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Melissa Erkens Date

Microbial Contamination and Consumption Patterns of Produce and Street Food Across Ten Cities in Africa, Asia, and the USA

By

Melissa Erkens
Master of Public Health

Epidemiology

_____

Christine L. Moe, PhD
Committee Chair

Microbial Contamination and Consumption Patterns of Produce and Street Food Across Ten Cities in Africa, Asia, and the USA

By

Melissa Erkens

B.A. German Studies, B.S. Biochemistry
Converse College
2018

Thesis Committee Chair: Christine L. Moe, PhD

An abstract of
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Abstract

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By Melissa Erkens

Foodborne diseases have been increasing globally, despite efforts to improve water, sanitation, and hygiene (WASH) conditions in areas with high disease burden. Food items can become contaminated at multiple points in the farm-to-fork pathway, and improperly prepared or uncooked foods can cause disease. Street food dishes and dishes with raw produce, such as salads, have also become increasingly popular. This analysis aimed to quantify the association between *E. coli* contamination and type of produce or street food, socioeconomic status (SES), and city where samples were collected, as well as characterize food consumption patterns for adults and children. Produce and street food samples were collected from 44 neighborhoods in ten cities in Africa, Asia, and the USA and analyzed for *E. coli* using membrane filtration or IDEXX. Sample type, SES, and city were modeled against *E. coli* concentration using logistic regression at multiple cutoffs. One member of each household surveyed in study sites was interviewed about household produce and street food consumption, and frequencies of behaviors were calculated for adults and children. Herbs, leafy, and root-underground vegetables had significantly higher odds of *E. coli* contamination above 2.93 $\log_{10}$ CFU/MPN compared to seeded vegetables. Mixed street food dishes had significantly higher odds of contamination above 2.67 $\log_{10}$ CFU/MPN and 2 $\log_{10}$ CFU/MPN compared to cooked dishes. Samples of street food from neighborhoods with higher SES had increased odds of *E. coli* contamination above 2.67 $\log_{10}$ CFU/MPN and 2 $\log_{10}$ CFU/MPN compared to low SES neighborhoods. Street food samples from Accra, Kampala, and Kumasi had significantly lower odds of *E. coli* contamination above 2.67 $\log_{10}$ CFU/MPN and 2 $\log_{10}$ CFU/MPN compared to Dhaka. Adults reported similar food consumption patterns between themselves and children in their household across study sites. There was a clear association between uncooked food items and increased odds of *E. coli* contamination, as well as different odds by SES or city. It is important to address poor food hygiene and understand how it is linked to inadequate sanitation in low-income settings in order to minimize the risk of contamination of popular food items and reduce the burden of foodborne diseases.

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CHAPTER I: LITERATURE REVIEW

Introduction

Produce and street food are staple foods in nearly all cultures. However, despite the nutritional benefit that these foods provide to the consumer, they can also be vehicles for transmission of numerous pathogens. Consumption of uncooked produce and contaminated street foods can lead to disease, but identifying where contamination is occurring and exercising sanitary practices in high-risk settings can decrease the risk of contamination and alleviate the disease burden. This literature review examines the global burden of foodborne diseases, prior research on which foods were associated with the highest risk for disease, studies of contamination along the farm-to-fork pathway in different settings, and ongoing research in some of the countries included in the data analysis. Predictors of fecal contamination of produce and street food (indicated by *E. coli* contamination) and trends in produce and street food consumption will be reviewed.

Global Burden of Foodborne Diseases

In 2007, the World Health Organization established the Foodborne Disease Burden Epidemiology Reference Group (FERG) to estimate the global burden of foodborne diseases of microbial, parasitic, or chemical origin and strengthen the capacity of countries to conduct foodborne disease burden assessments (1). Foodborne diseases are a major burden on the health of a population, but there are still numerous unknowns, including the different risks and associated burden from foods contaminated by parasites or chemicals. High burdens of foodborne disease can impact the socioeconomic development of countries worldwide. Globally, as of 2010, 31 identified foodborne hazards were estimated

to have caused 600 million illnesses and 420,000 deaths, with 40% of the disease burden among children under 5 years old. The 31 foodborne hazards resulted in nearly 33 million disability-adjusted life years (DALYs) globally, meaning 33 million healthy years of life were lost in the global population in 2010 (1, 2). The highest disease burden per population was noted in Africa D and E subregions (1,276 and 1,179 DALYs per 100,000 population, respectively), followed by South-East Asia B and D, and the Eastern Mediterranean B and D subregions. The African and South-East Asian subregions include several of the countries that are included in this research. These six subregions had the highest proportions of foodborne DALYs associated with diarrheal disease and invasive infectious disease agents, which included agents such as: norovirus; enteropathogenic, enterotoxigenic, and Shiga toxin-producing *Escherichia coli* (EPEC, ETEC, and STEC, respectively); *Vibrio cholerae*; *Shigella* spp.; non-typhoidal *Salmonella enterica*; *Giardia* spp.; *Cryptosporidium* spp.; hepatitis A; *Listeria monocytogenes*; *Salmonella* Typhi and Paratyphi A (1). This study found that the key hazards for disease were dietary risk factors, unimproved water and sanitation, HIV/AIDS, malaria, air pollution, and tuberculosis (1).

Produce Contamination Studies

Raw salad vegetables (RSV) are an integral part of a healthy diet, but they can introduce pathogen contaminants to humans by being consumed without proper preparation and cooking. In addition to providing necessary micronutrients, RSV also provide phytonutrients that can “act as an effective media for the transmission of pathogens” (3). Foodborne illnesses and outbreaks attributed to fresh produce have increased globally (3-7), and specific types of produce are “more frequently implicated” in

such outbreaks, including leafy greens, jalapeños, tomatoes, and melons (5). Pathogen contamination of RSV can occur at multiple points of the growth, harvesting, and consumption pathway through a variety of means, primarily including use of contaminated manure or water from livestock operations, through direct contact with animals, during harvesting, transport, processing, distribution, and marketing, or cross-contamination at home (3). Specifically, the use of wastewater for irrigation not only affects the produce itself, but also human health. Certain pathogens, including enterotoxigenic *E. coli* O157:H7, *Shigella*, *Salmonella*, *L. monocytogenes*, and *Campylobacter* can be introduced to RSV through wastewater and manure. In one study in Ghana, the key source of lettuce contamination was found to be at the farm, due to contamination of irrigation water, soil, and manure application (4). While lettuce is not traditionally part of the Ghanaian diet, it has increased in popularity in street food and restaurant dishes (4). Additionally, bacteria are able to evade decontamination measures, such as washing or sanitizing, by forming a biofilm on the surface of RSV with other bacteria (3). One reason for the observed increase in foodborne illnesses and outbreaks could be due in part to changes in "agronomic practices and an increase in the number of immune-compromised consumers" (3). The presence of *E. coli* on food is used as an indicator of fecal contamination, so its presence on produce can be a proxy for microbiological safety of produce (3).

Mritunjay and Kumar sought to "evaluate microbiological contamination" on the surfaces of produce consumed in India in response to the lack of adequate documentation of this in Dhanbad city. They collected 480 samples of RSV (cucumber, tomato, carrot, coriander, cabbage, beetroot, radish, and spinach) from various market vendors on different dates. Once samples were collected, they were serially diluted and cultured, then the

number of colony-forming units was determined. Real-time quantitative PCR (qPCR) was used to confirm the presence of *Salmonella*, *L. monocytogenes*, *E. coli* O157:H7, and *Exiguobacterium* (3). Forty-four samples out of 480 were positive for contamination by one of these bacteria. Spinach had consistently high mean values for all indicators tested. One hypothesis explaining this finding was that spinach leaves are open and have a large surface area, which makes the leaves "more susceptible to bacterial contamination and adhesions" compared to leafy greens with smaller leaves (like coriander) or more densely-leaved heads (like cabbage) (3). *Exiguobacterium* was found in spinach, which marks the first report of this bacteria on the surface of RSV. Spinach and beet-root were both positive for *E. coli*, and none of the samples of cabbage or coriander were positive for *E. coli* and *L. monocytogenes*, the latter of which had a relatively low occurrence compared to other studies quantifying microbacterial contamination of RSV. *Salmonella* were detected in 19 samples, which included all RSV except cabbage (3). Mritunjay and Kumar stated that no cases of salmonellosis or listeriosis were reported in Dhanbad during the study period, despite those two causative agents being most prevalent species of the ones detected. They emphasize that these results reveal a need for improved agricultural practices and hygiene practices by food vendors, processors, and consumers, and surveillance of vegetable vendors (3).

Amoah and colleagues aimed to investigate water contamination in "urban and peri-urban areas" in and around Accra, Ghana, where nearly all of the lettuce used for city consumption was produced (4). From April 2004 to June 2005, they studied the "farm to fork" pathway for lettuce, a food that is now commonly consumed with street food or restaurant dishes, by surveying farmers, wholesale and retail sellers, food vendors, and

consumers on agronomical practices, handling, and distribution. Additionally, in- and outflow of lettuce in markets (shipments and purchases), fast food purchases, and lettuce turnover (how quickly it was purchased once stocked) were observed and quantified, accompanied by survey results to identify the consumption risk groups (4). Microbiological analyses were done by periodically collecting lettuce samples at three critical steps in the farm-to-fork pathway: at the farm right before harvesting, from the stock where sellers acquire lettuce to sell, and from the shelves of retailers 2-3 hours after being put on display (this was the approximate turnover rate at the retailer) (4). Fecal coliform and total coliform levels were determined by culturing and using most probable number (MPN) tables to enumerate the number of bacteria. Helminth egg counts were determined using the concentration method and identified using the Bench Aid for the Diagnosis of Intestinal Parasites (4). All sources of irrigated water, except for piped water, were found to have fecal coliform levels exceeding the World Health Organization's standard for unrestricted irrigation. However, all samples were found to be contaminated, even if the water source for irrigation was piped water. The authors noted that post-harvest handling and marketing did not correlate with increased lettuce contamination, suggesting that "the initial contamination on the farm is so high that it hides any post-harvest contamination" (4). They found that in the cases of piped irrigation water, the soil itself was contaminated in the upper 5 cm of soil, and there was frequent use of "incompletely composted (poultry) manure" (4).

Amoah et al. concluded that, while the obvious solution to reduce contamination is to target sanitation efforts on farms and their respective water and manure sources, this may not be feasible, as farmers face numerous challenges including insecurity of land,

limited water treatment due to economic limitations, high demand, and frequent turnover of crops to meet that demand (4). Additionally, food vendors and households reported sanitation practices that are not sufficient for avoiding contamination, despite the fact that little contamination was noted to occur post-harvesting. Not only are alternative risk reduction strategies necessary for farms, but vendors' unsafe practices need to be corrected and consumers need to be knowledgeable of how to select produce that appears clean (4).

Bartz and colleagues aimed to quantify the association between microbial contamination of hands, soil, and water with produce contamination by testing for microbial indicators (coliforms, *Escherichia coli*, *Enterococcus* spp., and somatic coliphages) on both the sources and produce. Their study examined 11 farms and packing facilities in Mexico and tested produce rinses matched with "water, soil, and worker hand rinses during two growing seasons" (5). The logistic regression model yielded statistically significant odds ratios for the associations between detection of *E. coli* and coliphage on hands and detection on produce; if *E. coli* was detected on a farmworkers' hands, produce was "nine times more likely to contain *E. coli*" and if coliphage was detected, produce was "eight times more likely to contain coliphage" (5). No other statistically significant associations were noted (5). In the correlation analysis comparing Spearman correlation coefficients for the general association between source type and produce and source type stratified by type of produce, only the associations with hands remained statistically significant for contamination by coliforms, *E. coli*, and *Enterococcus* spp., affirming that the farmworkers' hands were an important source of contamination (5).

