

Efficacy of Two Hand Hygiene Interventions at Reducing Hand Contamination Among Produce Farmworkers in Northern Mexico

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Previous research has shown that produce can become contaminated through contact with farmworkers' hands and that hand-hygiene interventions are important for reducing contamination. It is unknown whether hand-hygiene intervention results are produce-specific. Research exploring other produce commodities is needed to prevent farm-level contamination and reduce the number of U.S. produce-related outbreaks. This study aims to assess hand-hygiene intervention efficacy on melon farms and compare findings to previous research conducted on jalapeño farms.

Two studies assessed the efficacy of two hand-hygiene interventions; one among jalapeño farmworkers and one among melon farmworkers. 129 melon and 159 jalapeño farmworkers in Mexico were randomly assigned to one of three groups: handwashing, two-step alcohol-based hand sanitizer (SaniTwice), or no hand-hygiene (control). After harvesting, hand-hygiene interventions were performed, and hand rinsate samples were collected and tested for soil (absorbance A_{600nm}) and bacterial indicators (coliforms, generic *E. coli*, *Enterococcus* spp., a universal *Bacteroidales* marker (AllBac), and a human-specific *Bacteroidales* marker (BFD). Melon groups were compared using linear and logistic regression models ($\alpha=0.05$) and Tukey's adjustment for multiple comparisons. Studies were compared using two-way, fixed-effects models. Spearman's correlations described outcome measurement relationships. Surveys regarding farmworker perceptions on hand-hygiene were also summarized.

Compared to controls (geomean A_{600nm} 0.138), handwashing (geomean A_{600nm} 0.014; $p<0.0001$) and SaniTwice (geomean A_{600nm} 0.043; $p<0.0001$) interventions yielded significantly lower absorbance levels on melon farmworkers' hands, with the handwashing group having the lowest ($p<0.0001$). Bacterial indicator concentrations on melon farmworkers' hands did not differ across intervention group ($p=0.1238$-0.4168). The efficacy of handwashing and SaniTwice, compared to controls, differed between melon and jalapeño farmworkers; fixed-effects interactions between intervention group and produce type were significant for absorbance ($p=0.0018$), *E. coli* ($p=0.0050$), and coliforms ($p=0.0005$), but not *Enterococcus* spp. ($p=0.2797$). Correlations between outcome measurements ranged from -0.14 to 0.56 for melon data and 0.06 to 0.49 for jalapeño data. Melon farmworker hand-hygiene perceptions varied.

Although handwashing and SaniTwice reduced soil on melon farmworkers' hands after one 30-minute harvest, neither intervention reduced indicator bacteria. The efficacy of handwashing and SaniTwice interventions differed for melon and jalapeño farmworkers, suggesting it may be necessary to develop produce-specific hand-hygiene interventions in agricultural settings.

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I. LITERATURE REVIEW

Foodborne Illness and Outbreak Trends in the U.S.

There is a high incidence of foodborne illness and outbreaks in the U.S. every year. The Centers for Disease Control and Prevention (CDC) estimates that about 48 million people annually in the US get sick, with 128,000 hospitalized, and 3,000 die from a foodborne illness (1). Furthermore, a review published using CDC's Foodborne Outbreak Online Database reports a mean of about 1,000 outbreaks causing 20,000 illnesses each year (2). In fact, certain types of outbreaks, such as fresh produce-related outbreaks, have increased in frequency over time (3).

Produce-Related Outbreak Trends

Fresh produce-related outbreaks are of growing public health concern due to their increasing frequency. From 2004 to 2013, at least 36% of foodborne illnesses were associated with contaminated produce (4). During 1998-2007, the percentage of fresh-produce related outbreaks increased from about 15% to about 23% (3). This increase may be influenced by guidelines proposed by the World Health Organization (WHO) in 1990, that recommend consuming ≥400 g of fruits and vegetables per day (5). Therefore, observed increases in fresh produce-related outbreaks may have been linked to an increase in the consumption of fresh produce based on these recommendations (6, 7). Additionally, the increasing number of produce-related outbreaks that occur annually are disproportionately distributed across commodity type; certain types of produce cause outbreaks more often than others. *Salmonella* outbreaks occur most commonly from melons, tomatoes, and sprouts while *E. coli* outbreaks occur most frequently due to consumption of leafy greens. *Cyclospora* and hepatitis A outbreaks often occur in raspberries and green onion (8, 9). A review of fresh produce-related outbreaks identified from 1973 to 1997 found that of the 85 outbreaks that implicated a single produce item, 89% were linked to just eight produce types with lettuce, melons, and seed sprouts being the most frequent culprits (29%, 15%, and 13% respectively) (7). More recently, of the 114 multistate foodborne outbreaks reported by the CDC from 2006 to 2017, the produce commodities most implicated were sprouts (12 outbreaks), lettuce (7 outbreaks), papaya (5 outbreaks), and melons (4 outbreaks). Tomatoes and jalapeños each only caused one outbreak during this period (9). In general, the frequency of produce-related outbreaks is increasing in the U.S., with lettuce, sprouts, and melons implicated as the most common sources.

One of the many concerns associated with fresh produce-related outbreaks is the impact they can have on local and global economies; economic losses due to produce-related outbreaks have been shown to be substantial (10). It is hard to estimate these losses given that costs incurred from fresh produce-related outbreaks depend on a wide variety of factors including economic climate, size of the outbreak, and commodity type, among others (10, 11). One study calculated the costs incurred on the farm and retail sides of three multistate produce-related outbreaks; a 2006 *E. coli* outbreak linked to bagged spinach, a 2008 outbreak of *Salmonella* attributed to muskmelons, and a 2008 *Salmonella* outbreak thought to be related to tomatoes. The farm side lost about $12 million in spinach sales, about $6 million in muskmelon sales, and $25 million in tomato sales during these outbreaks. The retail side lost $63 million in spinach sales, about $21 million in muskmelon sales, and $89 million in tomato sales during these outbreaks (11). It is clear that a single outbreak alone can have severe economic impact within the produce industry. The economic losses brought about by these three outbreaks impacted not only U.S. commerce, but foreign produce markets as well due to the fact that much of the produce consumed in the U.S. is imported from other countries. Muskmelon importers lost about $24 million over the course of the outbreak (11). It is clear that outbreaks related to both domestic and imported produce commodities can come at a significant cost to many sectors of the economy.

Imported produce plays a significant role in U.S. produce-related outbreaks. It is estimated that 15% of U.S. food is imported, with 80% of seafood, 50% of fresh fruit, and 20% of vegetables being imported (12). Much of this produce comes from Mexico since it is the leading importer of fresh fruits and vegetables for the U.S., according to the United States Department of Agriculture's Economic Research Service (13). The number of foodborne outbreaks in the U.S. traced back to imported foods have been increasing in recent years. A study published in Emerging Infectious Diseases in 2017 reports that the average annual number of foodborne outbreaks related to imported produce increased from one percent between 1996 - 2000 to five percent between 2009 - 2014 (14). This study also analyzed data to identify the most common locations where each outbreak originated. Among the 177 foodborne outbreaks with data on location of origin during the study period, Latin America and the Caribbean were most often involved. Mexico accounted for 42 of the 177 outbreaks alone, the most linked to any implicated country (14). CDC annual reports also suggests that Mexico is a large contributor to produce-related outbreaks in the U.S. In 2017, four (50%) of the multistate outbreaks related to fresh produce identified by the CDC were linked to farms in Mexico. All four outbreaks involved different strains of *Salmonella* and came from separate farms (9). Therefore, imported products, especially commodities imported from Latin America, contribute to the burden of fresh-produce related outbreaks reported in the U.S. annually.

Pathogens Associated with Produce-Related Outbreaks

With a high incidence of foodborne outbreaks in the United States annually, especially those linked to imported produce, it is important to understand the pathogens that cause such outbreaks in order to work towards the improvement of prevention efforts. CDC reports that of all foodborne outbreaks reported from 1998-2008, 45% were caused by bacteria, 45% were caused by viral agents, and 1% were caused by parasites. Among produce-related outbreaks specifically, 30% were caused by bacterial agents, 42% were caused by viral pathogens, and less than 1% were caused by parasites (15). The most common bacterial pathogens found on fresh produce are *Salmonella*, *E. coli* and *L. monocytogenes* (6, 16, 17) while norovirus and hepatitis A virus are the most common viral pathogens associated with fresh produce outbreaks (6, 17, 18, 19). *Cryptosporidium* and *Giardia* are the most common parasitic agents involved in fresh produce contamination, although parasitic infections are relatively uncommon in the U.S (20). Among all produce outbreaks in the US reported from 2004-2012, norovirus was the most common viral agent (59%) and *Salmonella* was the most common bacterial agent (18%) involved (6).

Recent outbreaks associated with these agents have generated frequent media attention. In 2017, there were four major outbreaks of involving four different strains of *Salmonella* linked to papayas grown at four different farms in Mexico (9). Additionally, in 2006, more than 200 people became ill with *E. coli* as a result of contaminated Dole (Dole Food Company, Westlake Village, CA) spinach (21) and in 2017, 130 people contracted norovirus while eating at a Chipotle in Sterling, VA. Finally, in 1990, 217 became ill from norovirus-contaminated fruit salad while on a cruise in Hawaii (22). Overall, it appears that *Salmonella*, *E. coli*, *L. monocytogenes,* and norovirus frequently cause produce-related foodborne outbreaks.

Microbial Indicators for Measuring Agricultural Contamination

Microbial indicators are often used as surrogates for measuring human and animal fecal contamination with pathogenic organisms such as *Salmonella, E. coli,* and norovirus. Testing for individual pathogens is expensive and impractical: there are too many potential pathogens and strains of pathogens that contaminate produce commodities and water sources in agricultural settings (23). As a way to circumvent this limitation, researchers use bacterial indicators to more broadly assess the presence of fecal contamination, as a surrogate for potential pathogenic contamination. Surrogates used to measure fecal contamination have previously included soil contamination as measured by absorbance values 600nm (A_{600nm}), generic *E. coli*, *Enterococcus* spp., coliforms, and *Bacteroidales* markers (24, 25).

Absorbance measures the ability of a substance to absorb light of a specific wavelength and is used to quantify particle density in a liquid sample (26). Indicator bacteria (*E. coli, Enterococci,* and coliforms) are useful in detecting and quantifying bacterial contamination. These indicators are non-pathogenic bacteria naturally found in the human gut, but the greater their presence is, the more likely it is that foodborne illness-causing pathogens are also present (27). Generic *E. coli* is an indicator bacterium that is often measured to quantify potential for pathogenic contamination because it is found in fecal matter from humans and other warm-blooded mammals (28, 29).

Enterococci are also used to measure the potential for pathogenic contamination from human fecal matter, because similarly to many pathogenic bacteria, they survive very well in salt water (29, 30). Coliforms are used to quantify the potential for pathogenic contamination more broadly. Coliforms are often found in human feces, animal manure, soil, and wood and thus, they indicate potential contamination from many possible sources (29, 31). Similarly, *Bacteroidales* markers are a promising method of fecal contamination source tracking because they are specific to the gut of warm-blooded animals and they do not grow in the environment and so they also indicate a potential for pathogenic contamination from human feces (32).

Literature regarding the association of bacterial indicators and pathogen presence is contradictory; some research shows significant correlations between indicators and pathogens while other show no significant correlations (30, 31, 33). However, a 2011 systematic review of this literature concluded that it is possible for indicators to be correlated with pathogen presence when enough data is available for analysis. Indicators cannot detect pathogens with certainty but do signal an increased likelihood of a pathogen being present (33). Researchers have used a combination of these indicators when studying environmental contamination routes (24, 25, 34, 35). Previous research on the failure rate of tests to detect *E. coli* in ocean recreational waters suggests that the strength of the correlation between bacterial indicators is highly dependent on environmental conditions. As a result, Noble et al. recommend the use of multiple indicator tests to detect and quantify contamination (36). Another study by Kinzelman et al., conducted in water from Lake Michigan, found that *E. coli* and *Enterococcus* spp. indicator correlations were low and therefore, were not interchangeable (37). However, other studies testing correlations of these indicator bacteria on produce commodities in agricultural settings found significant correlations between fecal coliforms and *E. coli* (38).