In a separate, earlier study also analyzing produce from Mexico, Johnston and colleagues sought to compare the "overall quality of domestic and Mexican produce

throughout the packing process", look at microbiological changes in produce "at each stage of production and processing", and "evaluate the prevalence of select pathogens on fresh produce" (6). They collected produce grown domestically in Texas or imported from Mexico from eight packing sheds, and targeted produce that is typically consumed raw: leafy greens, herbs, melons, and vegetables. Samples were collected in a manner that reflected the processing and packaging process, beginning with pre-processing, immediately following wash and rinse steps, and prior to distribution. Environmental swabs were also collected from the same areas produce samples were collected. Produce samples were tested for the presence of *Enterococcus faecium* and *faecalis* strains (these strains were assessed for antibiotic resistance), *Salmonella*, *L. monocytogenes*, *Shigella*, and *E. coli* O157:H7. Their analysis revealed that no produce samples contained *Salmonella*, *Shigella*, or *E. coli* O157:H7, but three cabbage samples were positive for *L. monocytogenes* (6). This was consistent with earlier research done by FDA that examined 1028 domestic produce samples; 99% of these samples were free of *Shigella*, *Salmonella*, and *E. coli* O157 (8). Additionally, they also looked at changes in microbial levels for the packing process for cantaloupe, and found that *E. coli* levels increased throughout this process and were higher in domestic samples versus imported samples, which could indicate "higher fecal contamination within domestic packing sheds" (6).

Johnston et al. concluded that the way produce is handled at harvest and during processing directly impacts the microbiological quality of that produce. If interventions to minimize contamination are not present, such as specific procedures for sanitation and washing practices, contaminants will persist to the final product and put consumers at risk for foodborne illnesses. Johnston et al. specifically encouraged adherence to the *Guide to*

Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables (6); while recommendations for food handling procedures are still evolving, emphasizing adherence to a set of guidelines in similar environments could promote consistency across producers. However, growth environments still demand tailored approaches to improving sanitation depending on regional- or produce-specific requirements.

Produce-Related Outbreaks

Machado-Moreira and colleagues compiled and reviewed literature on 571 ready-to-eat (RTE) produce-related outbreaks from MEDLINE and Web of Science Core collection databases in order to capture a comprehensive picture of these outbreaks globally from 1980 to 2016. They also conducted a separate analysis of data from "public health agencies in Europe and the United States" that may not have published outbreak data in a peer-reviewed journal (7). Their analysis revealed that leafy greens, which includes lettuce, basil, parsley, spinach, clover, cilantro, cress, and watercress, were attributed to the 51.7% of the 571 reported outbreaks included in this study. More than a quarter of cases included soft fruits (blueberries, strawberries, raspberries, other berries, and pomegranate arils). Sprouted plants (alfalfa, fenugreek, fennel seeds, mung beans, anise seeds, bean sprouts, clover sprouts, and other sprouts) were associated with the largest proportion of deaths (31.8%) (7).

The single food item with the largest number of reported cases linked to it was strawberries; Machado-Moreira and colleagues indicate that berries have a higher risk of contamination as they cannot handle intensive washing, and washing may impact "commercially valuable properties, such as shape and appearance". This puts

contamination of these soft fruits at the mercy of the harvesters, their personal hygiene, and the hygiene of the facilities. Bartz and colleagues concluded in their study in Mexico that contamination of the produce was most likely to occur from the hands of the farmworkers (5), which compounds the concern introduced by Machado-Moreira et al. Radishes were also associated with a large number of cases, though these cases were largely associated with a single outbreak that occurred in Japan in 1996 (7). This association emphasizes the role that contact with soil, manure, animal feces, and irrigation water may have on produce contamination. Similar to radishes, various species of edible sprouts are also at increased risk for contamination; because the sprouting step of that crop's life cycle occurs in conditions favorable for microbial growth, seeds and sprouts may become contaminated by fertilizer or water (7). Leafy greens are associated with nearly 24% of the reported cases of foodborne disease analyzed in this study. As with other produce, leafy greens are susceptible to contamination by water, soil, or manure, and the risk of contamination may be exacerbated by "extreme weather events" such as heavy rain leading to flooding (7). These associations emphasize the role that contact with soil, manure, animal feces, and irrigation water may have on produce contamination. Machado-Moreira et al. also highlighted the organisms primarily responsible for the outbreaks they analyzed, which included norovirus, *Salmonella*, *E. coli*, hepatitis A, *Cyclospora*, *Listeria monocytogenes*, *Shigella*, *Yersinia*, *Giardia*, and *Cryptosporidium*, several of which have been studied in other literature mentioned previously (7).

Another key issue raised by Machado-Moreira et al. is the ability to trace back to the origin of a food item. For example, seeds may be distributed worldwide, and batches of seeds may be combined prior to shipping, so the process finding the origin of a small lot

of contaminated seeds is incredibly difficult. The European Union and U.S. Food and Drug Administration have trace-back requirements in place in an attempt to make this process possible (7).

In their discussion of prevention and mitigation strategies, Machado-Moreira et al. emphasize that control measures are implemented in such a way that they can be put into practice by "all parties involved in the food production operation" (7). They cite Gil et al. and Julien-Javaux et al.'s recommendations at the primary level of production. Julien-Javaux et al. specify that "Good Agricultural Practices (GAPs)" should be instituted at the seven possible routes of microbial contamination at the farm level: growing field and adjacent land, hygiene and human health, worker harvesting practices, animal husbandry practices and intrusion of wild animals, manure-based soil amendments, harvesting equipment, storage areas and transportation, and agricultural water (9). They further emphasize that these GAPs need to be rolled out so that growers, in addition to auditors or assessors, fully understand "the impact of those strategies on the safety of the produce they are growing and/or how to implement these strategies in a practical, sustainable and cost-effective way" (9). Gil and colleagues highlight that one of the key strategies for prevention and intervention is the "training and education of the growers and handlers along the entire food chain" about personal hygiene, clean handling procedures, and control of cross-contamination, a conclusion that has been shared by many others (10).

Produce can become contaminated by numerous pathogens through multiple pathways. Produce items such as leafy greens and herbs were some of the most common items found to be contaminated, along with sprouted plants and soft fruits (7). In considering the farm-to-fork pathway, Bartz et al. found that the hands of farm workers

were the most significant cause of contamination in their study in Mexico (5). Amoah et al. found that contaminated water and soil were the primary cause of contamination in urban and peri-urban farms in Ghana, but also acknowledged that contamination can still occur during and after harvest (4). The researchers all agreed that produce contamination can be addressed through improved agricultural practices and hygiene practices by food vendors and consumers; Amoah et al. emphasized the importance of supporting farmers to ensure they don't face land insecurity or limited water treatment due to a lack of resources, which is likely a challenge that is shared in other low- and middle-income countries in addition to Ghana (4).

Street Food Contamination Studies

Street food, defined by WHO as "foods and beverages prepared and/or sold by vendors in streets and other public places for immediate consumption or consumption at a later time without further processing or preparation" (11), has increased in popularity recently for a number of reasons, mainly its affordability and convenience. However, street food vendors often fail to meet sanitation requirements and may lack knowledge about proper sanitary practices during food preparation (12). Islam and colleagues noted that the major sources of microbial contamination for street food are "infrastructure, preparation and storage, cooking, cleaning and serving utensils, quality of water and personal hygiene of food handlers" (13). Organisms commonly found in street food that can lead to foodborne illnesses are largely similar to those found in produce: *E. coli*, *Shigella*, *Salmonella*, *Vibrio cholerae*, *Campylobacter jejuni*, hepatitis A, hepatitis E, norovirus, and Nipah virus (14).

Bereda and colleagues conducted a cross-sectional study in Jigjiga, Ethiopia to examine the microbial contamination of street foods and the hygiene and sanitation practices of street food vendors (12). They defined street food as "foods and beverages prepared and/or sold by vendors and hawkers especially in streets around trading centers and other public places for immediate consumption or consumption at a later time without further processing or preparation" (12). Not only is street food typically not covered, but vendors operate from public places that usually fail to meet food and safety requirements, such as bus terminals, market places, and industrial areas. Factors contributing to the risk of contamination are the lack of vendors' education on proper food handling practices and keeping food at improper temperatures that favor microbial growth (12). In their study, Bereda et al. assessed 132 street vendors from four sites in Jigjiga using structured questionnaires, interviews, and observations. Of these, 33 vendors were selected for food sampling; 33 samples each of '*Fuol*', '*Ades*', '*Pasta*', and '*Sambusa*' were collected, totaling to 132 samples (12).

They found that majority of street food vendors were either self-taught (59.8%) or taught by parents (39.4%); only one vendor received formal training in food handling (12). Nearly 60% of vendors reported washing food prior to cooking, and around 14% warmed food prior to serving. Eighty-three percent of preparation surfaces were observed to be dirty, and 86% of vendors were observed to prepare food in "unhygienic conditions" (12). Three quarters of vendors handled food without using gloves, and all vendors observed also handled money while running their stalls. Microbiological analyses of food samples revealed that 72% of foods were contaminated, 68 samples were positive for *E. coli*, 85 samples were positive for *S. aureus*, and 26 were positive for *Salmonella spp* (12). The

'*Sambusa*' had the largest proportion of samples with *S. aureus* detection in 23 of 33 samples. *E. coli* was found in 24 '*Pasta*' samples out of 33. *Salmonella* was found in 8 '*Ades*' samples, but had the lowest detection rate of the three organisms. Overall, *S. aureus* detection was consistently greater than 60%, but *E. coli* was more common in '*Pasta*' dishes (12).

Bereda and colleagues reported results that were consistent with numerous other studies, including Muinde and Kuria's 2005 study in Nairobi, Kenya. They utilized similar methods of surveying vendors about food hygiene and sanitation practiced using structured interviews and observations. Muinde and Kuria found that most food was prepared at the stall, with 85% of preparation places noted as unhygienic upon observation. Nearly all vendors interviewed did not have garbage receptacles and disposed of wastewater beside the stall. Despite the expectation that level of education was related to sanitation practices, there was no statistically significant association between education and stall environment. Muinde and Kuria also found that many vendors did not wash foods due to a lack of water, oil was reused to cook multiple foods, and more than one type of food was usually prepared on the same surface (15). Bereda et al. had similar observations in Jigjiga (12). In their discussion, Muinde and Kuria noted that poor vendor personal hygiene, poorly constructed stalls, lack of covering food or utensils, and lack of water all contributed to the overall lack of hygiene in street food preparation (15). Bereda et al. concluded by encouraging increasing awareness of proper food handling and investment in social services such as education for vendors in response to the alarming level of microbiological contamination found in food samples.

Islam et al. conducted a study in Dhaka, Bangladesh to "identify the presence of common pathogens" that may be found in street food based on a previous study by Mohakhali and Aftabnagar in Dhaka (13). Foods included in sample collection were: deep fried and fried snacks; quick lunch items; pickles; fruit chutney; baked items; spicy, sour and hot snacks; juices, tamarind water and plain drinking water (13). Food samples were tested for *E. coli*, *Shigella*, *Salmonella*, and *Vibrio* spp. Half of the food samples were positive for *Salmonella,* and 46% were positive for *E. coli*; pathogenic *E.* coli strains were noted in 17% of samples (13). While there were initially some food samples positive for *Shigella* and one sample positive for *Vibrio*, further analyses were negative (13).

The investigators of studies of street food contamination (Islam et al., Bereda et al., and Muinde and Kuria) all recommend promoting education of food vendors on proper food handling procedures and person hygiene, as well as improving WASH infrastructure in areas with street food vendors, including access to water, toilets, and handwashing stations, to help minimize the risk of contamination of food and subsequent foodborne illness (12, 13, 15). Despite the risk of foodborne illness and the general lack of healthy options in street food offerings, many people still rely on street food because of its convenience, affordability, and lack of food preparation space and materials in some low-income households.

The SaniPath Approach

Robb and colleagues highlighted that some of the United Nations Sustainable Development Goals (SDGs), which replaced the Millennium Development Goals, capture water, sanitation, and hygiene initiatives: Goal Six aims to increase access to toilets and

safe fecal sludge management, and Goal Eleven aims to improve safety, resiliency, and sustainability of cities, which can be extended to urban sanitation (16). Scarcity of resources and space and rapid population growth, among other factors, adversely impact a low-income country's ability to provide "adequate water and sanitation services", leaving urban residents at an increased risk of exposure to fecal contamination (16). The SaniPath study was designed to "characterize risks from fecal contamination in low-income, urban environments and identify the dominant fecal exposure pathways". This approach used a wide breadth of data on exposure behavior, including focus groups, key informant interviews, structured observations, household surveys, and environmental microbiological data (16).

Two papers by Robb et al. and Wang et al. described the methods and results from the first SaniPath study, conducted over 16 months in Accra, Ghana from June 2011 to December 2012. Robb et al. reported that consumption of uncooked produce was the dominant exposure pathway, and the analyses by Wang et al. indicated that hands may be an important vehicle for transferring fecal microbes from contaminated surfaces to ingestion. They highlighted that urban agriculture has been an important contributor to food supply in this region, and that wastewater irrigation is often used, which correlated with the microbiological findings of produce contamination in this study. They concluded by emphasizing that produce contamination has impacts not solely on poor urban neighborhoods, but on all neighborhoods, as produce is grown and sold throughout the entire city. Furthermore, the shift towards increased produce consumption could indicate a transition from traditional diets and increased dependence on street-vended foods (which often included salads) by the poorer populations (16).

Wang and colleagues emphasized that in order to adequately quantify exposure, concentration of fecal microbes including *E. coli*, exposure behaviors and intake "must be quantified for each exposure pathway" (17). This study framework utilized structured observations of behaviors, environmental sample collection and analyses of water, food, and surfaces that children came in contact with, household surveys, and data on water usage, sanitation, and hygiene for children under age 5 in Accra. Observations included sanitary habits such as handwashing, bathing, and defecating, as well as playing and sleeping. The locations of observed activities were also recorded, which included dirt floors, open drains, stagnant water/trash areas, and concrete floors. Children's behavior was then modeled to show transitions from one behavior to another and concurrent transfer of fecal microbes (17). Environmental samples including tap water, household water, flood water, irrigated water, soil, raw produce, street food, and surface swabs were collected to assess fecal contamination by analyses for *E. coli* contamination (17).

Wang et al. found that exposure "depended not only on all the behaviors … but also on the order in which they occurred" (17). Numerous critical control points were identified, including exposure to fecal contamination in food; food was found to be the "greatest contribution of exposure to fecal contamination" (17). In order to lower the risk for fecal exposure via food, Wang et al. suggested minimizing the amount of uncooked food that children consume. Hands were also found to be an important part of the transfer of microbes from the environment to ingestion. Handwashing, among other child behaviors, when done in a particular frequency, duration, and order, will have different impacts on exposure to fecal contamination (17).

Green and colleagues utilized the SaniPath Exposure Assessment Tool to understand fecal contamination pathways in five neighborhoods in Siem Reap, Cambodia, which included analyzing drinking water, floodwater, raw produce, and ice. This field report focused on two informal settlements and three formal neighborhoods to identify trends and differences between neighborhood type (18). Green et al. noted that raw produce was a significant pathway of exposure to fecal contamination in each of the five neighborhoods included in the study; all participants were "exposed to high levels of fecal contamination (>10^4 [colony forming units (CFU)] of *E. coli* per month) from consuming produce" (18).

Conclusion

It is evident that some of the burden of foodborne disease can be attributed to poor water and sanitation, among other risk factors, and that microbial contamination of foods can occur at any number of steps along the pathway from farm to fork. Ingestion of uncooked produce, such as leafy greens, has been implicated more frequently than other foods as the cause for a foodborne illness or outbreak (5). Clear trends have been noted in the literature associating food handlers as a key source of contamination of produce and street food, and food vendor education is considered a critical measure for improving hygienic preparation of street food and street food safety. When considering control measures for minimizing food contamination, it is critical to consider a multifaceted approach that includes education of farmers and food handlers along the entire chain from production to consumption, promotion of personal hygiene and clean food preparation spaces, and use of clean water sources for irrigation, rinsing, and food preparation, and

general measures to prevent contact between produce and soil or water with human or animal feces. However, information on produce agricultural and marketing practices is lacking for many parts of the world, and solutions introduced in one country may not be relevant or applicable for other countries also dealing with produce- or street food-related foodborne outbreaks. Moreover, the foods attributed to outbreaks in one part of the world may not be consumed in a different region that also faces the burden of foodborne diseases.

More research is needed in order to understand which produce and street foods are more likely to be contaminated in various regions of the world, where contamination occurs in areas with differing agricultural practices and food consumption patterns, and how food consumption behaviors differ between adults and children in different contexts in order to help guide these regions in sustainably minimizing contamination of food. These points will be addressed in the analysis of the combined data from SaniPath deployments in Accra, Ghana; Atlanta, USA; Dakar, Senegal; Dhaka, Bangladesh; Kampala, Uganda; Kumasi, Ghana; Lusaka, Zambia; and Vellore, India.

Research Aims and Rationale

The overall goals of this research were to: 1) examine if city, type of produce or street food, and socioeconomic status of study neighborhoods are predictors for *E. coli* contamination in produce and street food samples collected in SaniPath deployments in different cities, and 2) characterize raw produce and street food consumption patterns across deployment sites and populations. The results of this analysis can inform water, sanitation, and hygiene interventions to improve food safety for populations at greatest risk of exposure to fecal-contaminated food.

CHAPTER II: MANUSCRIPT

Microbial Contamination and Consumption Patterns of Produce and Street Food Across SaniPath Study Sites

Melissa Erkens

Abstract

Foodborne diseases have been increasing globally, despite efforts to improve water, sanitation, and hygiene (WASH) conditions in areas with high disease burden. Food items can become contaminated at multiple points in the farm-to-fork pathway, and improperly prepared or uncooked foods can cause disease. Street food dishes and dishes with raw produce, such as salads, have also become increasingly popular. This analysis aimed to quantify the association between *E. coli* contamination and type of produce or street food, socioeconomic status (SES), and city where samples were collected, as well as characterize food consumption patterns for adults and children. Produce and street food samples were collected from 44 neighborhoods in ten cities in Africa, Asia, and the USA and analyzed for *E. coli* using membrane filtration or IDEXX. Sample type, SES, and city were modeled against *E. coli* concentration using logistic regression at multiple cutoffs. One member of each household surveyed in study sites was interviewed about household produce and street food consumption, and frequencies of behaviors were calculated for adults and children. Herbs, leafy, and root-underground vegetables had significantly higher odds of *E. coli* contamination above 2.93 $\log_{10}$ CFU/MPN compared to seeded vegetables. Mixed street food dishes had significantly higher odds of contamination above 2.67 $\log_{10}$ CFU/MPN and 2 $\log_{10}$ CFU/MPN compared to cooked dishes. Samples of street food from neighborhoods with higher SES had increased odds of *E. coli* contamination above 2.67 $\log_{10}$ CFU/MPN and 2 $\log_{10}$ CFU/MPN compared to low SES neighborhoods. Street food samples from Accra, Kampala, and Kumasi had significantly lower odds of *E. coli* contamination above 2.67 $\log_{10}$ CFU/MPN and 2 $\log_{10}$ CFU/MPN compared to Dhaka. Adults reported similar food consumption patterns between themselves and children in their household across study sites. There was a clear association between uncooked food items and increased odds of *E. coli* contamination, as well as different odds by SES or city. It is important to address poor food hygiene and understand how it is linked to inadequate sanitation in low-income settings in order to minimize the risk of contamination of popular food items and reduce the burden of foodborne diseases.

Introduction

Produce and street food are staple foods in nearly all cultures. The accessibility, affordability, and novelty of street food dishes make them appealing to everyone who happens to walk past a street vendor. Produce is a critical part of a healthy diet. However, uncooked produce and improperly prepared street food can become contaminated vehicles

for pathogen transmission and ultimately disease. The World Health Organization (WHO)'s Foodborne Disease Burden Epidemiology Reference Group (FERG) was established to estimate the global burden of foodborne diseases, and strengthen the capacity for countries to conduct outbreak assessments (1). In 2010, foodborne pathogens were estimated to have caused 600 million illnesses and 420,000 deaths, with 40% of the disease burden among children under five years old; the 31 foodborne hazards identified by FERG resulted in an estimated nearly 33 million disability-adjusted life years (DALYs) globally, meaning 33 million healthy years were lost in the global population in 2010 (1, 2). Africa D and E subregions, South-East Asia B and D subregions, and Eastern Mediterranean B and D subregions were found to have the three highest foodborne disease burdens per population (1).

Foodborne illnesses and outbreaks attributed to fresh produce have increased globally (3-7), and specific types of produce are "more frequently implicated" in such outbreaks, including leafy greens, jalapeños, tomatoes, and melons (5). Although these high-risk produce types have been identified, understanding where the produce becomes contaminated continues to be a challenge. Contamination can occur at nearly any point on the farm-to-fork pathway, including from the use of manure or contaminated water from livestock operations, through direct contact with animals, during harvesting, transport, processing, distribution, and marketing, or cross-contamination at home (3). Amoah et al. investigated the contamination of lettuce, which is commonly consumed raw as a part of salads or in street food dishes in Ghana. They found that contamination occurring prior to harvesting seemed to mask any downstream contamination, and contamination was associated with untreated water and incompletely composted manure (4). Bartz et al. found

that contamination from the hands of farm workers was the most significant contributor to contamination of produce (5). In their investigation of microbiological contamination on the surfaces of produce consumed in Dhanbad, India, Mritunjay and Kumar emphasized a need for improved agricultural practices, hygiene practices by food vendors, processors, and consumers, and surveillance of vegetable vendors (3). However, improving agriculture and hygiene practices is not always feasible, especially in low- and middle-income countries such as Ghana, as farmers may face competing challenges, such as land tenure insecurity, limited water treatment due to economic limitations, high demand, and frequent turnover of crops to meet that demand (4).

While street food provides a quick, convenient, and affordable food option in urban areas, vendors often fail to follow good hygiene practices and may lack knowledge about proper sanitary practices to prevent contamination during food preparation (12). Islam et al. noted that the major factors contributing to microbial contamination of street food in Dhaka are poor "infrastructure, preparation and storage, cooking, cleaning and serving utensils, quality of water and personal hygiene of food handlers" (13). Vendors often set up in populous areas, such as bus terminals, market places, and industrial areas, which do not have any food and safety requirements for street food vendors. This is of even greater concern when considering street food vendors that set up near schools to serve schoolchildren. Bereda et al. assessed numerous street vendors in Jigjiga, Ethiopia and found that only one vendor received formal training in food handling, and although most vendors reported washing food prior to cooking, most preparation surfaces were observed to be dirty, and vendors prepared food in "unhygienic conditions" (12). Nearly three quarters of food samples tested had microbial contamination (12). Muinde and Kuria's

study in Nairobi, Kenya also found that most food preparation places were unhygienic upon observation. Nearly all vendors interviewed did not have garbage receptacles; many disposed of wastewater beside the stall, did not wash foods due to a lack of water, reused cooking oil, and prepared more than one type of food on the same surface (15). Muinde and Kuria noted that poor vendor personal hygiene, poorly constructed stalls, lack of covering food or utensils, and lack of water all contributed to the overall lack of hygienic street food preparation (15). Bereda et al. encouraged increasing awareness of, and education on, proper food handling to minimize microbiological contamination of street food. Many investigators have recommended interventions to educate street food vendors on proper food handling and personal hygiene as well as improving sanitation infrastructure to minimize microbiological contamination of street food (12, 13, 15).

In order to better understand the impacts of poor WASH in urban contexts, the SaniPath study method was designed to "characterize risks from fecal contamination in low-income, urban environments and identify the dominant fecal exposure pathways". This approach includes collecting a wide breadth of behavioral data, through focus groups, key informant interviews, structured observations, household surveys, and environmental microbiological data (16). In the first SaniPath study in Accra, Robb et al. reported that food was the dominant exposure pathway, and the analyses by Wang et al. indicated that hands were an important vehicle for transferring fecal microbes from contaminated surfaces to ingestion. Wang et al. analyzed SaniPath behavioral data from structured observations of behaviors and microbiological data from environmental samples. They found that exposure "depended not only on all the behaviors … but also on the order in which they occurred" (17). Numerous critical control points were identified, including

exposure to fecal contamination in food, which was the "greatest contribution of exposure to fecal contamination" (17). In order to lower the risk of fecal exposure from food, Wang et al. suggested minimizing the amount of uncooked food that children consume. Contaminated hands were again found to be an important part of the transfer of microbes from the environment to ingestion (17). Green et al. utilized the SaniPath Exposure Assessment Tool to examine four different fecal exposure pathways in five neighborhoods in Siem Reap, Cambodia and found that raw produce posed a significant risk of exposure to fecal contamination in each of the five neighborhoods included in the study (18).

Robb et al. concluded by emphasizing that produce contamination has impacts not solely on poor urban neighborhoods, but on all neighborhoods, as produce is grown and sold throughout the entire city. Furthermore, the shift towards increased produce consumption indicate a transition away from traditional diets, corresponding with an increased dependence on street-vended foods (which often included salads) by poorer populations (16).