Indicators of fecal contamination have also commonly been used to assess the efficacy of hand hygiene interventions. For example, to quantify the amount of soil present on farmworker hands, two previous studies measured A_{600nm} levels of hand rinsate samples (24, 25). Previous research has also measured *E. coli*, *Enterococcus* spp., and coliform (hereafter referred to as indicator bacteria) concentrations from hand rinsate samples (24, 25, 34, 35, 39). Previous studies examining hand contamination in agricultural settings also tested for the universal and human-specific 16S rDNA markers for *Bacteroidales* using AllBac and BFD primers (25). More research is necessary to understand the relationship between these indicators at detecting contamination in agricultural settings. A better understanding of these measurement tools will help inform and improve produce safety policies aimed at reducing contamination at the agricultural level of the supply chain.

Farm to Fork Produce Contamination

Each step in the produce supply chain, from farm to fork, presents risk for contamination as the produce is subjected to various treatments and surfaces (40, 41). The produce supply chain begins in the field, moves through processing and packaging stage, and then to retail and purchasing. After purchasing, the produce is either prepared and consumed by individuals in their own homes or prepared by retail establishment workers and consumed by their customers (42). Transmission routes are generally categorized into two periods within the overall supply chain: pre-harvesting and post-harvesting. Pre-harvesting includes planting and growth while post-harvesting involves harvesting, processing, shipping, storage, handling, and consumption (40, 41). Pre-harvest contamination results from exposure to animal manure, soil treatments, wild animals and insects in the field, water from irrigation systems or flooding, harvesting equipment, and farmworkers (40, 41). During post-harvesting stages, produce is exposed to pathogens through contact with harvesting equipment, farmworkers' hands, collection bins and boxes, cutting and grating tools, rinse water, improperly sanitized surfaces, faulty or soiled storage equipment, etc. (40).

Agricultural Transmission via Human and Animal Pathogens

Foodborne illness is transmitted via the fecal-oral route. Consuming foods contaminated by human or animal feces leads to the introduction of pathogens to the human body (43). At the agricultural level, foodborne pathogens can attach to produce after contact with fecal matter from animal manure, animals roaming in fields, insects, water, and humans (40, 41). Previous research has shown that frequent application of animal manure or droppings to fields makes it possible for bacterial indicators to survive for two months or longer, increasing the likelihood that produce will become colonized during growth. Insects cause contamination of produce less frequently because fecal coliforms are not part of their normal flora. However, produce contamination from insect vectors can occur when bacteria attach to their hairy exoskeleton and transfer to the produce on which they feed (44). Irrigation water, often obtained from untreated sewage water or river water, can be contaminated with animal or human feces due to open defection. In turn, this water can transfer pathogens to growing produce (40, 41, 44).

Finally, humans are commonly cited as sources of produce contamination due to open defecation in fields and improper hand hygiene (34, 40, 41, 45).

Soil, water, and hands can all contain bacterial indicators and can therefore act as vehicles of pathogen transmission to produce (35). To determine which route of transmission is the most important driver of produce contamination, Heredia et al. conducted a study comparing the microbial profiles of farmworker hands, soil, water, and produce at various stages of production (pre- and post-harvest) on farms in Mexico. The study found that soil and water samples were similar in microbial profile but differed from that of produce and hand samples (35), indicating that hands were most important vehicle for transmission Additionally, when the authors compared contamination levels across production steps, produce and hand rinsate samples from the final farm production step had the highest concentrations and prevalence of microbial indicators, corroborating the findings of previous research (39).

The only other study that has investigated the contribution of hand contamination to produce contamination on farms in Mexico was conducted by Bartz et al. In this study the authors quantified the associations between indicator bacteria found on produce and farmworker hands (34). Results showed a significant positive correlation between all indicators found on produce and on hands. Furthermore, there was a significant association between the presence of two of the four indicators tested (*E. coli* and coliphage) on hands and produce. *E. coli* was nine times more likely to be detected on produce that was harvested by farmworkers whose hand rinsate samples contained *E. coli*. Coliphage was eight times more likely to be detected on produce that was harvested by farmworkers whose hands tested positive for coliphage. In contrast, relationships between indicators found in soil, irrigation water, or source water and indicators found on produce were insignificant. This data supported the hypothesis that hands harbor microorganisms and serve as vehicles for the transmission of these microorganisms to produce during harvesting and processing (34). While many potential routes of fecal contamination have been suggested, farmworker hand contamination plays the most significant role in produce contamination at the farm level.

Interventions to Reduce Produce Contamination

Many different safeguards are put in place at each stage of the supply chain in order to reduce produce contamination. During pre-harvest, some farms employ pellet forms of manure that have been thermally treated during production to decrease the likelihood of harboring harmful pathogens, although more research is necessary to understand its efficacy (40). Additionally, a systematic review of produce contamination prevention literature by Park et al. reports that multiple studies found less contamination from chicken manure than cattle manure (45). Cattle diets intended to reduce pathogens in manure and fences to keep wild animals out of fields have been found ineffective at reducing produce contamination during the pre-harvest stage (40, 45). Interestingly, there is some evidence to suggest that exposure of leafy produce to insects is protective against *E.coli* O157:H7 contamination (45).

Monitoring of irrigation systems should also be performed to detect potential human pathogens and indicator bacteria during pre-harvest (40). In locations where wastewater is used to irrigate fields, the World Health Organization recommends that fecal coliforms not exceed 1000 colony-forming units (46). In-line irrigation treatment systems which treat water immediately before field application are available, but despite their effectiveness at reducing contamination, they are not cost effective or suitable for use in remote locations (40). Many studies have shown an association between the method of irrigation used and the occurrence of produce contamination but there is no consensus regarding which method is most effect (40, 41, 45). Aside from irrigation systems, flood waters also contribute to produce contamination in the fields (40, 45). The U.S. Food and Drug Administration (USFDA) provides a series of recommendations for assessing produce affected by flood waters, categorized by whether or not the edible portion of the food was exposed. The USFDA also recommends crop segregation, avoidance of using harvesting equipment in both flooded and non-flooded fields, use of proper sanitation and protective gear, and 30-foot buffer zones between fields to prevent cross-contamination of crops (47).

During the post-harvesting stage at the farm level, many interventions are aimed at preventing contamination from contact with infected surfaces. To prevent cross-contamination, harvesting tools and machines should be regularly disinfected (40). Similarly, contamination from the soiled hands of farmworkers can be prevented with proper hand hygiene practices, including the use of soap and water and alcohol-based hand sanitizers (ABHS) (24, 25, 34, 40). While the USFDA's Food Safety Modernization Act Proposed Rule for Produce safety recommends that all farm personnel use soap and running water for hand hygiene (48), there is some evidence to suggest that ABHS might be a suitable alternative in locations where access to potable water is limited or nonexistent (25).

Many pathogens can survive at cold storage-level temperatures, but refrigeration has been shown to limit the spread of foodborne pathogens during the post-harvesting transportation, preparation, and storage phases (40, 41). Refrigeration is especially important if the produce commodity has been cut, because the edible portions become exposed to any pathogens living on the rind or outer surfaces of fruits and vegetables (41). Similar to refrigeration, rinsing produce is unlikely to remove pathogens that have already attached themselves to a commodity's surface but it can prevent attachment from occurring (41). Therefore, both of these practices should be used, especially during the preparation stage of the food supply chain. Every point in the supply chain introduces new opportunities for contamination but at the agricultural level, the majority of contamination comes from contact with humans and animals.

Hand Hygiene Practices Domestically and Internationally

The USFDA provides the agricultural industry with evidence-based rules and regulations aimed at reducing the spread of foodborne pathogens across the U.S. On January 26, 2016, the Food Safety Modernization Act's *Final Rule on Produce Safety* went into effect. The current rule requires farmworkers to "use hygienic practices" when working with produce and farms to provide adequate and accessible handwashing facilities (49). This rule applies to U.S. domestic and imported produce. While the *Final Rule on Produce Safety* does not specify what methods should be used to achieve these standards, the USFDA did initially propose including the use of soap and running water as a hand hygiene standard, but does not mention glove use (48). Although the USFDA currently suggests the use of soap and running water as best-practice, it is not required.

There is no universal international standard for regulating hand hygiene practices in the food supply chain. However, many multi-nation organizations and countries have their own rules and regulations regarding the best method of hand hygiene for the food industry. For example, the majority of nations in the Asia-Pacific Economic Cooperation mandate the use of hand hygiene generally, but do not define standard practice for said hand hygiene. There is often no mention of recommendations on details such as water temperature or length of washing (50). The European Food Safety Authority, which governs European Union member states, recommends that produce handlers and consumers use soap, water, and a disposable towel when performing hand hygiene in order to protect from foodborne illness (51). On the other hand, countries such as Canada clearly define what constitutes hand hygiene. In Canada's *Federal/Provincial/Territorial Food Safety Committee (FPTFSC) - Food Retail and Food Services Code*, hand hygiene is defined as vigorous rubbing of hands together with soap for 20 seconds and then rinsing hands with warm, potable water (52). In other countries, regulations on hand hygiene in agricultural settings are inaccessible or hard to find. For example, an internet search of Mexico's food safety regulations and guidelines for agricultural settings yielded no useful results. This does not mean they do not exist, it simply means they may be less widely publicized.

Although handwashing standards are not universal, all produce entering the U.S. must meet the Food Safety and Modernization Act (FSMA) regulations (49). FSMA does not set standards for hand hygiene or recommend the use of protective equipment such as gloves. The USFDA does, however, recommend performing hand hygiene using soap and water (48, 49). There is a wealth of information supporting the efficacy of handwashing with soap and water in food handling and agricultural settings. One study, published by Edmonds et al. in 2012, found that when hands were exposed to chicken broth containing norovirus and ground beef containing *E. coli* (pathogens commonly found in food service settings), handwashing with nonantimicrobial hand soap did significantly reduce pathogen loads (53). Another study published in 2013 found that handwashing with soap and water and ABHS was more effective than handwashing with degreasing cream and ABHS or ABHS alone among poultry catching crews (54). Research also exists regarding the effect of handwashing using soap and water on produce farms, specifically. Two studies published by Fabiszewski de Aceituno et al. investigated the efficacy of different hand hygiene interventions at reducing hand contamination on tomato and jalapeño farms in 2015. Both studies found that hand washing with soap and water removed soil more successfully than both the ABHS intervention and the control groups (24, 25). Additionally, the study conducted on tomato farms found that soap and water removed significantly more indicator bacteria compared to the control group (24). Overall, there is a bounty of evidence to support the use of handwashing with soap and water to reduce hand contamination in agricultural settings. Additionally, the USFDA's *Proposed Rule for Produce Safety* listed handwashing with soap and water as a best practice for farms cultivating produce for U.S. consumption (48). There are some challenges, however, to implementing handwashing in agricultural settings, especially handwashing with soap and water.

Although handwashing with soap and water seems to be a preferred method for hand hygiene, some produce suppliers around the world have limited access to potable running water for hygiene use. Lack of access to such a water supply impedes the ability of farmworkers to properly perform hand hygiene. Additionally, handwashing with soap and water takes time and involves multiple resources including soap, water, paper towels, sinks, and garbage bins. As a result, some researchers have suggested the use of ABHS as a viable alternative to handwashing with soap and water (24, 25, 55). However, only two studies have researched the effect of ABHS on reducing farmworker hand contamination on produce farms, specifically. Fabiszewski de Aceituno et al.'s study of hand hygiene interventions on tomato farms in Northern Mexico found that both ABHS interventions tested removed more soil from farmworker hands than the control group. Additionally, the result indicated that ABHS interventions were just as effective at removing indicator organisms from hands as handwashing using soap and water. The authors concluded that ABHS was an effective alternative to traditional handwashing, even on produce farmworkers' visibly soiled hands (24). The other study conducted on jalapeño farms drew similar conclusions. The two-step ABHS group had significantly lower amounts of soil and indicator bacteria than the control group. Fabiszewski de Aceituno et al. again concluded that the two-step ABHS procedure may be a viable alternative to handwashing with soap and water in locations where access to potable water is limited (25). However, studies testing the efficacy of ABHS methods in more heavily soiled produce, such as melons, have not yet been conducted. There is a need to test these methods on melon farms since melons are shown to have higher levels of baseline contamination, and to compare efficacy results across commodity types using random effects testing.