Microbial contamination of produce or street food can occur at any step along the farm-to-form pathway, and certain food items have been implicated more frequently with foodborne illness or outbreaks (5). There are also known associations with food handlers and vendors as key sources of food contamination. However, there are still many gaps in our knowledge of how foods become contaminated and the links between food contamination and poor urban WASH services. This study ultimately aims to increase understanding of which produce and street foods are more likely to be contaminated in various regions of the world and how food consumption behavior varies between adults and children in different contexts. Food consumption and microbial contamination trends

will be analyzed using the combined data from SaniPath deployments in Accra, Ghana; Atlanta, USA; Dakar, Senegal; Dhaka, Bangladesh; Kampala, Uganda; Kumasi, Ghana; Lusaka, Zambia; and Vellore, India. The results of this analysis can inform water, sanitation, and hygiene interventions to improve food safety for populations at greatest risk of exposure to fecal-contaminated food.

Methods

Data Collection

The SaniPath Tool is a systematic approach for estimating exposure to fecal contamination through multiple environmental pathways for both children and adults. This tool uses data from both behavioral observations and environmental samples and has been used in multiple field sites including: Accra, Ghana; Atlanta, USA; Dakar, Senegal; Dhaka, Bangladesh; Kampala, Uganda; Kumasi, Ghana; Lusaka, Zambia; and Vellore, India. Initial key informant interviews were conducted to inform which exposure pathways would be relevant, determine environmental sample sites, and provide the context for data collection. Transect walks through neighborhoods were done to identify sampling locations where the population is interacting with a potential exposure pathway, and to identify any sanitation risk factors. For produce and street food, the key informant interviews were important for identifying which uncooked produce items and street food were popular in the study neighborhood and where they were sold.

Exposure Behavior Surveys

Three approaches for collecting behavior data were conducted used at each of the field sites: community participatory meetings, household surveys, and school participatory surveys. Each survey collected the same information on frequencies of exposure behaviors. The community and school surveys were administered to groups of adults and children, respectively, and participants were recruited using convenience sampling. Households were selected using systematic random sampling, and the survey was administered to the adult that managed WASH at the household. Survey questions mainly focused on frequency of behavior. For example, participants were asked how frequently they consumed street food; adult groups were asked about consumption behavior among their children, and children were asked about their parents' behavior. For the analyses presented here, only the data from the household surveys at each field site were used.

Environmental Samples

Environmental samples were collected from up to ten exposure pathways drinking water, bathing water, surface water, ocean water, flood water, open drains, raw produce, street foods, public or shared toilets, and soil from public spaces. A minimum of ten samples per pathway was recommended. *E. coli* was selected as the indicator organism for fecal contamination. Surface swabs, soil samples, and food samples all required a processing step prior to analysis. Produce samples were rinsed using 500 mL of phosphate buffer saline (PBST), and the rinse solution was analyzed for *E. coli* concentration. Street food samples were homogenized using 100 mL of distilled water, and the solution was analyzed for the concentration of *E. coli*. Concentrations of *E. coli* in samples were either

measured as colony-forming units (CFU) using membrane filtration and m-ColiBlue24 ® broth media (Hach Company, Loveland CO) or Chromocolt ® Coliform Agar (EMD MilliporeSigma, Burlington, MA), or as most probable number (MPN) using IDEXX-Colilert-24® and the Quanti-Tray/2000 (IDEXX Laboratories, Westbrook, ME) (HACH 1999; ISO 2014; U.S. EPA 2017). M-ColiBlue24 and Chromocolt Coliform media and Colilert-24 media were incubated at 37°C for 20 to 24 hours per media manufacturer's guidelines. Two or three dilutions were analyzed for each sample to yield a reliable concentration estimate. Concentrations were expressed as CFU or MPN per serving of produce or street food.

Data Analysis

This analysis focused exclusively on responses and results from household exposure behavior surveys and environmental samples of produce and street food. For both the produce and street food samples, there were samples which did not pass data quality checking for determining *E. coli* concentration; 40 produce samples and 31 street food samples were excluded from analysis. The number of samples collected were reflected in the summary statistics data, but only samples with a quantifiable *E. coli* concentration were included in subsequent analyses.

The environmental sample data was used to model the odds of *E. coli* contamination above a specific cut-off value for produce type, street food type, socioeconomic status (SES), and city. *E. coli* concentration was dichotomized using two cut points: the median *E. coli* concentration for produce (2.93 $\log_{10}$ CFU) and street food (2.67 $\log_{10}$ CFU) as well as a 100 CFU (or 2 $\log_{10}$ CFU) cut-off used in Ireland as their standard for unsatisfactory

food (19); the 2 $\log_{10}$ CFU cut point was only modeled using street food data. Produce types were categorized into five groups using the Center for Disease Control and Prevention's Interagency Food Safety Analytics Collaboration food categorization scheme: fruits, herbs, seeded vegetables, leafy vegetables, and root-underground vegetables (Appendix A) (20). Street food was dichotomized into fully cooked dishes ("cooked") and dishes that were a mix of cooked and uncooked food items ("mixed"); categorizations were informed by research staff at Centers for Global Safe WASH (CGSW). Socioeconomic status was assigned for each neighborhood included in SaniPath deployments; neighborhoods were identified as high, middle, low, or very low income. Categorizations were informed by research staff at CGSW. Data were initially analyzed using the frequency procedure and Fisher's exact test, as some expected cell counts were below five.

Data were further analyzed using logistic regression models for produce and street food. During model selection for the produce model, socioeconomic status was removed from the model; there was no evidence of statistically significant interaction, and city was identified as a confounder. For the first street food model, city was removed from the model during model selection, and SES was retained. There was no evidence of statistically significant interaction or confounding between street food category and SES. In order to understand how *E. coli* contamination of street food varied by city, a second street food model was designed to force the inclusion of a subset of cities that had street food samples collected with observations above and below both *E. coli* concentration cut points: Accra, Ghana; Dhaka, Bangladesh; Kampala, Uganda; and Kumasi, Ghana. Odds ratios were calculated for *E. coli* contamination by 1) produce type, controlling for city as a confounder, 2) street food type and SES, and 3) city, controlling for street food type. Exact

logistic regression methods were used for data where expected cell counts fell below five. Household exposure behavior survey responses for produce and street food consumption were compared for adults and children using the frequency procedure, and plots were generated for each group by food type using the SGPANEL procedure. SAS (version 9.4; SAS Institute) was used to conduct all analyses.

Ethical Considerations

The study protocol was approved by the Institutional Review Board at Emory University, GA, USA (Protocol number: IRB00051584). These protocols were also reviewed and approved by local ethics boards in each deployment location.

Results

Summary Statistics

Environmental sample collection and exposure behavior surveys were conducted throughout twelve deployments in ten cities: Accra, Ghana; Atlanta, USA; Dakar, Senegal; Dhaka, Bangladesh; Kampala, Uganda; Kumasi, Ghana; Lusaka, Zambia; Maputo, Mozambique; Siem Reap, Cambodia; and Vellore, India. Samples of produce were collected from every deployment site, and street food samples were collected from 30 neighborhoods in six cities (Accra, Ghana; Dakar, Senegal; Dhaka, Bangladesh; Kampala, Uganda; Kumasi, Ghana; and Lusaka, Zambia). Dhaka contributed the largest proportion of samples for both sample types (21.5% of produce samples, 32.8% of street food samples) (Table 1a).

Among the produce samples (Table 1a), samples from Maputo yielded the highest mean *E. coli* concentration (4.30 $\log_{10}$ CFU, SD 1.65) per serving, and samples from Atlanta had the lowest mean concentration (1.40 $\log_{10}$ CFU, SD 0). Very low socioeconomic status (SES) neighborhoods made up the smallest proportion of samples stratified by SES, but yielded the highest mean *E. coli* concentration (3.91 $\log_{10}$ CFU, SD 1.10). There were 19 different produce items samples in this study. Nearly 70% of produce samples were categorized as seeded vegetables (69.0%) (Appendix A). Tomatoes were the most frequently sampled produce item (34.9%), with at least one tomato sample collected from all ten cities, followed by cucumbers (15.7%) and peppers (14.3%) (Appendix B). Herb samples, which included coriander, had the highest mean *E. coli* concentration per serving (4.67 $\log_{10}$ CFU, SD 0.86), and fruit samples, which included apples, watermelon, and guava, had the lowest mean *E. coli* concentration (2.03 $\log_{10}$ CFU, SD 1.03).

There were 18 different street food dishes sampled for this study, with two thirds of samples being completely cooked dishes (66.7%) (Appendix A). Street food samples (Table 1b) from Dhaka had the highest mean *E. coli* concentration (3.78 $\log_{10}$ CFU, SD 1.31), and the samples from the 2018 Lusaka deployment had the lowest mean *E. coli* concentration (1.34 $\log_{10}$ CFU, SD 0.53). In contrast to the results from produce samples, street food samples from high SES neighborhoods had the highest mean *E. coli* concentration (3.23 $\log_{10}$ CFU, SD 1.25). Cooked street food samples, such as fried yams and fritters, had a lower mean *E. coli* concentration compared to street foods that were mixed dishes (such as fuska with chotpoti) that included both cooked and uncooked food items (2.38 $\log_{10}$ CFU, SD 1.03 for cooked dishes; 3.37 $\log_{10}$ CFU, SD 1.37 for mixed dishes). Fuska with chotpoti was the most frequently sampled street food item (20.1%),

and all samples were from Dhaka (Appendix B). Puffed rice, a mixed dish from Dhaka, had the highest mean *E. coli* concentration of all street food dishes (3.84 $\log_{10}$ CFU, SD 1.32). Belpuri, another mixed dish from Dhaka had the lowest mean *E. coli* concentration, but there was only one sample of this dish (0.69 $\log_{10}$ CFU).

Household surveys were administered to over 4500 households, with approximately 60% of homes being identified as compounds as opposed to single-family homes (Table 1c). Households reported an average population of 8.38 people per household (SD 8.68), and 86% of households reported having children between ages five and twelve years.

Logistic Regression Model – Produce

Model 1: $\ln(odds\ of\ E.coli\ contamination) = \alpha + \beta_1 Produce\ Type + \beta_2 City$

In assessing the association between produce type, socioeconomic status (SES), and *E. coli* concentration using exact methods, herbs were associated with significantly higher odds of being contaminated above the median cut point of 2.93 $\log_{10}$ CFU of *E. coli* per serving compared to seeded vegetables, after controlling for city (OR = 62.86, 95% CI: 9.03, >999.999) (Table 2a). The odds of leafy vegetables being contaminated above the median were 2.86 times higher than the odds for seeded vegetables, controlling for city (OR = 2.86, 95% CI: 1.33, 5.39). Likewise, the odds of contamination among root-underground vegetables was 4.48 times higher than for seeded vegetables, controlling for city (OR = 4.48, 95% CI: 1.14, 25.34). The odds of contamination above the median among fruits was 43% lower than the odds of contamination in seeded vegetables, controlling for city, but was not statistically significant (OR = 0.57, 95% CI: 0.01, 5.67).

Logistic Regression Model – Street Food and SES

$$\text{Model 2: } \ln(odds\ of\ E.coli\ contamination) = \alpha + \beta_1 Street\ Food\ Type + \beta_2 SES$$

Using the median cut point of 2.67 $\log_{10}$ *E. coli* CFU, there were statistically significantly higher odds of mixed street food samples with *E. coli* concentrations above the median compared to samples of cooked dishes, controlling for SES (OR = 3.73, 95% CI: 2.18, 6.37) (Table 2a). Samples from high SES neighborhoods had 83% higher odds of being contaminated over the median *E. coli* concentration compared to low SES neighborhoods, controlling for street food type, though this association was not statistically significant (OR = 1.83, 95% CI: 0.92, 3.62). The odds of samples from middle SES neighborhoods being contaminated above the median were 2.72 times higher than samples from low SES neighborhoods, controlling for street food type (OR = 2.72, 95% CI: 1.18, 6.23).

Using Ireland's 2 $\log_{10}$ CFU cut point for *E. coli*, the odds of contamination in mixed street food dishes remained significantly higher compared to cooked dishes, controlling for SES (OR = 2.83, 95% CI: 1.59, 5.03). Street food samples from high SES neighborhoods were associated with 2.32-times higher odds of contamination compared to samples from low SES neighborhoods, controlling for street food type (OR = 2.32, 95% CI: 1.04, 5.15), and the odds of contamination above 2 $\log_{10}$ CFU in samples from middle SES neighborhoods were 7.54 higher than low SES neighborhoods, controlling for street food type (OR = 7.54, 95% CI: 2.16, 26.26).

Logistic Regression Model – City and Street Food

Model 3: $\ln(odds\ of\ E.coli\ contamination) = \alpha + \beta_1 Street\ Food\ Type + \beta_2 City$

In the subset of cities included in this model, Dhaka had the highest proportion of street food samples above the median cut point of 2.67 $\log_{10}$ *E. coli* CFU (66 of 84, 78.6%). In Accra, only 35% of samples (7 of 20) had *E. coli* concentrations above the median. One-third of samples from Kampala had *E. coli* concentrations above the median (11 of 33), and just over 15% of samples from Kumasi were contaminated above the median (6 of 39). The odds of street food samples from Accra being contaminated above the median were 92% lower than the odds of contamination from Dhaka, controlling for street food type (OR = 0.08, 95% CI: 0.02, 0.53) (Table 2c). Similarly, the odds of contamination above the median among samples from Kampala were 91% lower than Dhaka, controlling for street food type (OR = 0.09, 95% CI: 0.02, 0.37), and the odds of contamination in samples from Kumasi were 97% lower than Dhaka, controlling for street food type (OR = 0.03, 0.004, 0.18).

Using the 2 $\log_{10}$ *E. coli* CFU cut point, nearly 93% of street food samples from Dhaka were contaminated above 2 $\log_{10}$ CFU (78 of 84). Eighty-five percent of samples from Accra (17 of 30), 61% of samples from Kampala (20 of 33), and 59% samples from Kumasi (23 of 39) were contaminated above this cut point. The odds of contamination above 2 $\log_{10}$ CFU in street food samples from Accra were 93% lower compared to samples from Dhaka, controlling for street food type (OR = 0.07, 95% CI: 0.005, 1.00). The odds of samples from Kampala being contaminated above 2 $\log_{10}$ CFU were 96% lower than the odds from Dhaka, controlling for street food type (OR = 0.04, 95% CI: 0.007, 0.20). The odds of samples from Kumasi being contaminated above 2 $\log_{10}$ CFU were 98% lower than

the odds compared to Dhaka, controlling for street food type (OR = 0.02, 95% CI: 0.002, 0.16).

Household Exposure Behavior Surveys

The results from the household surveys of self-reported behavior revealed that the majority of adult participants reported that they consumed raw produce less than five times in the past week (Accra, 44.4%; Atlanta, 45.5%; Dakar 2019, 71.2%; Dhaka, 49.5%; Kampala, 47.5%; Kumasi, 40.8%; Maputo, 88.2%; Siem Reap, 88.1%; Vellore, 49.5%) (Figure 1). Over half of participants from Lusaka reported never consuming raw produce in the past week (53.8%). Participants in all cities, except Vellore, reported similar results when asked about any children between ages five and twelve living in the house. In Vellore, the majority of participants indicated children never consumed produce in the past week (41.0%), followed by reporting the same frequency of consumption of less than five times in the past week (39.5%).

In Lusaka, nearly half of participants indicated that they never consumed street food in the past week (47.5%); participants also reported similar frequencies of consuming street food among children in their household (Figure 1). In Accra, Dakar, Dhaka, and Kumasi, the majority of participants reported consuming street food less than five times in the past week (Accra, 53.0%; Dakar, 30.0%; Dhaka, 47.1%; Kumasi, 37.3%). In Kampala, the majority of participants reported consuming street food six to ten times in the past week (44.0%), and reported the similar frequencies for children. Participants in Dakar and Kumasi reported that the majority of children in the households consumed street food more

than ten times in the past week (Dakar, 30.2%; Kumasi, 38.8%). In all cities, the majority of participants reported washing raw produce prior to consuming it.

Discussion

Summary Statistics

Produce samples from Maputo had the highest levels of *E. coli* contamination (4.30 $\log_{10}$ *E. coli* CFU, SD 1.65) (Table 1a). Only three produce types were collected from Maputo (cucumber, lettuce, and tomato); lettuce (4.01 $\log_{10}$ CFU, SD 1.33) and cucumber (3.08 $\log_{10}$ CFU, SD 1.42) both had mean *E. coli* concentrations above the median cut point of 2.93 $\log_{10}$ CFU, and the mean for tomatoes was just below this cut point (2.69 $\log_{10}$ CFU, SD 1.33) (Appendix B). It is possible that the high mean contamination of produce samples from Maputo is attributable to the types of samples collected, including two highly contaminated produce types, but this could also indicate that hygiene in Maputo is poorer than the other cities. Vellore also had a high mean level of *E. coli* contamination (4.12 $\log_{10}$ CFU, SD 1.11), and also had produce samples with high mean values, such as coriander (4.67 $\log_{10}$ CFU, SD 0.86), green chili peppers (3.79 $\log_{10}$ CFU, SD 1.54), okra (3.76 $\log_{10}$ CFU, SD 0.29), and tomatoes (2.69 $\log_{10}$ CFU, SD 1.33) (Appendix B). The high mean *E. coli* concentrations in produce samples from Vellore could be attributable to the produce types sampled or to poor hygiene. Atlanta had the lowest mean level of *E. coli* contamination (1.40 $\log_{10}$ CFU, SD 0), despite having samples of more highly contaminated produce items as highlighted above (cucumber, lettuce, and tomatoes). This implies that the different hygiene standards in the cities (or at the farms and packing plants where the produce originated) are a critical factor for *E. coli* contamination. High

contamination of produce samples from the countries in the AFRO and SEARO WHO regions correlates with a greater risk for foodborne diseases due to microbiological contamination, which is consistent with previous estimates of the burden of foodborne disease (1).

Produce samples from very low SES neighborhoods had the highest mean *E. coli* contamination (3.91 $\log_{10}$ *E. coli* CFU, SD 1.10), but overall the distribution of *E. coli* contamination by SES was similar for low, middle, and high SES (Table 1a). Produce samples from low SES neighborhoods had a mean *E. coli* concentration of 3.06 $\log_{10}$ CFU (SD 1.44), samples from middle SES neighborhoods had a mean concentration of 3.61 $\log_{10}$ CFU (SD 1.68), and samples from high SES neighborhoods had a mean concentration of 3.10 $\log_{10}$ CFU (SD 1.31). This result was expected, as many different neighborhoods in urban areas may share the same produce market. Additionally, produce samples at different markets may come from the same farm where they may initially be contaminated, so the burden of produce contamination can impact all neighborhoods, which was emphasized by Robb et al. (16).

Produce types with the highest *E. coli* contamination mean value were consistent with reports from the literature. Coriander (herbs) had the highest mean value for *E. coli* concentration (4.67 $\log_{10}$ *E. coli* CFU, SD 0.67), followed by eggplant (seeded vegetables) (4.47 $\log_{10}$ CFU, SD 0.51), lettuce (leafy vegetables) (4.01 $\log_{10}$ CFU, 1.33), and salad (leafy vegetables) (4.21 $\log_{10}$ CFU, 1.47) (Appendix B). Leafy greens and herbs have frequently been associated with previous foodborne outbreaks, as noted by Machado-Moreira et al. (7). Additionally, Amoah et al.'s study in Ghana quantifying the presence of microbiological contamination of lettuce showed high levels of fecal coliforms in lettuce

samples, so leafy greens were expected to be highly contaminated in this study (4). Seeded vegetables such as tomatoes and peppers have also been associated with foodborne outbreaks, so these produce items were also expected to be highly contaminated (5). Eggplant was not noted in the literature as being highly contaminated, although it had the second highest mean value for *E. coli* contamination. There were only two samples of eggplant taken from Siem Reap, Cambodia, so it is possible that these samples were incidentally highly contaminated. Eggplant is also not commonly consumed raw around the world, which is likely the primary reason behind not finding outbreaks related to eggplant in the literature.

Street food samples from Dhaka had the highest mean *E. coli* concentration (3.78 $\log_{10}$ *E. coli* CFU, SD 1.31), followed by samples from Accra (3.14 $\log_{10}$ CFU, SD 1.63). Samples from Lusaka in 2018 had the lowest mean *E. coli* concentration (1.34 $\log_{10}$ CFU, 0.53) (Table 1b). Street food dishes sampled in Lusaka (egg wraps, fritters, roasted maize, and scones) had the lowest means for *E. coli* contamination (egg wraps, 1.56 $\log_{10}$ CFU, SD 0.21; fritters, 1.38 $\log_{10}$ CFU, SD 0.43; roasted maize 1.30 $\log_{10}$ CFU; scones 1.92 $\log_{10}$ CFU) (Appendix B). Fritters, roasted maize, and scones were all fully cooked dishes, and egg wraps are mixed dishes (Appendix A). Fuska with chotpoti and puffed rice dishes were the two most common dishes sampled (20.1% and 12.5% of samples, respectively), and had the two highest mean *E. coli* contamination (3.80 $\log_{10}$ CFU, SD 1.26 and 3.84 $\log_{10}$ CFU, SD 1.32, respectively) (Appendix B). Both of these dishes were mixed dishes and were only sampled in Dhaka; mixed street food dishes appear to be more common in Dhaka compared to the other cities. The inclusion of uncooked food items in these dishes is likely the main reason for higher *E. coli* contamination.

Street food samples from low SES neighborhoods made up the largest proportion of samples (71.2%), but had the lowest mean *E. coli* concentration (2.66 $\log_{10}$ *E. coli* CFU, SD 1.27) (Table 1b). Samples from high SES neighborhoods had the highest mean *E. coli* concentration (3.23 $\log_{10}$ CFU, SD 1.25). Many street food dishes sampled from low SES neighborhoods were not sampled from high or middle SES neighborhoods, such as chapati with beans (3.06 $\log_{10}$ CFU, SD 1.14), egg wraps (1.56 $\log_{10}$ CFU, SD 0.21), fritters (1.38 $\log_{10}$ CFU, SD 0.43), Rolexes (2.48 $\log_{10}$ CFU, SD 1.02), and samosas (2.28 $\log_{10}$ CFU, SD 1.33) (Appendix B). Most of these dishes had lower mean *E. coli* concentrations compared to other street food samples, which is likely the main reason why the overall mean for street food samples from low SES neighborhoods is lower than for samples from middle or high SES neighborhoods.

Logistic Regression Model – Produce

Herbs were associated with significantly higher odds of contamination about the 2.93 $\log_{10}$ *E. coli* CFU threshold compared to fruits; the odds of herbs being contaminated above this cut point were nearly 63 times greater than that of seeded vegetables, after controlling for city (OR = 62.86, 95% CI: 9.03, >999.999) (Table 2a). This is not surprising, as herbs such as coriander (the same plant as cilantro) have been associated with foodborne outbreaks in the past (7). The leaves from the herbs can easily come in contact with *E. coli*-contaminated water or soil, subsequently contaminating the food and, if the leaves are not thoroughly rinsed, putting the consumer is at risk for disease. The width of the confidence interval is most likely due to sample size, as herbs had only one observation that fell below the median cut point, despite using exact methods to compute the odds ratio.

The odds of leafy vegetables, which included lettuce and cabbage, being contaminated above the median cut point were 2.86 times higher than seeded vegetables (OR = 2.86, 95% CI: 1.33, 5.39). Just over 70% of leafy vegetable samples (53 of 75) were contaminated above the cut point, compared to nearly 40% of seeded vegetable samples (117 of 295) (Table 2a). Findings for leafy vegetables were consistent with what was reported in the literature, as lettuce is commonly associated with foodborne outbreaks and lettuce was previously found to be highly contaminated in Amoah et al.'s study in Ghana (4, 7). Similar to herbs, the leaves are easily contaminated since these crops grow on the ground, have large surface area, and can easily come in contact with contaminated soil and surface water (7). Interestingly, Mritunjay and Kumar found that cabbage was not as highly contaminated as other leafy greens in their study due to the more densely-leaved heads (3); in this study, the mean *E. coli* concentration for cabbage was 3.22 $\log_{10}$ CFU (SD 1.52), which was higher than both cut points used in analysis, indicating a high level of contamination. Similar results were seen with root-underground vegetables, which included carrots and spring onions; the odds of *E. coli* contamination above the median was 4.48 times higher for root-underground vegetables compared to seeded vegetables (OR = 4.48, 95% CI: 1.14, 25.34). Contamination of root-underground vegetables could likely be caused from contact with contaminated soil or water, both on the ground and on the surface, for sprouting vegetables.