Aside from access to potable water, another barrier to implementing effective hand hygiene practices in agricultural settings is inherent differences between commodity type and farm environment, which might impact the efficacy of hand-hygiene interventions targeted at reducing contamination. Some produce commodities may be better at harboring microorganisms than others. A more effective hand hygiene method might therefore be necessary for more highly contaminated commodities. For example, there is a wealth of research suggesting that melons are more contaminated than other produce types due to their rope-like rinds. A study by Ukuku et al. explored the relationship between cell surface charges and hydrophobicity to test the ability of *Salmonella*, *E. coli*, and *L. monocytogenes* to attach to cantaloupe rinds and remain attached after rinsing. They found a positive correlation between cell surface charge and hydrophobicity with the strength of bacterial attachment (56). This suggests that the biological properties of cantaloupe melon rinds facilitate bacterial attachment. Another study by Bartz et al. published in 2016 compared somatic coliphages sampled from melons, jalapeños, and tomatoes at four stages in the farm production chain. Cantaloupe samples contained more coliphages than either jalapeños or tomatoes, suggesting that melons harbor more microorganisms than the other two produce commodities (57). Because melons may harbor more soil and microorganisms than other produce commodities, it would be helpful to test the efficacy of previously validated hand hygiene interventions at reducing hand contamination among farmworkers who harvest melons. To date, there have not been any studies directly assessing differences in efficacy of hand hygiene interventions at reducing farmworker hand contamination between melon and jalapeño farms.

Farmworkers' perceptions of handwashing interventions may also prove challenging to the implementation of good hygiene practices. Compliance with key guidelines and regulations may be influenced by perceptions on the perceived benefits and pitfalls, such as the time not spent working, of carrying out these hand-hygiene procedures. Moreover, compliance may be impacted by lack of farmworker knowledge on why hand washing is important. Several studies show that overall, farmworkers seem to appreciate the use of hand hygiene practices in agricultural settings. One study was conducted in Wales in 2014 and provided chicken farmworkers with access to pamphlets containing advice on various biosecurity practices. Almost all participants agreed that biosecurity influences foodborne illness rates and the hand hygiene advice card was among the most used pamphlets (58). Another observational study published by Odo et al. in 2015 found that hand hygiene was the most commonly observed farmworker hygiene practice on farms in the middle-western U.S. and Thailand (59). Additionally, Fabiszewski de Aceituno et al. surveyed farmworkers who harvest jalapeños to understand how they thought hand hygiene influenced their health, the quality of the produce, and the time it took to perform their jobs. Of those surveyed, 96% wished to continue performing hand hygiene and 81% felt that hand hygiene improved the quality of the product as well as farmworker health (25). However, possible barriers to performance may include perceptions on how hand hygiene measures affect work time, which method is preferred, and how many times per day hands should be washed. When asked how hand hygiene would affect their work, 42% of respondents from this study population felt that it would not affect their work, while 10% thought it would worsen their work. Responses to which intervention they preferred were diverse; 38% preferring handwashing and 33% preferring ABHS methods. Less than half of respondents claimed that they could perform hand hygiene 2-4 times per day (25). Therefore, farmworkers may see value in hand hygiene, but barriers such as time lost, conflicting preferred methods, and feasibility may prove difficult in achieving improved compliance.

Study Goals and Aims

There is a need to identify effective alternatives to handwashing with soap and water in locations where access to potable water is limited, such as in Mexico, and to determine whether produce-specific hand hygiene interventions are needed to prevent produce contamination during harvesting by farmworkers in Mexico. The goal of this study is to compare the efficacy of two hand hygiene interventions at reducing farmworker hand contamination on melon farms in Northern Mexico in 2014 and to compare these results to a similar study conducted among jalapeño farmworkers in Northern Mexico in 2013. Four specific aims were developed to address this goal. The first aim is to quantify hand contamination among farmworkers harvesting melons after one harvest cycle and to compare these values between those who used soap and water, those who used a two-step ABHS procedure, and those who did not perform any hand hygiene. The second of these aims is to assess any relationship between the presence of soil, indicator bacteria, and *Bacteroidales* overall and within each of the three hand hygiene groups. Third, this study aims to assess whether differences in the efficacy of each hand hygiene intervention exist between farmworkers on melon and jalapeño farms. Finally, this study aims to analyze any trends in the perceptions melon farmworkers hold related to the two hand hygiene interventions.

Significance

While research exists regarding the use of alternatives to handwashing with soap and water, studies have not yet been conducted on melon farms. Because previous research suggests that melons harbor more bacteria than other types of produce, testing alternative hand hygiene solutions on melon farms could determine whether produce-specific interventions are necessary or support the use of existing alternatives in locations where access to potable water is lacking. This information could be used to improve farm-level hygiene practices related to produce harvesting and provide the USFDA with guidance on how best to modify the FSMA produce rule. With more comprehensive guidelines for hand hygiene in agricultural settings, farmworker training could lead to an increase in compliance. The improvement of farm-level hygiene practices and compliance could reduce contamination of the produce that is available for consumption in the U.S. and could therefore reduce the burden of produce-related, foodborne illness. In turn, healthcare and economic costs associated with such illnesses and outbreaks could be reduced.

II. MANUSCRIPT

Title: Efficacy of Two Hand Hygiene Interventions at Reducing Hand Contamination Among Produce Farmworkers in Northern Mexico

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ROLES AND CONTRIBUTIONS

I, Molly Nace, was responsible for developing an analysis plan for the existing study data, performing that analysis, and writing this manuscript in its entirety after completing a review of relevant literature. As Committee Member, Dr. Prince-Guerra reviewed drafts of each section and provided feedback regarding content, grammar, clarity, and organizational structure. As Committee Chair, Dr. Leon granted me permission to access existing study data and reviewed drafts to provide feedback regarding content, grammar, clarity, and organizational structure. Drs. Leon and Prince-Guerra were responsible for signing off on the completion of this thesis.

ABSTRACT

Previous research has shown that produce can become contaminated through contact with farmworkers' hands and that hand-hygiene interventions are important for reducing contamination. It is unknown whether hand-hygiene intervention results are produce-specific. Research exploring other produce commodities is needed to prevent farm-level contamination and reduce the number of U.S. produce-related outbreaks. This study aims to assess hand-hygiene intervention efficacy on melon farms and compare findings to previous research conducted on jalapeño farms.

Two studies assessed the efficacy of two hand-hygiene interventions; one among jalapeño farmworkers and one among melon farmworkers. 129 melon and 159 jalapeño

farmworkers in Mexico were randomly assigned to one of three groups: handwashing, two-step alcohol-based hand sanitizer (SaniTwice), or no hand-hygiene (control). After harvesting, hand-hygiene interventions were performed, and hand rinsate samples were collected and tested for soil (absorbance A_{600nm}) and bacterial indicators (coliforms, generic *E. coli*, *Enterococcus* spp., a universal *Bacteroidales* marker (AllBac), and a human-specific *Bacteroidales* marker (BFD)). Melon groups were compared using linear and logistic regression models ($\alpha=0.05$) and Tukey's adjustment for multiple comparisons. Studies were compared using two-way, fixed-effects models. Spearman's correlations described outcome measurement relationships. Surveys regarding farmworker perceptions on hand-hygiene were also summarized.

Compared to controls (geomean A_{600nm} 0.138), handwashing (geomean A_{600nm} 0.014; $p<0.0001$) and SaniTwice (geomean A_{600nm} 0.043; $p<0.0001$) interventions yielded significantly lower absorbance levels on melon farmworkers' hands, with the handwashing group having the lowest ($p<0.0001$). Bacterial indicator concentrations on melon farmworkers' hands did not differ across intervention group ($p=0.1238$-0.4168). The efficacy of handwashing and SaniTwice, compared to controls, differed between melon and jalapeño farmworkers; fixed-effects interactions between intervention group and produce type were significant for absorbance ($p=0.0018$), *E. coli* ($p=0.0050$), and coliforms ($p=0.0005$), but not *Enterococcus* spp. ($p=0.2797$). Correlations between outcome measurements ranged from -0.14 to 0.56 for melon data and 0.06 to 0.49 for jalapeño data. Melon farmworker hand-hygiene perceptions varied.

Although handwashing and SaniTwice reduced soil on melon farmworkers' hands after one 30-minute harvest, neither intervention reduced indicator bacteria. The efficacy of handwashing and SaniTwice interventions differed for melon and jalapeño farmworkers, suggesting it may be necessary to develop produce-specific hand-hygiene interventions in agricultural settings.

INTRODUCTION

Produce-Related Outbreaks and Hand Contamination

There are a large number of produce-related foodborne outbreaks in the U.S. annually (3). An increase in produce consumption has led to an observable increase in produce-related foodborne outbreaks from roughly 15% in 1998 to 23% in 2007 (3). The pathogens that cause these outbreaks are transmitted via the fecal-oral route (43). Produce contamination, specifically, occurs through exposure to animal feces from manure and droppings, exposure to contaminated irrigation and flood waters, and exposure to pathogens from human hands and open defecation (40, 41). In agricultural settings, produce contamination from farmworker hands may be the most important transmission route. Our group previously published two studies exploring the most influential routes of produce contamination (34, 35). Both studies found that the levels of soil and bacteria present on farmworkers' hands were associated with the levels found on produce, implicating hands as the vehicle of transmission (34, 35). As such, effective hand hygiene practices may reduce produce contamination, thereby decreasing the consumer's risk of developing a foodborne illness. To this end, the U.S. Food and Drug Administration (USFDA) includes hand hygiene in their *Final Rule on Produce Safety* in the 2016 *Food Safety and Modernization Act* (FSMA).

The FSMA rule states that all farms that harvest produce for U.S. consumption, both domestically and internationally, must use hand hygiene practices, such as washing and drying hands regularly, when handling produce (49). The use of gloves is not included in this recommendation. The rule also requires that staff notify supervisors if they are ill with a communicable illness that could contaminate produce or surfaces and that staff be trained on the importance of health and hygienic (49). While the FSMA rule does not specify a standard protocol for hand hygiene, the use of soap and water has been suggested as a best practice (48). Previous studies have reported that handwashing with soap and water significantly reduces soil and bacterial indicators on hands (60, 61, 62). Although existing research supports the use of handwashing with soap and water to reduce farmworker hand contamination, performing this method is not always feasible. Many locations, especially farms outside of the U.S., have little to no access to potable, running water for handwashing purposes. Therefore, it is necessary to identify effective alternatives to handwashing with soap and water that will reduce farmworker hand contamination, and in turn, fresh produce contamination.

Alternatives to Handwashing with Soap and Water

Much of the research exploring effective alternatives to handwashing with soap and water has been conducted in healthcare settings, but may not apply to agricultural settings. Healthcare providers use gloves more often than farmworkers, so baseline hand contamination among providers may be lower than that of farmworkers. As a result, more literature regarding the efficacy of such practices in agricultural settings is necessary. Only two studies testing the efficacy of alternative approaches to hand hygiene on produce farms could be identified, both of which were published by Fabiszewski de Aceituno et al. (24, 25). The first study tested the efficacy of two alcohol-based hand sanitizer (ABHS) methods at reducing hand contamination among farmworkers on tomato farms in Northern Mexico (24). This study found that ABHS interventions significantly reduced soil and bacterial contamination on farmworker hands (24). The second study tested the efficacy of a two-step ABHS method among farmworkers on jalapeño farms (25). The two-step ABHS intervention effectively removed soil and bacteria from farmworkers' hands (25). In both studies, researchers concluded that ABHS interventions are an efficacious alternative to handwashing with soap and water among produce handlers (24, 25). More evidence supporting the efficacy of handwashing alternatives at reducing farmworker hand contamination on produce farms is necessary in order to prevent produce contamination in locations where access to potable, running water is limited. This research may also be able to inform decisions on which outcomes are best for measuring intervention efficacy.