Fruits had the most similar distribution of *E. coli* contamination compared to seeded vegetables; 90% of fruit samples (9 of 10) had *E. coli* concentration below the median of 2.93 $\log_{10}$ CFU, and just over 60% of seeded vegetable samples (178 of 295) fell below the median (Table 2a). The odds of fruits being contaminated above the median cut point was

43% lower than the odds of seeded vegetables (OR = 0.57, 95% CI: 0.01, 5.67). This was not surprising, as the seeded vegetable category contained some produce items previously associated with foodborne-related outbreaks or found to be contaminated in prior studies, including peppers, cucumbers, and tomatoes (5). Fruits that were included in this study (apples, watermelon, and guavas) were not noted in the literature as being common vehicles for foodborne outbreaks, although there have been outbreaks attributed to these fruits (7). The lack of statistical significance is most likely due to the large difference between the sample sizes for seeded vegetables (N = 295) compared to fruits (N = 10).

Logistic Regression Model – Street Food and SES

Mixed street food dishes, containing cooked and uncooked food items, were associated with 3.73-times higher odds of contamination above the median *E. coli* concentration of 2.67 $\log_{10}$ *E. coli* CFU compared to fully cooked street food dishes, controlling for SES (OR = 3.73, 95% CI: 2.18, 6.37) (Table 2b). This relationship between street food types was retained when Ireland's 2 $\log_{10}$ CFU cut point was used (OR = 2.83, 95% CI: 1.59, 5.03) (Table 2c). This 2 $\log_{10}$ CFU cut point is used to determine if microbiological contamination of a food item is unsatisfactory or acceptable for consumption. If a food item is found to be contaminated above this threshold, actions such as food recalls and investigations are taken to protect the health of consumers and improve the hygiene practices of the producer or packer. This standard is used across different food items, such as nuts, seeds, vegetables, and meat in Ireland (19). These results are consistent with expectations that the inclusion of uncooked food items could lead to higher contamination. Street food dishes may include uncooked foods in a number of ways: dishes

may simply have an herb garnish, include a salad, or have raw produce such as tomatoes incorporated. The results of the street food analysis after controlling for SES correspond with the increased odds of contamination associated with raw produce items. If a street food vendor acquires produce, such as herbs or salad, that is contaminated and uses it in a street food dish without cooking it, then the entire dish becomes contaminated.

Interestingly, samples from high or middle SES neighborhoods were associated with increased odds of contamination above the median compared to samples from low SES neighborhoods, controlling for street food type (High SES OR = 1.83, 95% CI: 0.92, 3.62; Middle SES OR = 2.91, 95% CI: 1.24, 6.82) (Table 2b). Of the 195 samples of street food collected from low SES neighborhoods, only 45.6% of samples (89 of 195) had an *E. coli* concentration above the median. Sixty-six percent of samples from high SES neighborhoods (33 of 50) and 58.6% of samples from middle SES neighborhoods (17 of 29) had *E. coli* concentrations above the median.

When the 2 $\log_{10}$ CFU of *E. coli* cut point was used, this association was even stronger, as a higher proportion of samples from high SES neighborhoods (41 of 50) and middle SES neighborhoods (26 of 29) were above this cut point (Table 2c). The odds of samples from high SES neighborhoods being contaminated above the 2 $\log_{10}$ CFU of *E. coli* cut point is more than two-fold higher compared to the odds for samples collected from low SES neighborhoods, controlling for street food type (OR = 2.32, 95% CI: 1.04, 5.15). Likewise, the odds of samples from middle SES neighborhoods being contaminated above this cut point were more than seven times higher compared to samples from low SES neighborhoods (OR = 7.54, 95% CI: 2.16, 26.26). These results were intriguing, as it was

not expected that higher SES would correlate with increased likelihood of contaminated street food.

Many street food dishes sampled from low SES neighborhoods were not sampled from high or middle SES neighborhoods, such as chapati with beans, egg wraps, fritters, Rolexes, and samosas; with the exception of chapati with beans, all of these dishes had mean *E. coli* concentrations below the median (egg wraps, 1.56 $\log_{10}$ CFU, SD 0.21; fritters, 1.38 $\log_{10}$ CFU, SD 0.43; Rolex, 2.48 $\log_{10}$ CFU, SD 1.02; samosas, 2.28 $\log_{10}$ CFU, SD 1.33) (Appendix B). The mean *E. coli* concentration for egg wraps and fritters also fell below Ireland's 2 $\log_{10}$ CFU cutoff (Appendix B). The samples collected only in low SES neighborhoods with mean concentrations below the median and 2 $\log_{10}$ CFU cut points are most likely driving the association between SES and *E. coli* contamination. Additionally, street food vendors are often set up in public spaces that may be shared by individuals from high and low SES neighborhoods, regardless of the income of the neighborhood the vendor is in. As noted in the literature, street food vendor sanitation is reliant on the availability of water or waste disposal at their stall or in the area they are in, so despite the fact that a vendor may be set up in a high-income location, they may not have adequate waste disposal practices, proper food storage capacities, or access to clean water to wash food items. They may also reuse cooking oil and preparation space throughout the day, increasing the risk of contamination of dishes.

Logistic Regression Model – City and Street Food

Data from a subset of cities was included in this model to see if there was a significant difference in street food sample contamination by cities. While the frequencies

of samples falling above both the median cut point of 2.67 $\log_{10}$ *E. coli* CFU and 2 $\log_{10}$ CFU cut point indicated samples from Dhaka were more contaminated, the strength of the associations were surprising. At both cut points, all other cities included in this subset had more than 90% lower odds of contamination of street food compared to Dhaka (Tables 2b and 2c). This result was also interesting as Dhaka is located in south Asia and Accra, Kampala, and Kumasi are located in Africa. In one study in Ethiopia, 72% of street food samples were contaminated, 68 of which were contaminated with *E. coli* (51.5%) (12). This study identified any microbiological contamination, whereas this analysis is looking at *E. coli* concentration above two cut points. Using the 2 $\log_{10}$ CFU cut point, all four cities included in this subset had a higher frequency of samples contaminated above this cut point compared to *E. coli* contamination of samples in Ethiopia (Accra, 85.0%; Dhaka 92.9%; Kampala, 60.6%; Kumasi, 59.0%) (Table 2c). Further research is needed to understand the role of geography in street food contamination, but this data suggests that samples included in this study are more contaminated than what has been noted in Ethiopia, although comparisons are not being made on the same scale. This also suggests that the odds of contamination are quite different between the different food cultures (although the data in this subset were limited to only three countries).

Household Exposure Behavior Surveys

The household behavior surveys collected data from individuals who managed WASH for the household. In nearly all cities, participants reported the same frequency in consumption of produce between themselves and children living in the household (Figure 1). In Dakar and Kumasi, the majority of participants reported that children consumed

street food more frequently in the past week than adults did. Majority of participants in Accra, Dhaka, Kampala, and Lusaka reported the same frequency of street food consumption for children and adults. Looking at all cities, there was an increased number of participants who reported that children consumed street food six to ten or more than ten times per week compared to what participants indicated about themselves, meaning that although majority of children consumed street food as frequently as adults did, overall children are consuming street food more frequently than adults.

These results are consistent with the expectation that food consumption patterns are likely to be similar between individuals living in the same household, especially given that the individual interviewed in the household surveys was the one who managed WASH, which likely included food preparation. Understanding the frequencies at which households consume produce and street food is critical for advising on sanitation practices. Households that consume street food more frequently, for example, could have a higher risk of being exposed to contaminated foods. Street food vendors may choose to set up their stalls near schools to attract the crowds of both children and adults, so it would be vital to ensure vendors were handling foods properly to avoid contamination. It was reassuring to see that an overwhelming majority of households reported washing produce prior to consumption, so that brings encouragement that families are practicing safe food handling at home. However, their methods of washing produce items were not assessed in this research, and washing of produce may not be sufficient for decontaminating produce (3, 4).

Strengths and Limitations

This analysis was designed to examine the associations between produce type, street food type, socioeconomic status, and *E. coli* contamination of produce and street food as well as describe the trends in produce and street food consumption among children and adults. The data from each of the SaniPath deployments were combined into a single dataset, and the project analyzed the aggregate data to review these trends and associations. While other studies exist quantifying the presence of microbiological contamination of certain food items (3-6, 12, 13), this study's greatest strength is that it quantifies and compares microbiological contamination of 37 produce and street food items across multiple neighborhoods in ten different cities and nine countries.

There are numerous limitations to consider in interpreting these results. For the environmental samples, there were small sample sizes for several different food items. There were also many samples that had to be excluded from analysis due to data quality issues, which further decreased the sample size. Additionally, one of the produce types, "long plant", was not able to be categorized and was subsequently excluded from analysis.

While confounding by city was controlled for in the produce logistic regression model, there was no further assessment of residual confounding using bias analysis that could then be corrected. In the street food model including city, only the four cities from three countries with observations above and below the two cut points were included, limiting the generalizability of these results to only those three countries. Bias analyses were not performed for the data, so any bias noted is not quantified or corrected.

The household exposure behavior surveys record self-reported behavior, and participants were asked to recall produce and street food consumption from the past week

to study staff. These data could be susceptible to recall bias and bias due to social desirability. This analysis only used the data from the exposure behavior household survey which may not accurately capture the behavior of children (especially when they are not at home) because it is based on the adult's beliefs about the behavior of children in their household. The results from the community and school exposure behavior surveys, respectively administered to groups of adults and children, could have yielded different results, and the results may not be consistent across survey types. The household survey also does not take into account the types of street food dishes being consumed by adults and children.

Conclusion

E. coli contamination of raw produce and street foods, particularly dishes including uncooked components, poses a considerable public health threat to consumers. This study examined a wide range of produce and street foods in ten cities in nine countries. It is essential to understand more about how the diverse foods in these settings become contaminated and what could be done to prevent or reduce contamination before consumption. Previous studies have recommended education interventions focused on food handlers to help prevent contamination of food items (3, 10, 12, 13, 15). Street food vendors should be offered educational resources on proper handling of food, food storage, minimizing reuse of cooking oils, sanitizing food preparation surfaces, and washing food items prior to cooking or incorporating in dishes, especially foods that are not cooked. Likewise, it is important to ensure farmers are equipped to grow and handle produce safely, practice personal hygiene, and use clean water, soil, and fertilizer. Knowing that children

consume street food more times throughout the week than adults, potentially because vendors are located near schools, there should be targeted messaging at schools for children to understand their risk of consuming contaminated foods and how to choose safer foods and prevent contamination. It is also critical to continue promoting education for communities on safe food handling practices at home.