Measurements of Hand Contamination

Contamination in agricultural environments is measured using many different indicators. Researchers can assess outcomes such as absorbance level (soil), bacterial indicator concentrations (fecal contamination), and *Bacteroidales* marker concentrations (fecal contamination) (23). Researchers commonly debate whether testing for multiple outcomes is necessary, or if one measure could adequately detect and quantify contamination (63). Previous studies have shown that some bacterial indicators underperform at detecting potential pathogenic contamination and that indicators are not always predictive of one another in agricultural environments (64, 65). These studies suggest that indicators of fecal contamination perform differently depending on environment and that the use of one single indicator to test for fecal contamination may not be sufficient. However, other studies conducted in agricultural settings sampling produce surfaces have reported significant positive correlations between fecal coliforms and *E. coli* (38, 66). Even if these indicators are predictive of one another, they are not indicative of pathogenic contamination. Presence of indicator variables suggest a higher possibility for pathogenic contamination, but they do not confirm pathogenic contamination (27, 64). Evidence also exists to suggest that visible soil can predict turbidity and absorbance levels, but that it does not predict indicator bacteria levels (67). Given the contrasting findings regarding the relationship between bacterial indicators, and due to the limited research on the correlation of indicators on farmworker hands, more research is necessary to understand whether multiple outcome measures are necessary in assessing hand contamination and whether hand hygiene interventions equally reduce all indicators. If multiple outcome measures are not necessary for assessing contamination, extended research could support the use of one single indicator in best assessing hand hygiene interventions. Furthermore, indicator correlations have been tested on a limited number of produce commodities. Thus, research sampling different produce commodities could prove useful in determining whether produce commodity type influences the correlation of these outcomes. Correlations between outcome variables may not be the only findings influenced by produce type. The efficacy of hand hygiene interventions at reducing hand contamination may also differ by produce commodity.

Efficacy of Hand Hygiene Interventions by Produce Type

The efficacy of hand hygiene interventions may depend on the produce commodity being tested. Existing research suggests that some produce commodities are more likely to harbor pathogens than others are. Prior studies have shown that the surface characteristics of cantaloupe melons provide pathogens with excellent conditions for attachment and survival. Ukuku et al. explored the role of cell surface charge and hydrophobicity in enabling *Salmonella*, *E. coli*, and *L. monocytogenes* to attach to cantaloupe melon rinds and to remain attached after rinsing with potable water. Both cell surface charge and hydrophobicity showed significant correlation with the strength of bacterial attachment (56). This suggests that rind properties of melons facilitate pathogenic attachment. Another study by Bartz et al. found that compared to tomatoes and jalapeños, melons harbored more somatic coliphages on produce farms in Northern Mexico (57). In fact, because melons are so often contaminated with foodborne pathogens, the USFDA released specific hygiene guidelines for melon production as part of the FSMA (68). If melons truly harbor more pathogens than other produce commodities, stronger, more effective hand hygiene interventions may be necessary to prevent melon contamination. Currently, there have not been any studies that have tested the efficacy of alternatives to handwashing with soap and water on melon, therefore there is a need to test alternative hand-hygiene interventions, such as ABHS methods, among farmworkers who harvest melons. Results could either verify previous findings regarding the efficacy of such practices or suggest a need for produce-specific hand hygiene interventions. In addition to efficacy testing, understanding farmworker perceptions on hand hygiene is also important in assessing the feasibility of implementing alternatives to handwashing with soap and water in agricultural settings.

Farmworker Perceptions of Hand Hygiene

Farmworkers' perceptions of hand hygiene play a large role in the ability of a farm to implement effective hand hygiene procedures. Hand hygiene techniques shown to reduce farmworker hand contamination are not useful unless staff are willing and able to correctly follow the designated procedures. Previous surveys of produce farmworkers' perceptions of hand hygiene practices are limited. Multiple studies have demonstrated that farmworkers in general tend to appreciate hand hygiene (25, 58, 59). However, some farmworkers believe that hand hygiene increases work time and there is limited to no consensus regarding which methods are preferred (25). Such barriers necessitate the conduction of more surveys in order to understand different populations' perceptions of hand hygiene and to understand how compliance is affected by these barriers.

Study Goals

To address these needs, the goal of this study is to compare the efficacy of two hand hygiene interventions at reducing hand contamination among farmworkers harvesting melons and to compare these results with those of a similar study conducted among farmworkers harvesting jalapeños on farms in Northern Mexico. Presence and concentration of contamination indicators were compared between hand rinsate samples from three farmworker intervention groups; those performing handwashing with soap and water, those performing a two-step ABHS method, and those performing no hand hygiene practices. These results were then compared to those found during a previous study conducted among farmworkers on jalapeño farms to detect potential differences in efficacy by produce type. Both the handwashing and two-step ABHS groups significantly reduced soil levels on melon farmworkers' hands compared to the control group but did not reduce bacterial indicator or *Bacteroidales* presence or concentrations. The efficacy of these interventions differed significantly between farmworkers harvesting melons and farmworkers harvesting jalapeños, suggesting that commodity-specific hand hygiene interventions may need to be developed for use in agricultural settings.

MATERIALS AND METHODS

Study Location and Participants

Data used for analysis were collected from two melon farms (Santa Paulina and Santa Elena) and three jalapeño farms (La Bolsa, La Pila, and General Trevino) in Nuevo Leon, Mexico from 2013 to 2014. Results regarding the efficacy of hand hygiene interventions among farmworkers on jalapeño farms have been previously published (25). However, data from that study have been utilized for comparison of contamination levels and in fixed-effects models. Enrollment of 129 study participants from the melon farms and 120 from the jalapeño farms occurred over six non-consecutive days. To be eligible for participation, individuals were required to work for one of the four participating farms, have been assigned to harvesting either jalapeños or melons, and give oral consent for participation. Before enrollment, the study was explained to potential participants, and consent obtained. Study protocols were reviewed and approved by the Institutional Review Boards of Emory University in Atlanta, GA and Universidad Autónoma de Nuevo Leon in Monterrey, Mexico (IRB#00035460). Permission to access the available data was granted by Dr. Juan Leon, Emory University.

Study Design

At the start of the melon study, participants were randomly assigned to one of three groups; handwashing with soap and water (handwashing), two-step ABHS (SaniTwice), or no hand hygiene (control). Participants on jalapeño farms were randomized to one of five groups; those who performed handwashing with soap and water immediately after harvesting, those who performed SaniTwice immediately after harvesting, those who performed no hand hygiene (control), those who performed handwashing with soap and water before harvesting, and those who performed SaniTwice before harvesting. Only the first three of these groups were included in the analysis presented here.

First, the handwashing and SaniTwice groups were trained to execute their designated procedure by watching a demonstration and completing a trial performance under the supervision of study staff. Then, after one harvest cycle (duration ~30 minutes), hand hygiene groups completed their assigned intervention and provided study staff with hand rinsate samples. Descriptions of each hand-hygiene group is detailed below.

Handwashing Group

Handwashing was performed as previously described (25). Members of the handwashing group rinsed their hands under potable, room-temperature, water and rubbed 2 mL of non-antimicrobial foaming hand soap (GOJO® Green Certified Foam Hand Cleanser, GOJO Industries, Akron, OH)) into their hands for 20 seconds. They then rinsed their hands again with potable water and dried them off with a single-use paper towel. Water used for cleansing hands was previously tested and found to be free of *E. coli*, *Enterococcus* spp., and coliforms.

SaniTwice Group

Two-step ABHS was performed as previously described (25). SaniTwice participants were given 3 mL of ABHS (disinfectant gel, active ingredient 70% ethyl alcohol, Desinfectantes y Aromatizantes, S.A., Monterrey, Mexico) and participants rubbed their hands together for 15 seconds. Excess ABHS was removed with a single-use paper towel. Finally, an additional 1.5 mL of ABHS was dispensed and participants rubbed their hands together again, this time until dry.

Control Group

The control group did not receive any hand hygiene training. Their hands were sampled directly after the 30-minute harvest cycle without performing any hand hygiene interventions.

Hand Rinsate Sample Collection and Testing

For all groups, hand rinsate samples were collected as previously described (25). Participants placed one hand in a Whirl-Pak bag, which contained 750 mL of sterile 0.1% peptone water. Workers then stirred the contents of the bag with their hand for 30 seconds, with a study member massaging their hand in the bag for an additional 30 seconds to remove any excess material from the fingers. The procedure was repeated for the other hand, using the same Whirl Pak bag. All samples were transported to the study lab at Universidad Autónoma de Nuevo Leon and samples were stored at 4ºC until further analysis. Along with hand rinsate samples, study staff recorded demographic information (age, gender, harvest time) for each participant and invited participants to complete an optional survey described below. All study participants were compensated for their time with a cold beverage and a t-shirt or baseball cap.

Hand Rinsate Testing

Hand rinsate samples were processed as previously described within 24 hours of collection (25). Prior to testing, all samples were inverted multiple times to resuspend all particulates. An aliquot of each hand rinsate sample was tested for absorbance at 600nm using a spectrophotometer (Sequoia Turner, Mountain View, CA). A second aliquot from each of the hand rinsate sample was sent overnight on ice to North Carolina State University's Department of Food, Bioprocessing, and Nutritional Sciences to test for the presence and concentration of AllBac and BFD *Bacteroidales* as described (32). The remaining sample was processed for the presence and concentration of *E. coli*, *Enterococcus* spp., and coliforms as previously described (69). Samples were filtered and processed as previously discussed, using a 0.45 μm pore size cellulose filter (EMD Millipore Corporation, Billerica, MA) and a vacuum-manifold filtration system (Pall Corporation, Port Washington, NY) (25). Each effective volume of rinsate samples was filtered through a duplicate membrane and then membranes were placed on individual Petri dishes containing solidified agar. For the study conducted on jalapeño farms, effective volumes ranged from 0.01 mL to 50 mL and limits of detection were 0.01 CFU per mL and 50,000 CFU per mL (25). For the study conducted on melon farms, effective volumes ranged from 0.01 mL to 1 mL and limits of detection were 0.5 CFU per mL and 500,000 CFU per mL. Remaining rinsate samples were stored at 4°C for no more than 72 hours and reprocessed if necessary.

Participant Survey

The optional participant survey addressed perceived benefits and barriers to each intervention, intention to continue performing hand hygiene, and suggestions for improvement. Among melon farmworkers, 39 of the 129 participants completed an optional survey.

Data Entry

A Microsoft Excel spreadsheet (Microsoft, Seattle, Washington) was created in which demographic information and laboratory results from each participant in the melon study was manually entered by two independent individuals. After the double data entry, all observations were checked against original forms by a third party and discrepancies were reconciled. Data utilized from the jalapeño study was obtained from an existing excel spreadsheet that had gone through the same data quality checks as above (25). The two data sets were later merged using SAS 9.4 (SAS Institute Inc., Cary, NC) to allow for comparisons between the studies. Survey data collected from farmworkers harvesting melons was extracted from original forms and entered into a separate Excel spreadsheet (Microsoft, Seattle, Washington). Personal identifying information was removed for each participant and replaced by a unique study identification number in all databases.

Statistical Analysis

All statistical tests were carried out using SAS 9.4 (SAS Institute Inc., Cary, NC). Shapiro-Wilk tests (70) showed that the age of participants, absorbance of hand rinsate samples, CFU per hand concentrations of indicator bacteria, and GEC per hand concentrations of *Bacteroidales* were non-normally distributed (data not shown). Therefore, these values were $\log_{10}$-transformed when running linear and logistic regression models. Geometric mean and standard deviation are used to describe non-normally distributed data. Kruskal-Wallis testing (71) showed that age was not equally distributed across intervention groups among farmworkers harvesting melons (Table 1). Therefore, statistical models that controlled for the age variable were used. Pearson's chi-square tests (72) revealed that gender and farm location did not differ across intervention group and therefore, were not controlled for during the modeling stage (Table 1).

Linear regression models (73) controlling for age were used to identify significant differences in absorbance, indicator bacteria, and *Bacteroidales* concentrations across intervention group. Logistic regression models (74) controlling for age were used to identify significant differences in the proportion of samples positive for indicator bacteria and *Bacteroidales*. Because none of the control group rinsate samples were positive for *E. coli*, Firth's correction was used to make comparisons between this group and the other two intervention groups. Statistically significant correlations between absorbance, indicator bacteria, and *Bacteroidales* concentrations for both the melon and jalapeño study data were identified using Spearman's partial correlation (75), controlling for age. Correlations were then classified by the strength of association (Spearman's rho value) using four categories; very weak (0.00 to 0.09), weak (0.10 to 0.29), moderate (0.30 to 0.49), and strong (0.50 to 1.00). This scale was chosen because of its previous use in assessing correlations between variables across multiple disciplines (76, 77). Linear regression models controlling for farm location, gender, harvest time, and age were also used to assess baseline differences in absorbance and indicator bacteria among melon and jalapeño farmworker controls. Farm location, gender, and harvest time were controlled for here to adjust for differences between the two study populations. *Bacteroidales* concentrations were not compared between melon and jalapeño farmworkers due to lack of access to that data among farmworkers harvesting jalapeños. Finally, two-way fixed-effects ANOVAs (78) were used to identify significant interactions between intervention group and produce type for absorbance and indicator bacteria concentrations. Proc GLM in SAS 9.4 (SAS Institute Inc., Cary, NC) was used to depict graphs of interaction between intervention group and produce type. Significant differences were identified using an alpha level of 0.05 and Tukey's procedure (79) was used to correct for multiple comparisons made during linear and logistic regression.