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TABLES

Table 1a. Summary statistics for *E. coli* contamination in produce samples[a,b]

		Produce Samples		
	Total N (%)[c]	**N (%)[c]**	**Mean (SD)[d]**	**Median (Range)[d]**
Location of Deployments (Year)				
Accra, Ghana (2016)	70 (15.05)	59 (13.88)	3.30 (1.74)	2.78 (1.40-6.00)
Accra, Ghana (2018)	20 (4.30)	20 (4.71)	3.97 (1.61)	3.84 (1.40-6.00)
Atlanta, USA (2016)	10 (2.15)	10 (2.35)	1.40 (0)	1.40 (1.40-1.40)
Dakar, Senegal (2019)	50 (10.75)	50 (11.76)	3.18 (1.17)	2.40 (2.40-6.00)
Dhaka, Bangladesh (2017)	100 (21.51)	87 (20.47)	3.13 (1.45)	2.82 (1.40-6.06)
Kampala, Uganda (2018)	50 (10.75)	41 (9.65)	2.97 (1.30)	3.18 (1.30-4.97)
Kumasi, Ghana (2018)	39 (8.39)	38 (8.94)	3.47 (1.19)	3.59 (1.40-5.59)
Lusaka, Zambia (2018)	20 (4.30)	19 (4.47)	2.28 (1.11)	2.18 (1.40-5.50)
Lusaka, Zambia (2019)	30 (6.45)	30 (7.06)	2.05 (0.99)	1.55 (1.40-5.18)
Maputo, Mozambique (2016)	23 (4.95)	22 (5.18)	4.30 (1.65)	3.78 (2.40-7.00)
Siem Reap, Cambodia (2016)	33 (7.10)	29 (6.82)	4.09 (1.26)	4.13 (1.40-6.00)
Vellore, India (2014)	20 (4.30)	20 (4.71)	4.12 (1.11)	3.90 (2.40-6.00)
Total	465	425		
SES[e]				
Very Low	20 (4.52)	16 (4.96)	3.91 (1.10)	3.91 (2.48-6.00)
Low	290 (65.61)	268 (71.96)	3.06 (1.44)	2.78 (1.40-6.06)
Middle	62 (14.03)	54 (15.38)	3.61 (1.68)	2.98 (1.40-6.00)
High	70 (15.84)	65 (17.37)	3.10 (1.31)	2.70 (1.40-6.00)
Produce Samples[f]				
Vegetables				
Root-underground	14 (3.01)	12 (2.82)	3.74 (1.03)	4.01 (2.40-5.22)
Seeded vegetables	321 (69.03)	295 (69.41)	2.98 (1.44)	2.69 (1.40-7.00)
Herbs	35 (7.53)	30 (7.06)	4.67 (0.86)	4.74 (2.70-6.06)
Leafy	81 (17.42)	75 (17.65)	3.74 (1.47)	3.83 (1.40-7.00)
Fruits	11 (2.37)	10 (2.35)	2.03 (1.03)	1.40 (1.40-5.50)
Uncategorized[b]	3 (0.65)	3 (0.71)	2.70 (1.13)	3.22 (1.40-3.48)

[a] "-" values indicate data was not collected on that variable for the respective group

[b] Includes entries where correct sample_type is specified but food name entered as "NA"

[c] Total N includes all samples collected, N includes samples with a calculated *E. coli* concentration

[d] Concentration in $\log_{10}$ colony forming units ($\log_{10}$ CFU) of *E. coli* per serving

[e] Excludes samples from neighborhood UID 801 and 802 as these were repeat samples from a single neighborhood

[f] Categories assigned per CDC designations of food groupings

Table 1b. Summary statistics for *E. coli* contamination in street food samples[a,b]

		Street Food Samples		
	Total N (%)[c]	**N (%)[c]**	**Mean (SD)[d]**	**Median (Range)[d]**
Location of Deployments (Year)				
Accra, Ghana (2016)	0	0	-	-
Accra, Ghana (2018)	20 (6.65)	20 (7.30)	3.14 (1.63)	2.29 (1.95-6.55)
Atlanta, USA (2016)	0	0	-	-
Dakar, Senegal (2019)	50 (16.39)	49 (17.88)	2.78 (0.21)	2.70 (2.67-3.83)
Dhaka, Bangladesh (2017)	100 (32.79)	84 (30.66)	3.78 (1.31)	3.56 (0.69-6.44)
Kampala, Uganda (2018)	45 (14.75)	33 (12.04)	2.73 (1.17)	2.18 (1.54-5.56)
Kumasi, Ghana (2018)	40 (13.11)	39 (14.23)	2.28 (0.64)	2.15 (1.32-4.34)
Lusaka, Zambia (2018)	20 (6.56)	19 (6.93)	1.34 (0.53)	1.38 (0.70-1.99)
Lusaka, Zambia (2019)	30 (9.84)	30 (10.95)	1.50 (0.23)	1.53 (0.98-1.99)
Maputo, Mozambique (2016)	0	0	-	-
Siem Reap, Cambodia (2016)	0	0	-	-
Vellore, India (2014)	0	0	-	-
Total	305	274		
SES[e]				
Very Low	0	0	-	-
Low	215 (70.49)	195 (71.17)	2.66 (1.27)	2.30 (0.69-6.44)
Middle	30 (9.84)	29 (10.58)	2.94 (1.22)	2.69 (1.32-6.55)
High	60 (19.67)	50 (18.25)	3.23 (1.25)	2.80 (1.22-5.94)
Street Food Samples[f]				
Cooked	165 (54.10)	156 (56.93)	2.38 (1.03)	2.26 (0.70-6.55)
Mixed cooked and uncooked	138 (45.25)	116 (42.34)	3.37 (1.37)	3.25 (0.69-6.44)
Uncategorized[b]	2 (0.66)	2 (0.73)	1.52 (0.23)	1.52 (1.36-1.69)

[a] "-" values indicate data was not collected on that variable for the respective group

[b] Includes entries where correct sample_type is specified but food name entered as "NA"

[c] Total N includes all samples collected, N includes samples with a calculated *E. coli* concentration

[d] Concentration in $\log_{10}$ colony forming units ($\log_{10}$ CFU) of *E. coli* per serving

[e] Excludes samples from neighborhood UID 801 and 802 as these were repeat samples from a single neighborhood

[f] Categories assigned in consultation with SaniPath team

Table 1c. Summary statistics for household surveys of produce and street food consumption

	Number of Homes Surveyed N (%)	Living in Single Family Home N (%)	Household Size Mean (SD)	Participants with Children ages 5-12 N (%)
Location of Deployments (Year)				
Accra, Ghana (2016)	821 (17.90)	377 (45.92)	14.47 (12.90)	467 (56.88)
Accra, Ghana (2018)	200 (4.36)	123 (61.50)	5.07 (1.61)	200 (100)
Atlanta, USA (2016)	23 (0.50)	-	3.65 (2.06)	6 (26.09)
Dakar, Senegal (2019)	500 (10.90)	322 (64.40)	12.82 (6.53)	500 (100)
Dhaka, Bangladesh (2017)	823 (17.95)	183 (22.24)	4.57 (2.33)	801 (97.33)
Kampala, Uganda (2018)	548 (11.95)	75 (13.69)	5.27 (2.23)	548 (100)
Kumasi, Ghana (2018)	400 (8.72)	44 (11)	12.90 (14.95)	400 (100)
Lusaka, Zambia (2018)	100 (2.18)	20 (20.00)	5.96 (2.20)	100 (100)
Lusaka, Zambia (2019)	300 (6.54)	57 (19.00)	5.91 (1.86)	299 (99.67)
Maputo, Mozambique (2015)	125 (2.73)	-	-	50 (63.29)
Maputo, Mozambique (2016)	136 (2.97)	-	-	57 (57.58)
Siem Reap, Cambodia (2016)	410 (9.94)	405 (98.78)	5.13 (2.60)	321 (78.29)
Vellore, India (2014)	200 (4.36)	-	-	124 (62.00)
Total	4586	1606 (39.15)	8.38 (8.68)	3873 (86.01)

Table 2a. Results from unconditional and exact logistic regressions using median cut point for produce data (2.93 $\log_{10}$ *E. coli* CFU[a]).

Variable	N	*E. coli* Concentration < 2.93 $\log_{10}$ CFU	*E. coli* Concentration ≥ 2.93 $\log_{10}$ CFU	Odds Ratio	95% Confidence Interval	p-value
Unconditional Logistic Regression						
Produce Type						
Fruit	10	9	1	0.57	0.06, 5.26	0.6188
Herbs	30	1	29	66.18	8.34, 525.1	<0.0001*
Leafy	75	22	53	2.71	1.41, 5.22	0.0029*
Root-Underground	12	4	8	5.07	1.34, 19.19	0.0168*
Seeded	295	178	117	Ref		
Exact Logistic Regression[b]						
Produce Type						
Fruit	10	9	1	0.57	0.01, 5.67	1.0000
Herbs	30	1	29	62.86	9.03, >999.999	<0.0001*
Leafy	75	22	53	2.36	1.33, 5.39	0.0042*
Root-Underground	12	4	8	4.88	1.14, 25.34	0.0302*
Seeded	295	178	117	Ref		

[a] CFU = colony forming units

[b] Exact logistic regression used for expected cell counts less than 5

* Statistically significant at alpha = 0.05

Table 2b. Results from unconditional logistic regressions using median cut point for street food data (2.67 $\log_{10}$ *E. coli* CFU[a]).

Variable	N	*E. coli* Concentration < 2.67 $\log_{10}$ CFU	*E. coli* Concentration ≥ 2.67 $\log_{10}$ CFU	Odds Ratio	95% Confidence Interval	p-value
Model 1: Street Food Type and SES						
Street Food Type Alone						
Mixed	116	37	79	3.73	2.18, 6.37	<0.0001*
Cooked	156	96	60	Ref		
SES Alone						
High	50	17	33	1.83	0.92, 3.62	0.0854
Middle	29	12	17	2.72	1.18, 6.23	0.0184*
Low	195	106	89	Ref		
Model 2: Street Food Type and Location of Deployment						
Location of Deployment Alone						
Accra, Ghana (2018)	20	13	7	0.08	0.02, 0.53	0.0089*
Kampala, Uganda (2018)	33	22	11	0.09	0.02, 0.37	0.0008*
Kumasi, Ghana (2018)	39	33	6	0.03	0.004, 0.18	0.0002*
Dhaka, Bangladesh (2017)	84	18	66	Ref		

[a] CFU = colony forming units

* Statistically significant at alpha = 0.05

Table 2c. Results from unconditional and exact logistic regressions using 2 $\log_{10}$ *E. coli* CFU[a] cut point for street food data.

Variable	N	*E. coli* Concentration ≤ 2 $\log_{10}$ CFU	*E. coli* Concentration > 2 $\log_{10}$ CFU	Odds Ratio	95% Confidence Interval	p-value
Model 1: Street Food Type and SES – Unconditional Logistic Regression						
Street Food Type Alone						
Mixed	116	24	92	2.83	1.59, 5.03	<0.0001*
Cooked	156	61	95	Ref		
SES Alone						
High	50	9	41	2.32	1.04, 5.15	0.0391*
Middle	29	3	26	7.54	2.16, 26.26	0.0015*
Low	195	75	120	Ref		
Model 2: Street Food Type and Location of Deployment – Unconditional Logistic Regression						
Location of Deployment Alone						
Accra, Ghana (2018)	20	3	17	0.07	0.008, 0.59	0.0146*
Kampala, Uganda (2018)	33	13	20	0.04	0.009, 0.17	<0.0001*
Kumasi, Ghana (2018)	39	16	23	0.02	0.003, 0.11	<0.0001*
Dhaka, Bangladesh (2017)	84	6	78	Ref		
Model 2: Street Food Type and Location of Deployment – Exact Logistic Regression[b]						
Location of Deployment Alone						
Accra, Ghana (2018)	20	3	17	0.07	0.005, 1.00	0.0500*
Kampala, Uganda (2018)	33	13	20	0.04	0.007, 0.20	<0.0001*
Kumasi, Ghana (2018)	39	16	23	0.02	0.002, 0.16	<0.0001*
Dhaka, Bangladesh (2017)	84	6	78	Ref		