RESULTS

Participant Demographics

The goal of this study was to compare the efficacy of two hand hygiene interventions at reducing soil and indicator contamination on melon farmworkers' hands in Mexico and to determine if intervention efficacy differs between melon and jalapeño farmworkers. Of the 129 participants from the melon farms, 42 produce farmworkers were assigned to the handwashing group, 45 were assigned to the SaniTwice group, and 42 were assigned to the control group. Among farmworkers harvesting jalapeños, 40 participants were assigned to each of the three intervention groups. Among farmworkers harvesting melons, 90% were male, 93% worked at Santa Paulina (as opposed to Santa Elena), and geometric mean age was 29 years old. Gender and farm location did not differ significantly across intervention groups (Table 1). However, age differed significantly across intervention groups and therefore, the age variable was control for in subsequent analyses.

Effect of Hygiene Interventions on Soil Contamination on Melon Farmworker Hands

Absorbance (A_{600nm}) of hand rinsate samples was measured as a proxy to quantify the level of soil contamination on farmworkers' hands. The control group had the highest geometric mean absorbance of all three groups, indicating that those individuals had the greatest amount of soil contamination on their hands (Figure 1 and Table 2). The handwashing group had the lowest geometric mean absorbance of the three groups (A_{600nm} = 0.01), differing significantly from both the control (A_{600nm} = 0.14, $p<0.0001$) and SaniTwice (A_{600nm} = 0.04, $p<0.0001$) groups. The SaniTwice group also had a significantly lower geometric mean absorbance than the control group ($p < 0.0001$). These findings indicate that both interventions significantly reduce soil contamination on the hands of farmworkers harvesting melons.

Effect of Interventions on Indicator Bacteria Concentrations Among Farmworkers Harvesting Melons

Presence of soil does not necessarily indicate the presence of bacteria or pathogenic organisms. As such, hand rinsate samples from farmworkers harvesting melons were tested for concentrations of *E. coli*, *Enterococcus* spp., and coliforms in order to quantify potential bacterial contamination (32). The presence and concentrations of *E. coli*, *Enterococcus* spp., and coliforms from hand rinsate samples were compared across all intervention groups (Table 2a and 2b). Table 2a presents the proportions of samples positive for each indicator by melon study intervention group. *E. coli* bacteria were only present in 10% of handwashing and 2% of the SaniTwice group samples (none of the control samples contained *E. coli*). However, *Enterococcus* spp. was found in the majority of samples; 120 of the 129 samples (93%) collected from farmworkers harvesting melons contained *Enterococcus* spp. Coliform bacteria were present in 67% of control, 62% of handwashing, and 42% of SaniTwice samples. Table 2b presents the mean concentrations of each indicator by melon study intervention group. None of the proportions of samples positive for or concentrations of indicator bacteria differed significantly across any of the hand hygiene groups ($p = 0.1238 – 0.9992$). This suggests that neither handwashing with soap and water nor the ABHS method tested here reduced indicator bacteria on the hands of farmworkers harvesting melons. These results were in contrast to the previously published study on jalapeño farms (25).

Bacteroidales were also tested for, in addition to bacterial indicators, because this indicator is perhaps a better indicator of fecal contamination. *E. coli* measures are indicative of quality or hygiene problems whereas presence of *Bacteroidales* can indicate potential fecal contamination. Previous research showed no correlation between these measures (32). In addition to quantifying hand contamination using bacterial indicators, hand rinsates were tested by qPCR to determine the concentration of AllBac and BFD *Bacteroidales* concentrations (Table 2b). Table 2a also presents the proportion of samples positive for the *Bacteroidales* by melon study intervention group. Testing of AllBac and BFD *Bacteroidales* was completed for 128 of the 129 hand rinsate samples collected from farmworkers harvesting melons. The universal AllBac marker was detected in 67% (86/128) of all rinsate samples tested while the BFD marker was detected in 88% (113/128) of samples. The proportion of samples positive for and concentrations of both the AllBac and BFD markers did not differ significantly across any of the hand hygiene groups ($p = 0.3699 – 0.9922$).

Correlations Between Absorbance, Bacterial Indictors, and *Bacteroidales*

The relationship between soil (absorbance) and bacterial indicators (*E. coli*, *Enterococcus* spp., coliforms, and AllBac and BFD *Bacteroidales* markers) were also of interest in order to determine whether measures could act as proxies for one another. The relationships between soil and indicator bacteria on melon farmworker hands were evaluated using Spearman’s correlations correcting for age. Associations were tested for all 129 melon farmworker hand samples, irrespective of hand-hygiene group (Table 3), but associations were also tested for each hand hygiene group (Tables 4-6). Correlations between the different soil and bacterial indicators exhibited similar trends, regardless of whether all observations were used, or correlations were generated by intervention group. Table 3 displays the correlations between bacterial indicators sampled from all melon farmworkers. The strongest significant correlations among melon farmworkers' samples were seen between coliforms and absorbance (ρ=0.40, p<0.0001) and coliforms and *Enterococcus* spp. (ρ=0.56, p<0.0001). Statistically significant, but weak correlations, were observed between *Enterococcus* spp. and absorbance (ρ=0.21, p=0.0199), BFD and absorbance (ρ=0.28, p=0.0015), and coliforms and *E. coli* (ρ=0.18, p=0.0445). Tables 4-6 depict correlations between indicators within each of the three melon study hand hygiene groups. The largest significant indicator correlations among controls occurred between absorbance and coliforms (ρ=0.560, p=0.0002), although no *E. coli*-positive samples were available for analysis (Table 4). Within the handwashing group (Table 5), the largest significant correlation occurred between coliforms and *Enterococcus* spp. (ρ=0.390, p=0.0116). Within the SaniTwice group (Table 6), the largest significant correlation also occurred between coliforms and *Enterococcus* spp. (ρ=0.679, p<0.0001). Correlations between soil and indicator bacteria were also tested using observations from the jalapeño study in order to assess whether or not relationships between measures depend on environment. These results are shown in Table 7. Among farmworkers harvesting jalapeños, the only significant correlations occurred between *E. coli* and absorbance (ρ=0.447, p<0.0001) and coliforms and *Enterococcus* spp. (ρ=0.487, p<0.0001). Coliforms and *Enterococcus* spp. are correlated in both datasets, but not all correlations were the same between produce. Soil and bacterial indicators, while somewhat correlated, were not 100% predictive of one another. Overall, the indicators used to measure produce contamination in this study were not highly correlated.

Comparison of Hand Contamination Between Melon and Jalapeño Farmworkers

Efficacy results of the hand hygiene interventions on melon farms differed from the results of the previous study conducted on jalapeño farms. Given this difference, and that contamination has been found to be greater on melons, we wanted to investigate this further. Comparisons of control group absorbance and indicator bacteria concentrations from both studies were made using a linear regression model controlling for farm location, gender, harvesting time, and age to assess baseline contamination levels (Figures 2 and 3). As depicted in Figure 2, mean $\log_{10}$ absorbance among controls was significantly higher among jalapeño farmworkers than melon farmworkers ($p=0.0037$). In contrast, Figure 3 shows that *E. coli* and *Enterococcus* spp. concentrations were significantly higher among melon farmworkers as compared to jalapeño farmworkers ($p<0.0001$ and $p=0.0313$, respectively). Coliform concentrations among controls were not statistically significantly different between jalapeño and melon farmworkers ($p=0.5029$). Therefore, hand rinsate samples collected from jalapeño farmworkers contained more soil while hand rinsate samples collected from melon farmworkers contained more indicator bacteria.

To illustrate how and which outcome measures differed by produce type, mean concentrations were compared across all intervention groups. Comparisons were made using a linear regression model controlling for farm location, gender, harvesting time, and age. Table 8 presents mean differences in soil (absorbance A_{600}) and indicator bacteria (coliforms, *E. coli*, *Enterococcus* spp.) concentrations by hand hygiene group and produce type. Absorbance concentrations are significantly higher among farmworkers harvesting jalapeños than farmworkers harvesting melons for the SaniTwice group ($p=0.0002$), but not for the handwashing group ($p=0.3640$). *E. coli* and *Enterococcus* spp. concentrations are significantly greater among melon farmworkers than jalapeño farmworkers across all three intervention groups ($p=0.0001$-0.0313). Coliforms only differ significantly between produce type for the SaniTwice group, with samples obtained from melon farms having higher mean concentration than samples obtained from jalapeño farms ($p<0.0001$). It is clear that there are significant differences between indicator concentrations by intervention group and produce type.

Understanding that there are differences between outcomes by produce type and intervention group, statistical analysis was necessary to quantify interaction between these two variables. Two-way fixed-effects ANOVA models were used to identify the presence of interaction between intervention group variables and produce type for absorbance and bacterial indicators (Figure 4). The efficacy of SaniTwice and handwashing, compared to controls, differed between melon and jalapeño farmworkers; fixed-effects interactions between intervention group and produce type were significant for absorbance ($p=0.0018$), *E. coli* ($p=0.0050$), and coliforms ($p=0.0005$), but not for *Enterococcus* spp. ($p=0.2797$). Figure 4 depicts an interaction plot for each of the indicators of contamination that were compared across studies. The diverging lines in 4a, 4b, and 4d indicate that hand hygiene efficacy results differ based on produce type whereas the parallel lines in 4c indicate that melons have higher baseline levels of contamination but there is no significant difference in efficacy across produce type (Figure 4). This suggests that the efficacy of each intervention at reducing farmworker hand contamination, compared to the control group, differs by produce type. Random effects two-way ANOVAs were also conducted for comparison purposes, and the results were found to be similar (data not shown).

Melon Farmworkers' Hand Hygiene Perceptions

Optional surveys regarding perceptions of hand hygiene were administered in order to identify potential barriers or incentives for compliance. Of the 129 participants, 39 melon farmworkers – three from Santa Elena farm and 36 from Santa Paulina farm – chose to complete the optional survey assessing their perceptions on hand hygiene interventions. Of the 39 respondents, one was a foreman, two were supervisors, and 36 harvesters. About half (46%) had already completed the study before answering the survey. When asked about which method participants preferred using, responses varied; 33% preferred handwashing with soap and water, 13% preferred ABHS, 33% said both, and 21% did not provide a response (Table 9). Therefore, there was no clear majority as to which method was preferred. The majority of respondents believed that the interventions improved the quality of produce (82%) as well as the health of workers (67%). Most participants thought that hand hygiene interventions either improved or did not affect work time (85%), with only 3% believing that hand hygiene negatively impacted work time. Out of the 39 respondents, 85% expressed a desire to continue performing hand hygiene interventions at work. When participants were asked how many times they thought that hand hygiene should be performed during an 8-hour work shift, responses varied but the most common answer was 3 times per shift (49%). Overall, responses varied a good deal.

DISCUSSION

The goal of this study was to compare the efficacy of two hand hygiene interventions (handwashing with soap and water and SaniTwice) at reducing contamination from soil and microbes on farmworkers' hands from melon farms in Nuevo Leon, Mexico from May to July of 2014 and to compare these results to a similar study conducted on jalapeño farms in Northern Mexico in May of 2013. Analysis showed that the handwashing group had significantly less soil on their hands than the SaniTwice or control groups. None of the percentages of samples positive for or concentrations of indicator bacteria differed by hand hygiene group. Data also indicated that soil (absorbance) and indicator bacteria (*E. coli*, *Enterococcus* spp., and coliforms) were not necessarily predictive of one another. Results also suggested that baseline concentrations of soil and indicator bacteria differed on melon and jalapeño farmworkers' hands and fixed-effects models demonstrated that the efficacy of hand hygiene interventions differed based on produce type. Finally, farmworker perceptions regarding the effects of performance on work time, feasibility of frequent use, and preferred method varied.

Both handwashing with soap and water and the SaniTwice method reduced soil on farmworkers' hands compared to the control group, with soap and water removing the most soil. However, neither intervention significantly reduced bacterial indicators or *Bacteroidales* markers on farmworkers' hands. The fact that the handwashing group had the lowest soil levels is consistent with prior studies and suggests that handwashing with soap and water effectively removes soil from farmworker hands (24, 25). This is likely due to the fact that soap acts as an emulsifying agent, absorbing and suspending dirt particles on contaminated hands. When hands are rinsed with potable water, the soap and the dirt particles are removed (80, 81). The success of the SaniTwice method at removing dirt from farmworkers' hands in this study, however, is contrary to the findings of many previous studies (61, 82), but is consistent with the previously published study conducted on jalapeño farms (25). The SaniTwice method uses an excess of sanitizer in the first step which is then removed using a paper towel. Current ABHS research suggests that the friction from the paper towels is likely to remove soil from the hands (82). The greater success of handwashing with soap and water than SaniTwice at reducing soil levels may be due to the fact that handwashing with soap and water combines the emulsifying effect of soap with the friction of the paper towel (80, 81, 82). Overall, both handwashing and SaniTwice methods are efficacious at removing soil from farmworker hands on melon farms, but they do not remove bacterial indicators. While soap may remove dirt particles, it does not necessarily inactivate pathogens on farmworkers' hands. Therefore, farmworkers harvesting melons should not assume that their hands are free from pathogens just because they appear to be clean and unsoiled. The dose-response principle may be responsible for the poor efficacy of these interventions at reducing bacterial indicators. The dose-response principle states that as the level of an exposure (i.e. baseline contamination levels) increases, the outcome being measured (i.e. bacterial concentration) also increases (83). Melons have been shown to have high baseline levels of bacterial indicator presence and concentration due to the biological properties of their rope-like rinds (56, 57). Because baseline contamination of melons is high, stronger and more effective hand hygiene routines may need to be developed in order to remove higher levels of bacterial contamination. With these results in mind, it should be noted that the efficacy of the two tested interventions at removing soil from farmworkers' hands was observed after 30 minutes of harvest, indicating that other melon-harvesting farmworkers would need to wash their hands every 30 minutes to achieve similar efficacy.

In order to assess the need for multiple measurements of hand contamination in agricultural settings, correlations between outcome measures such as soil (absorbance), indicator bacteria (*E. coli*, *Enterococcus* spp., coliforms), and *Bacteroidales* (AllBac and BFD markers) were tested. Results showed few strong, significant correlations between these outcome measures. Furthermore, the correlations that were significant for the data collected among farmworkers harvesting melons were different from data collected among farmworkers harvesting jalapeños. Although previous studies have assessed the relationship of these variables in the context of recreational lake and ocean waters (36, 37), no previous research exploring the relationships between these outcome measures on melon farms could be identified. Our results, combined with previous research, may indicate that outcome measures cannot speak for each other and that multiple measures are necessary when testing the contamination of produce in agricultural settings. Previous research showed that soil was not necessarily indicative of pathogenic contamination and that bacterial indicators were not correlated. Morrill et al. found that while visible soil assessment could act as a proxy for turbidity measures of rinsate samples, it was not a good indicator of microbial contamination (67). Furthermore, Kinzelman et al. found that *E. coli* and *Enterococcus* measures were not interchangeable (37). The lack of correlation between these indicators may be due to the fact that each indicator has a unique set of optimal biological conditions for survival. The success of the bacterial indicator depends on the environment it is living in and therefore, certain indicators may survive better in soil or on hands than other indicators, resulting in inconsistent measurements and weak correlations (29, 84). Given that these outcome measures are not correlated or interchangeable, it is important for future research to explore which methods work best for agricultural settings, especially among produce farmworkers. A better understanding of which indicators work best in agricultural settings will improve the quality of hand hygiene efficacy and intervention studies.

Baseline contamination was compared between hand rinsate samples collected from farmworkers harvesting melons and farmworkers harvesting jalapeños in order to predict and explain potential differences between the effect of interventions on each of these commodities. Data from this study indicated significant differences in baseline contamination between hand rinsate samples collected from farmworkers harvesting melons and farmworkers harvesting jalapeños. Interestingly, results showed higher levels of soil contamination among farmworkers harvesting jalapeños (25) while, in contrast, farmworkers harvesting melons had higher levels of bacterial indicators. This finding contradicts previous research that found that melons had higher amounts of soil and indicator bacteria than other produce types tested (35, 57). These findings may be explained by the fact that hand rinsate samples from the study conducted on jalapeño farms were plated using a wider range of effective volumes than the samples collected on melon farms. Because indicator concentration calculations include largest effective volume plated in the denominator, concentrations of bacterial indicators among jalapeño samples appear to be lower than that of melon samples (25, 85). Differences in soil property by region may also explain the differences in baseline contamination. A study conducted by Hathaway-Jenkins et al. found that the shear strength of soil was dependent on geographic region and land properties (86). Another study found that microbial profiles of soil collected in the Cuatro Cienegas Basin of Mexico were dependent on the type of vegetation and environmental characteristics present (87). Even though farm location was controlled for in this analysis, more specific factors such as soil classification and nutrient distribution may have influenced the differences between contamination on jalapeño and melon farms. On the other hand, findings on the differences in bacterial indicators between melon and jalapeño farms are in line with many studies suggesting that melons have biological properties promoting greater bacterial indicator attachment (56). The cell surface charge and the hydrophobicity of a melon's rope-like rinds have been shown to promote attachment and harboring of organisms such as fecal coliforms (56). Because jalapeños have smoother surfaces than melons, this might explain why farmworkers harvesting jalapeños had significantly lower levels of hand contamination as compared to melon farmworkers. Because the results are contrary to findings from previous studies, there is a need to conduct more comprehensive studies in diverse environments among multiple agricultural populations. Such research would better inform whether handwashing practices are equally efficacious at reducing farmworker hand contaminations across different environments and indicate whether certain protocols deploying various hand hygiene methods are best for different environments.

Analysis of data collected from both melon and jalapeño farms indicated that the efficacy of the two interventions at reducing hand contamination differed by produce type. This finding can be partially explained by the fact that baseline contamination of produce varies (56, 57) potentially due to the fact that certain produce surfaces harbor bacteria better than others (56). Additionally, jalapeños contain a substance called capsaicin which has been shown to successfully inactivate bacterial pathogens such as *Streptococcus pyogenes* and *H. pylori* (88, 89). It is possible that transfer of capsaicin to hands from jalapeños during harvest could act as an additional protective barrier against bacterial contamination. Because melons do not contain capsaicin, hand hygiene methods may appear to more effectively remove bacteria from jalapeño farmworkers' hands than melon farmworkers, resulting in an apparent difference in efficacy. Finally, these results may again be influenced by the fact that hand rinsate samples collected on jalapeño farms were plated using a wider range of effective volumes than samples collected from melon farms, artificially increasing melon farm concentrations (25, 85). Previous research suggests that ABHS methods may be efficacious alternatives to handwashing with soap and water in locations where access to potable water is limited or nonexistent. However, based on this study, more comprehensive research among a wide variety of produce farms is warranted. SaniTwice does not appear to be an efficacious alternative to handwashing with soap and water among farmworkers harvesting melons.

Finally, to help inform researchers on how to improve hand hygiene compliance in agricultural settings, optional surveys on barriers to and drivers of performance were administered. Responses to survey questions varied greatly. Because the response rate for our study was fairly low (30%), responses may not have been representative of all of the farmworkers enrolled in the study and responses appear to vary more than one would expect. Responses regarding preferred method of hand hygiene and the number of times handwashing activities should occur varied the most, which may be due to a lack of standardized recommendations and differences in education level (90). The FSMA Final Rule on Produce safety is the only recommendation in place regarding hand hygiene activities, and it simply states that hand hygiene should be performed (49). Standardized recommendations regarding hand hygiene activities may foster a greater consensus regarding hand hygiene perceptions and improve compliance. However, based on the findings reported here, creating standard recommendations could prove difficult given the discrepancies in efficacy of each measure at reducing hand contamination. Future research should deploy more comprehensive surveys, using a larger sample size. More specific analysis of such surveys could allow for a better understanding of farmworkers' true perceptions and could be used to assess compliance with each intervention method.

This study was able to assess efficacy of hand hygiene at reducing farmworker hand contamination in a comprehensive manner using several different measures of contamination. Therefore, a strength of this study was its ability to make conclusions using a wide variety of different measures of contamination. This study is also the first to compare the efficacy of hand hygiene interventions reducing contamination across different produce farms. Additionally, although two different populations were compared, we were able to take into account potential confounding factors such as farm location, gender, harvesting time, and age due to the availability of many demographic factors. Despite these strengths, this study only compared two produce commodities necessitating future research testing more diverse commodities in order to apply conclusions based on the comparison of these two studies to other produce farms. Furthermore, the two studies were compared used different ranges of effect volumes, which made comparisons of hand contamination between studies difficult due to possible artificial inflation of melon concentrations and reduced the generalizability of results (85). Further analysis normalizing these data for more accurate comparison is necessary. Finally, analyses conducted using the data from these studies did not assess efficacy at reducing indicators of viral or parasitic pathogens. Some pathogens, such as norovirus, are much more difficult to inactivate using hand sanitizers, in place of soap and water (91). Therefore, guidance on what handwashing practice to adopt needs to take into account pathogen type, in addition to produce type or environment.

Based on this study, reduction of soil on farmworkers' hands can be achieved by performing hand hygiene using soap and water or SaniTwice methods. Results presented herein can also assist future researchers in developing more comprehensive hand hygiene methods and protocols to reduce produce contamination and therefore, reduce produce-related illnesses. Results can also help inform which measurements should be used to assess soil and bacterial presence on produce farms in order to more comprehensively examine contamination routes and mechanisms.

The results of this study do not provide supporting evidence that SaniTwice is an efficacious alternative to handwashing with soap and water on produce farms, especially melon-harvesting farms. Different formulations of ABHS or different protocols that employ the use of ABHS might be more effective in these settings. Results also validate the need for using multiple bacterial indicators to evaluate farmworker hand contamination and support the notion that melons may harbor more bacteria than other produce types. Intervention efficacy may differ by produce commodity but more research, involving more produce commodities, is necessary to support this conclusion. Overall, future research should focus on further exploring hand hygiene interventions on produce farms in order to validate the use of ABHS methods in order to reduce hand contamination. Furthermore, future studies should focus on more systematic approaches to evaluating perceptions of farmworkers on hand hygiene practices in order to determine if perceptions influence hand hygiene compliance.

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TABLES

Table 1: Demographics of 129 Farmworkers by Hand Hygiene Intervention Group at Melon Farms in Nuevo Leon, Mexico

Measure	**Control**	**Handwashing**	**SaniTwice**	**p-value**[a]
Number of Males (%)	38/42 (90%)	38/42 (90%)	40/45 (89%)	0.9601
Number at Santa Paulina (%)[b]	39/42 (93%)	39/42 (93%)	42/45 (93%)	0.9949
Age in years[c]	24.4±1.5	32.8 ±1.7[d]	30.4±1.4[d]	0.0004

[a]Pearson X^2 was used to compare the proportion of males and the proportion working at Santa Paulina across groups. Kruskal-Wallis was used to compare age across groups and Steel-Dwass was used to make pairwise comparisons of age across groups.
[b]Number at Santa Paulina/number at Santa Paulina and Santa Elena
[c]Geometric mean ± standard deviation
[d]Statistically significantly different from the control group only

Table 2a: Proportions of Positive Samples of Absorbance, Indicator Bacteria, and Bacteroidales from Farmworker Control and Intervention Group Hand Rinsate Samples Collected on Melon Farms in Nuevo Leon, Mexico

Sample Type	Control[a]	Handwashing[a]	SaniTwice[a]
Absorbance	42/42 (100%)	42/42 (100%)	45/45 (100%)
E. coli[b]	0/42 (0%)	4/42 (10%)	1/45 (2%)
Enterococcus spp.	41/42 (98%)	40/42 (95%)	39/45 (87%)
Coliforms	28/42 (67%)	26/42 (62%)	19/45 (42%)
AllBac	28/41 (68%)	26/42 (62%)	32/45 (71%)
BFD	35/41 (85%)	38/42 (90%)	40/45 (89%)

[a]Proportion of samples positive/total number of samples collected (%)
[b]Firth correction used in comparison of proportions of positive samples

Table 2b: Concentrations of Absorbance, Indicator Bacteria, and *Bacteroidales* from Farmworker Control and Intervention Group Hand Rinsate Samples Collected on Melon Farms in Nuevo Leon, Mexico

Sample Type	Control[a]	Handwashing[a]	SaniTwice[a]
Absorbance	0.14 ± 0.01[c]	0.01 ± 0.00[b,c,d]	0.04 ± 0.01[b]
E. coli	2.27 ± 0.00[e]	2.38 ± 0.06	2.28 ± 0.01
Enterococcus spp.	5.15 ± 0.23	5.03 ± 0.25	4.56 ± 0.30
Coliforms	3.88 ± 0.30	3.17 ± 0.18	3.62 ± 0.29
AllBac	4.32 ± 0.40	3.74 ± 0.38	4.08 ± 0.34
BFD	5.59 ± 0.24	5.50 ± 0.20	5.15 ± 0.18

[a]Geometric mean of absorbance. Mean $\log_{10}$ CFU/hand for *E. coli*, *Enterococcus* spp., and coliforms. Mean $\log_{10}$ GEC/hand values for AllBac and BFD *Bacteroidales*.
[b]Statistically significantly different from the control group using Tukey's correction for multiple comparisons
[c]Statistically significantly different from the SaniTwice group using Tukey's correction for multiple comparisons
[d]Standard deviation is actually 0.002
[e]No control group samples were positive for *E. coli*. All values were outside the limit of detection and therefore have the same concentration

Correlation Value	Strength of Relationship
0.50 to 1.00	Strong
0.30 to 0.49	Moderate
0.10 to 0.29	Weak
0.00 to 0.09	Very weak

Table 3. Correlations Between Concentrations of Absorbance, Indicator Bacteria, and *Bacteroidales* in Melon Farmworker Hand Rinsate Samples (n=128)[a]

	Absorbance	*E. coli*	*Enterococcus* spp.	Coliforms	AllBac	BFD
Absorbance	1.00					
E. coli	-0.03	1.00				
Enterococcus spp.	0.21[b]	0.06	1.00			
Coliforms	0.40[b]	0.18[b]	0.56[b]	1.00		
AllBac	0.05	-0.01	-0.09	-0.14	1.00	
BFD	0.28[b]	0.03	0.10	0.16	0.12	1.00

[a]Light gray = very weak (0.00 to 0.09), gray = weak (0.10 to 0.29), medium gray = moderate (0.30 to 0.49), dark gray = strong (0.50 to 1.00)[1,2]
[b]Statistically significant result at α=0.05 using Spearman's partial correlation controlling for age

Table 4. Correlation Among Melon Control Group of Absorbance, Indicator Bacteria, and Bacteroidales (n=41)[a]

	Absorbance	*E. coli*[b]	*Enterococcus* spp.	Coliforms	AllBac	BFD
Absorbance	1.00					
E. coli[b]	.	.				
Enterococcus spp.	0.13	.	1.00			
Coliforms	0.56[c]	.	0.42[c]	1.00		
AllBac	0.07	.	-0.07	-0.10	1.00	
BFD	0.11	.	0.00	0.15	0.16	1.00

[a]Light gray = very weak (0.00 to 0.09), gray = weak (0.10 to 0.29), medium gray = moderate (0.30 to 0.49), dark gray = strong (0.50 to 1.00)[1,2]
[b]No *E. coli*-positive samples available for correlation analysis
[c]Statistically significant result at α=0.05 using Spearman's partial correlation controlling for age

Table 5. Correlations Among Melon Handwashing Group of Absorbance, Indicator Bacteria, and Bacteroidales (n=42)[a]

	Absorbance	*E. coli*	*Enterococcus* spp.	Coliforms	AllBac	BFD
Absorbance	1.00					
E. coli	0.20	1.00				
Enterococcus spp.	0.09	0.17	1.00			
Coliforms	0.30	0.31[b]	0.39[b]	1.00		
AllBac	-0.13	0.13	-0.27	-0.15	1.00	
BFD	0.06	0.07	0.03	0.13	0.07	1.00

[a]Light gray = very weak (0.00 to 0.09), gray = weak (0.10 to 0.29), medium gray = moderate (0.30 to 0.49), dark gray = strong (0.50 to 1.00)[1,2]
[b]Statistically significant result at α=0.05 using Spearman's partial correlation controlling for age

Table 6. Correlation Among Melon SaniTwice Group of Absorbance, Indicator Bacteria, and Bacteroidales (n=45)[a]

	Absorbance	*E. coli*	*Enterococcus* spp.	Coliforms	AllBac	BFD
Absorbance	1.00					
E. coli	0.25	1.00				
Enterococcus spp.	0.31[b]	-0.09	1.00			
Coliforms	0.56[b]	0.20	0.68[b]	1.00		
AllBac	-0.19	-0.07	-0.06	-0.24	1.00	
BFD	0.45[b]	0.04	0.10	0.16	0.12	1.00

[a]Light gray = very weak (0.00 to 0.09), gray = weak (0.10 to 0.29), medium gray = moderate (0.30 to 0.49), dark gray = strong (0.50 to 1.00)[1,2]
[b]Statistically significant result at α=0.05 using Spearman's partial correlation controlling for age

Table 7. Correlation Among Jalapeño Farmworkers of Absorbance and Indicator Bacteria (n=89)[a]

	Absorbance	*E. coli*	*Enterococcus* spp.	Coliforms
Absorbance	1.00			
E. coli	0.45[b]	1.00		
Enterococcus spp.	0.10	0.20	1.00	
Coliforms	0.20	0.06	0.49[b]	1.00

[a]Light gray = very weak (0.00 to 0.09), gray = weak (0.10 to 0.29), medium gray = moderate (0.30 to 0.49), dark gray = strong (0.50 to 1.00)[1,2]
[b]Statistically significant result at α=0.05 using Spearman's partial correlation controlling for age

Table 8. Mean Difference in Concentrations of Absorbance and Indicator Bacteria by Intervention Group Between Melon and Jalapeño Farmworker Hand Rinsate Samples

Sample Type[a]	Intervention	Jalapeño	Melon	Difference
Absorbance	Control	0.24	0.14	0.10[b]
	Handwashing	0.00	0.01	-0.01
	SaniTwice	0.10	0.04	0.06[b]
E. coli	Control	0.84	2.27	-1.43[b]
	Handwashing	0.73	2.38	-1.65[b]
	SaniTwice	0.90	2.28	-1.39[b]
Enterococcus spp.	Control	4.09	5.15	-1.05[b]
	Handwashing	3.99	5.03	-1.04[b]
	SaniTwice	3.11	4.56	-1.44[b]
Coliforms	Control	3.28	3.88	-0.60
	Handwashing	2.76	3.17	-0.40
	SaniTwice	1.47	3.62	-2.15[b]

[a]Geometric mean absorbance and mean $\log_{10}$ CFU/hand for *E. coli*, *Enterococcus* spp., and coliforms.

[b]Significant difference between jalapeño and melon farm samples at $\alpha=0.05$ using linear regression controlling for farm location, gender, harvest time, and age.

Table 9. Perceptions of Hand Hygiene Intervention Methods Reported by 39 Farmworkers Harvesting Melons in Nuevo Leon, Mexico

Topic	Control % (n=3)[a]	Handwashing % (n=18)[a]	SaniTwice % (n=18)[a]	Total % (n=39)[a]
Farm				
Santa Elena	33.3%	5.6%	5.6%	7.7%
Santa Paulina	66.7%	94.4%	94.4%	92.3%
Job				
Manager/Forman	33.3%	0.0%	0.0%	2.6%
Supervisor	33.3%	5.6%	0.0%	5.1%
Worker	33.3%	94.4%	100.0%	92.3%
Already Participated?				
Yes	33.3%	38.9%	55.6%	46.2%
No	66.7%	61.1%	44.4%	53.8%
Preferred Method				
Hand Washing	33.3%	55.6%	11.1%	33.3%
Gel	0.0%	11.1%	16.7%	12.8%
Both	66.7%	27.8%	33.3%	33.3%
Do not know/ Did not answer	0.0%	5.6%	38.9%	20.5%
Effect of Methods on Produce				
Improves	66.7%	94.4%	72.2%	82.1%
Worsens	0.0%	0.0%	0.0%	0.0%
Does not affect	33.3%	5.6%	22.2%	15.4%
Do not know/Did not answer	0.0%	0.0%	5.6%	2.6%
Effect of Methods on Worker Health				
Improves	66.7%	83.3%	50.0%	66.7%
Worsens	0.0%	0.0%	0.0%	0.0%
Does not affect	33.3%	5.6%	44.4%	25.6%
Improves and does not affect	0.0%	11.1%	0.0%	5.1%
Do not know/Did not answer	0.0%	0.0%	5.6%	2.6%
Effect of Methods on Work Time				
Improves	0.0%	22.2%	44.4%	30.8%
Worsens	0.0%	0.0%	5.6%	2.6%
Does not affect	66.7%	77.8%	27.8%	53.8%
Do not know/ Did not answer	33.3%	0.0%	22.2%	12.8%
Desire to Continue Hand Hygiene Practices				
Yes	100.0%	94.4%	72.2%	84.6%
No	0.0%	5.6%	5.6%	5.1%

Do not know/ Did not answer	0.0%	0.0%	22.2%	10.3%
How Many Times Hand Hygiene Methods Should Be Used per Work Day				
1	33.3%	5.6%	11.1%	10.3%
2	0.0%	11.1%	22.2%	15.4%
3	33.3%	72.2%	27.8%	48.7%
4	0.0%	5.6%	11.1%	7.7%
5	0.0%	5.6%	0.0%	2.6%
3 or 4	0.0%	0.0%	5.6%	2.6%
Every half hour or hour	0.0%	0.0%	5.6%	2.6%
Do not know/Did not answer	33.3%	0.0%	16.7%	10.3%

[a]Percentages may not add to 100% due to rounding

FIGURES

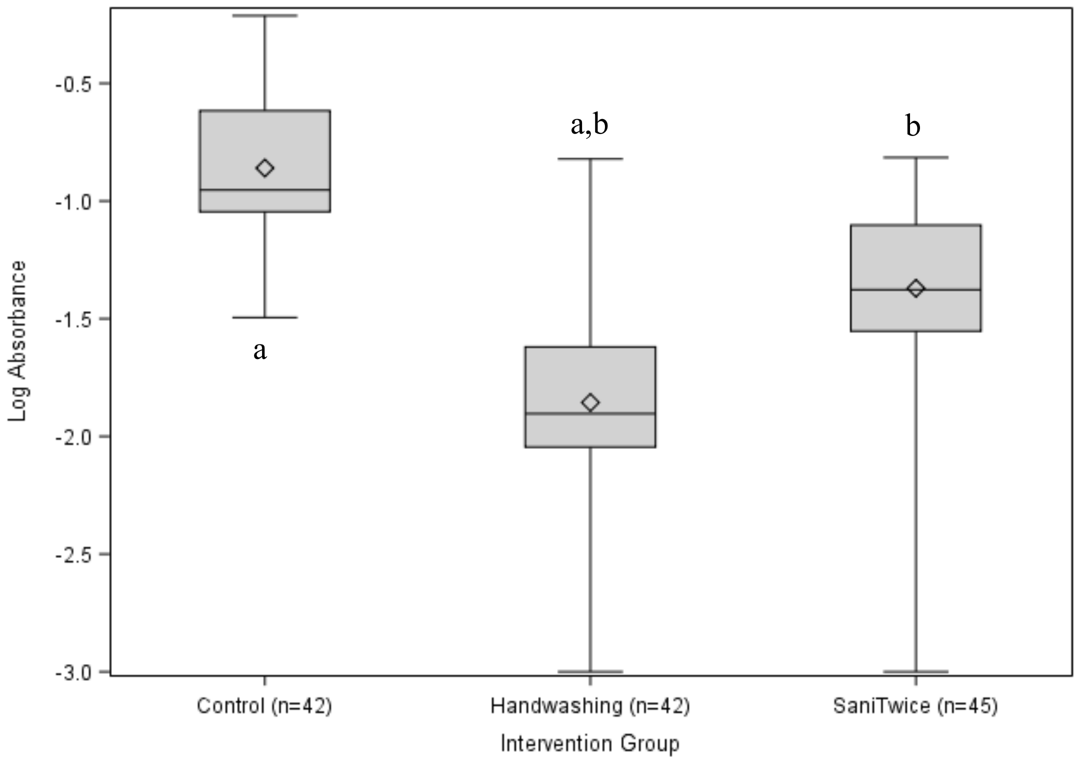

Figure 1. Absorbance (600 nm) levels measured from melon farmworker hand rinsate samples differ significantly across both intervention groups and the control group. The boxes display the 25^{th}, 50^{th}, and 75^{th} percentiles and diamonds denote the mean for absorbance data. Whiskers depict minimum and maximum values. Subscripts identify significant differences in log-transformed absorbance values across intervention group from a linear regression model controlling for farmworker age (a=significantly different from the SaniTwice group, b=significantly different from the control group). Tukey's procedure was used to adjust for multiple comparisons.

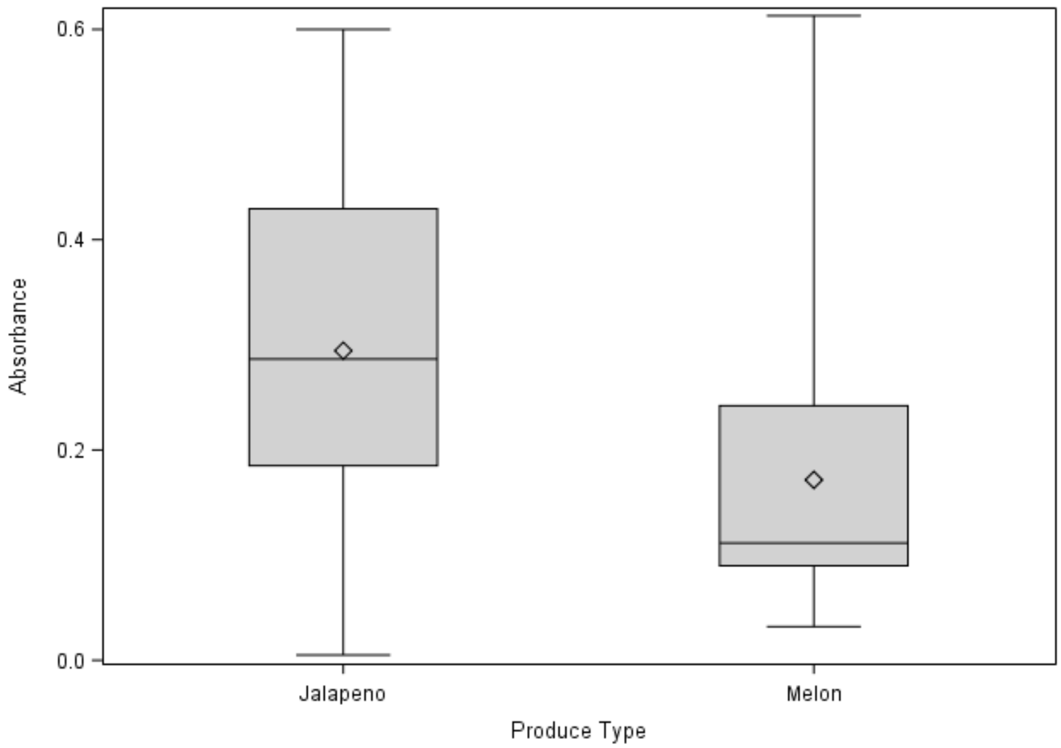

Figure 2. Absorbance (600 nm) levels measured from control group hand rinsate samples are significantly lower among farmworkers harvesting melons compared to farmworkers harvesting jalapeños. The boxes display the 25th, 50th, and 75th percentiles and diamonds denote the mean for absorbance data. Whiskers depict minimum and maximum values. Linear regression was used to compare mean log_{10} absorbance by produce group, controlling for farm location, gender, harvest time, and age.

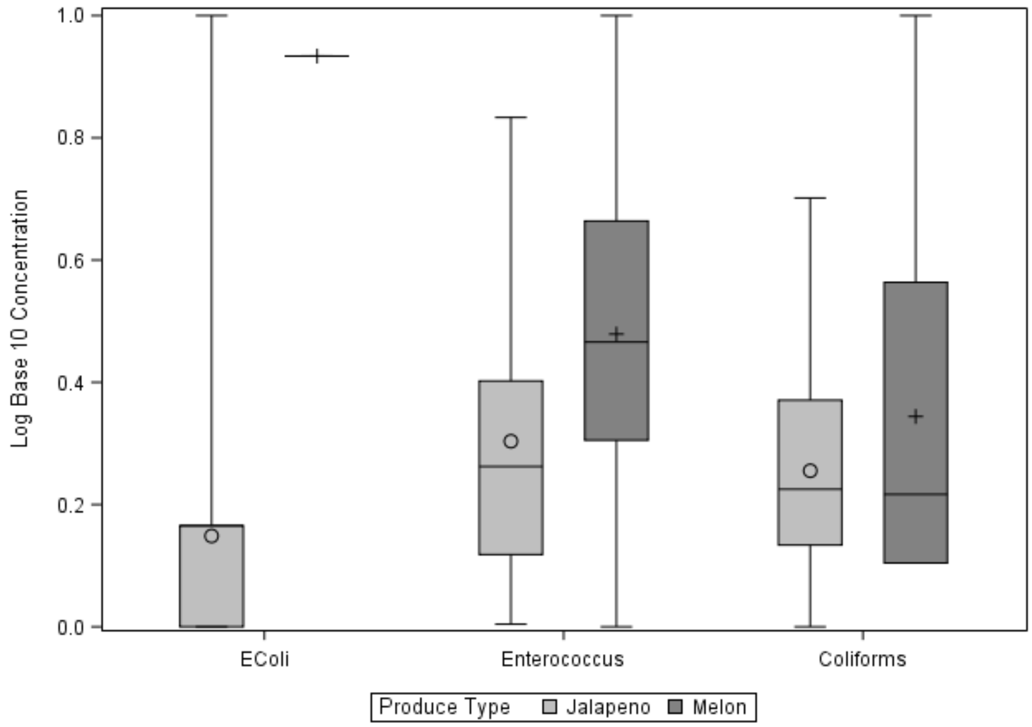

Figure 3. Concentrations of *E. coli* and *Enterococcus* spp. measured from control group hand rinsate samples are significantly lower among farmworkers harvesting jalapeños than farmworkers harvesting melons. *E.coli* samples among the melon control group were above the limit of detection, so concentration appears to be higher among melon controls than jalapeño controls. The boxes display the 25th, 50th, and 75th percentiles and circles and plus signs denote the mean for absorbance data. Whiskers depict minimum and maximum values. Linear regression was used to compare mean $\log_{10}$ CFU/hand concentrations for *E. coli*, *Enterococcus* spp., and coliforms by produce group, controlling for farm location, gender, harvest time, and age.

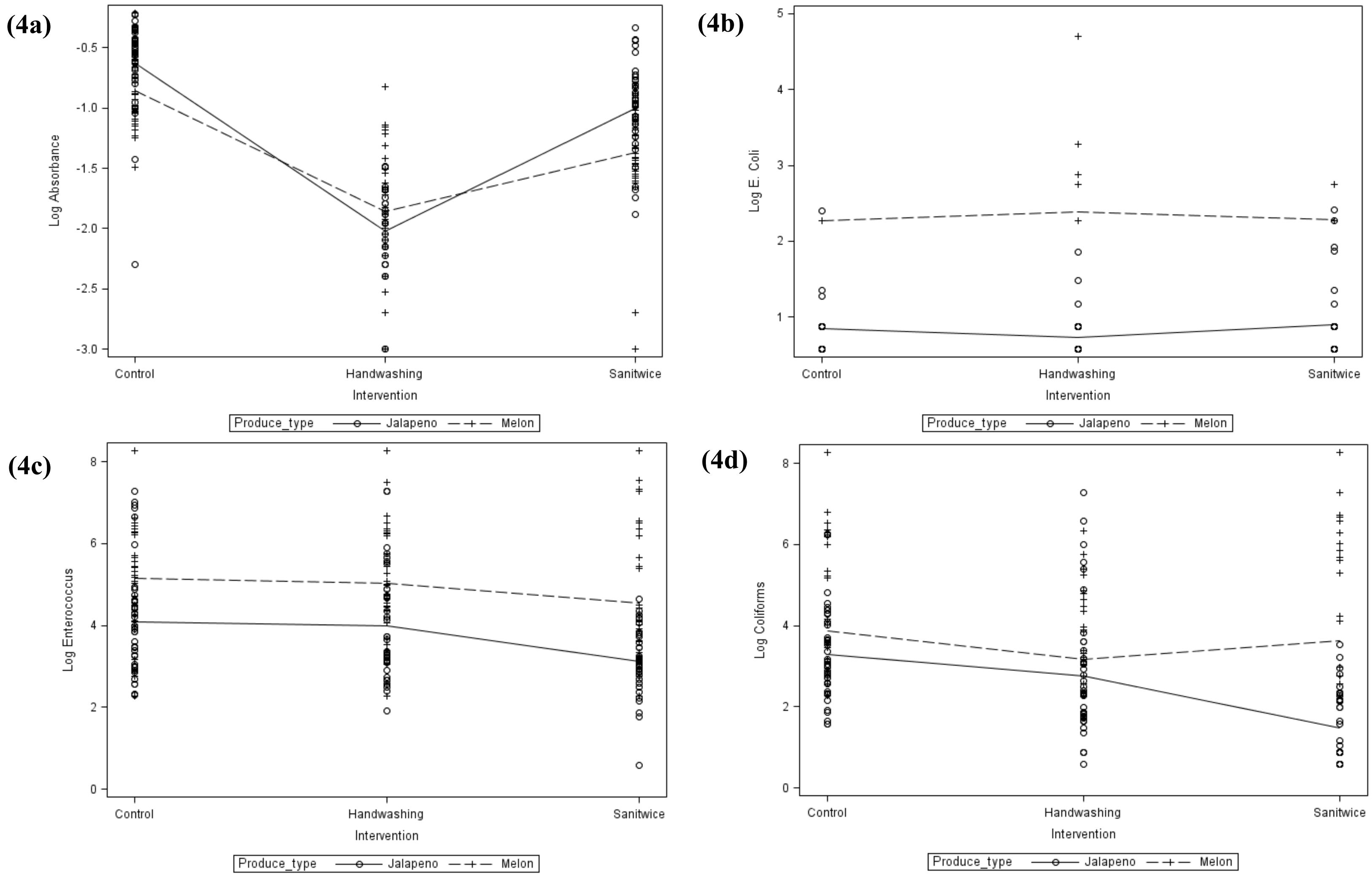

Figure 4. The effect of each intervention group on absorbance, *E. coli*, and coliform concentrations differs significantly by produce type. (4a) Absorbance interaction plot (4b) *E. coli* interaction plot (4c) *Enterococcus* spp. interaction plot (4d) coliforms interaction plot. Two-way fixed-effects models were used to identify significant interaction between intervention group and produce type for absorbance and bacterial indicators. Models controlled for farm location, gender, harvest time, and age.

III. PUBLIC HEALTH IMPLICATIONS

- The two interventions tested, handwashing with soap and water and SaniTwice, reduced soil on hands but not indicator bacteria. The FSMA produce rule currently suggests using soap and water for hand hygiene in agricultural settings but this may not remove bacterial contamination, so the risk to consumers is not equal in all settings
- More comprehensive strategies for the reduction of hand hygiene contamination on produce farms are necessary to improving produce quality and reducing produce-related outbreaks and illnesses
- Fixed-effects model results support the development of commodity-specific interventions for the reduction of produce contamination
- Our results showed that bacterial indicators were not all correlated with one another, so better guidance is necessary regarding which indicator measures should be used to assess produce contamination in agricultural settings
- Results regarding preferred method of hand hygiene and time impacts varied, necessitating more detailed and extensive surveys of farmworkers' perceptions to understand the drivers of and barriers to hand hygiene compliance.
- Survey results highlight the fact that there is no standardized method for handwashing frequency, which could lead to higher levels of contamination in certain settings based on compliance and hand hygiene perceptions
- Future interventions based on the implications above will reduce produce contamination by catering to the unique characteristics of different commodities

and environments. This will further reduce produce contamination levels and risk of illness to the consumer.