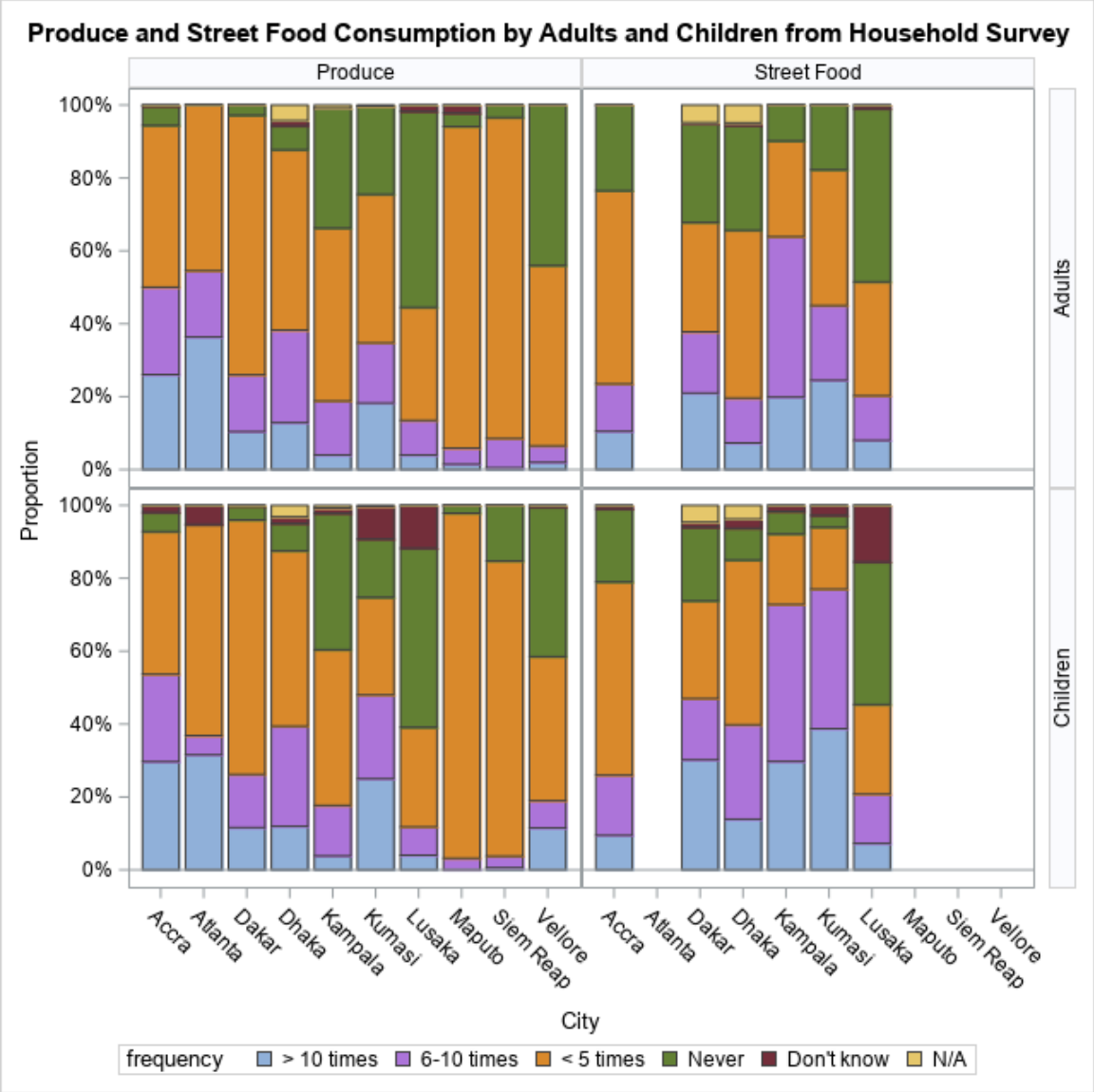
[a] CFU = colony forming units

[b] Exact logistic regression used for expected cell counts less than 5

* Statistically significant at alpha = 0.05

FIGURES

Figure 1. Plots of household survey responses on produce and street food consumption by participants and children in their household. Frequency of consumption over the past week is noted as more than ten times, six to ten times, less than five times, never, do not know, or not applicable.



CHAPTER III: CONCLUSIONS AND RECOMMENDATIONS

Conclusions

Raw produce and street food items are key vehicles for exposure to microbial contamination that could ultimately lead to illness in the individual consuming these food items. Produce types, including herbs, leafy vegetables, and root-underground vegetables had statistically significantly higher odds of contamination compared to seeded vegetables. Street food dishes that included uncooked food items also had significantly higher odds of being contaminated compared to fully cooked street food dishes. It is also important to note that the cut points used in these analyses were above any satisfactory level of *E. coli* contamination of a food item intended for consumption. Because *E. coli* indicates the presence of fecal contamination and possible enteric pathogens, *E. coli* should ideally not be detected at any level in food items. The results from this study suggest that these food items, without being cooked, are highly contaminated, which could lead to an increased risk of foodborne illness after consuming raw produce or street food dishes containing uncooked foods. These findings highlight the importance of improving sanitation and hygiene along the entire farm-to-fork pathway, and educating food vendors on proper food handling, storage, and preparation.

While identifying the source of contamination of a food item is useful in providing targeted intervention strategies, a whole-of-path approach is also valuable. The farm-to-fork pathway includes multiple sources of contamination and multiple points where contamination may be introduced. Contact between produce crops and wild and domestic animals, contaminated workers' hands, irrigation water, manure used for fertilizer, soil, wash tanks and surfaces in produce facilities have all been identified as risk factors for

produce contamination (3-6, 12, 13, 15). Agricultural workers need to be trained to minimize contamination through regular handwashing and in ensuring that water, soil, and fertilizers used for food crops are not contaminated.

Once food items are shipped, though, they may become contaminated during the process of transport and distribution due to lack of proper food handling and storage. If food vendors that receive items at local markets do not practice proper sanitation and hygiene, they also run the risk of contaminating their food items. Street food vendors may purchase contaminated food items and not have the means to maintain proper hygiene at their food stalls, whether it be not cleaning food preparation surfaces or not having access to water to wash their hands or rinse foods. Food vendors should be trained to handle foods hygienically and given assistance in doing so (for example, working with the local governments to improve access to clean water or dedicated waste disposal sites for used oil and old food). Some street food vendors may also prepare certain foods in their homes prior to bringing them to their stalls; hygiene practices should be present both in their homes and at their stalls.

Community-level improvements to WASH may help mitigate the risk of contaminated food items. The majority of participants in the SaniPath study reported washing raw produce before consuming it. Good food hygiene practices may become more feasible as access to adequate quantities of safe water, sanitation services, and fecal sludge management practices improve around the globe. If these improvements are made throughout the farm-to-fork pathway, there may be a marked decrease in the frequency and magnitude amount of microbial contamination of produce, including produce items

commonly consumed raw or food items used in preparing street food dishes. This decrease in contamination may subsequently reduce the burden of foodborne diseases.

Recommendations

Based on the findings from this study, farms growing produce items that are typically consumed without cooking, such as herbs, leafy vegetables, and root-underground vegetables, should be targeted for WASH improvement interventions. These items had the highest odds of *E. coli* contamination, which could lead to a higher risk of foodborne disease. Street food dishes that include uncooked components likely incorporate some of these produce items, so interventions targeted at the farms will likely have positive downstream effects for improving food items purchased from street vendors. While washing of produce items should continue to be promoted, this should not be the only intervention used to minimize contamination, as this exclusively is not sufficient for eliminating contamination (4).

The data presented for *E. coli* concentrations were quantified as concentration per serving size of the food item. In the future, sample sizes and concentrations should be converted to grams so these data are more easily comparable to other research and international standards for microbiological contamination. It is also recommended that bias analyses be performed in future analyses of these data, so any residual biases can be quantified and corrected. The sanitation and hygiene needs of farms in each of the cities may be different, so it will be critical to understand the current agricultural practices at the farms in each city. It will also be important to know if the farms where most of a city's produce originates are or urban, peri-urban, or rural areas. Additional studies similar to

those of Amoah et al., Bartz et al., and Johnston et al. where samples of produce are collected and analyzed at each step of the farm-to-fork pathway would be beneficial for understanding the critical points where there is the greatest risk of contamination and where to target interventions (4-6). Further research is also needed to identify what types of street food dishes adults and children are consuming. Knowing that children consume street food more frequently than adults, if children are found to primarily consume mixed street food dishes, they are at a higher risk of ingesting contaminated food items that could lead to disease. This information would allow for interventions tailored to street food vendors located near schools that primarily serve children. Understanding the different practices and trainings for street food vendors all cities will also be important, as they may differ along with agricultural practices. A study similar to Bereda et al.'s design would be beneficial for obtaining this information among SaniPath study sites and making specific recommendations for each city (12).

APPENDICES

Appendix A. Categorizations of produce and street food.

	Food Types Included
Produce Category[a]	
Vegetables	
Fungi	none
Sprouts	none
Root-underground	carrot, spring onion
Seeded vegetables	cucumber, okra, long bean, water mimosa, wing bean, tomato, pepper, eggplant, green chilly
Herbs	coriander
Leafy (Vegetable Row Crops)	lettuce, salad, cabbage
Fruits	watermelon, apple, guava
Nuts-Seeds	none
Uncategorized	long plant, "NA"
Street Food Category[b]	
Cooked	beans, chapati with beans, fried yam, fritters, kenkey, pasta, peas, roasted maize, samosas, scones, stew, waakye
Uncooked	none
Mixed cooked and uncooked	belpuri, egg wrap, fuska with chotpoti, puffed rice, rolex, tuna sandwich
Uncategorized	"NA"

[a] Categories assigned per CDC designations of food groupings

[b] Categories assigned in consultation with SaniPath team

Appendix B. Line listing of produce and street food items and the distribution of *E. coli* for each item.

	N (%)[a]	Mean (SD)[b,c]	Median (Range)[b]	Cities where samples were taken
Produce Items				
Apple	6 (1.3)	1.57 (0.42)	1.40 (1.40-2.41)	Accra, Lusaka
Cabbage	33 (7.1)	3.22 (1.52)	3.46 (1.40-5.73)	Accra, Kampala, Siem Reap
Carrot	11 (2.4)	3.76 (1.04)	4.01 (2.40-5.22)	Accra, Dakar
Coriander	35 (7.5)	4.67 (0.86)	4.74 (2.70-6.06)	Dhaka, Vellore
Cucumber	73 (15.7)	3.08 (1.42)	2.71 (1.40-7.00)	Accra, Atlanta, Dakar, Dhaka, Lusaka, Maputo, Siem Reap
Egg plant	2 (0.4)	4.47 (0.51)	4.47 (4.11-4.83)	Siem Reap
Green chili pepper	10 (2.2)	3.79 (1.54)	3.29 (2.40-6.00)	Dakar, Vellore
Guava	4 (0.9)	3.16 (2.11)	2.57 (1.40-5.50)	Lusaka
Lettuce	34 (7.3)	4.01 (1.33)	3.83 (1.40-7.00)	Accra, Atlanta, Kumasi, Maputo, Siem Reap
Long bean	2 (0.4)	2.24 (0.34)	2.24 (2.00-2.48)	Siem Reap
Long plant	2 (0.4)	3.35 (0.18)	3.35 (3.22-3.48)	Siem Reap
Okra	3 (0.6)	3.76 (0.29)	3.60 (3.57-4.10)	Vellore
Pepper	66 (14.2)	3.37 (1.61)	2.78 (1.40-6.00)	Accra, Atlanta, Dakar, Kumasi
Salad	14 (3.0)	4.21 (1.47)	5.06 (2.40-5.82)	Dakar, Siem Reap
Spring onion	3 (0.6)	3.60 (1.41)	3.60 (2.60-4.60)	Accra
Tomato	162 (34.9)	2.69 (1.33)	2.44 (1.40-7.00)	Accra, Atlanta, Dakar, Dhaka, Kampala, Kumasi, Lusaka, Maputo, Siem Reap, Vellore
Water mimosa	1 (0.2)	4.80	4.80 (4.80-4.80)	Siem Reap
Watermelon	1 (0.2)	1.40	1.40 (1.40-1.40)	Lusaka
Wing bean	2 (0.4)	3.81 (1.41)	3.81 (2.81-4.81)	Siem Reap
Total	464			
Street Food Items				
Beans	6 (2.0)	2.41 (0.63)	2.00 (2.00-3.30)	Accra
Belpuri	1 (0.3)	0.69	0.69 (0.69-0.69)	Dhaka
Chapati with beans	17 (5.6)	3.06 (1.14)	2.31 (2.00-5.31)	Kampala
Egg wrap	10 (3.3)	1.56 (0.21)	1.55 (1.12-1.84)	Lusaka
Fried yam	11 (3.6)	2.21 (0.64)	1.94 (1.59-3.38)	Kumasi
Fritters	34 (11.2)	1.38 (0.43)	1.53 (0.70-1.99)	Lusaka
Fuska with chotpoti	61 (20.1)	3.80 (1.26)	3.56 (0.72-6.12)	Dhaka
Kenkey	20 (6.6)	2.27 (0.08)	2.29 (2.12-2.39)	Accra, Kumasi
Pasta	20 (6.6)	2.77 (0.10)	2.80 (2.69-3.00)	Dakar
Peas	10 (3.3)	2.67 (0.01)	2.67 (2.67-2.70)	Dakar
Puffed rice	38 (12.5)	3.84 (1.32)	4.12 (1.22-6.44)	Dhaka

Roasted maize	1 (0.3)	1.30	1.30 (1.30-1.30)	Lusaka
Rolex	18 (5.9)	2.48 (1.02)	1.96 (1.90-4.93)	Kampala
Samosas	12 (4.0)	2.28 (1.33)	1.71 (1.50-5.56)	Kampala, Lusaka
Scones	1 (0.3)	1.92	1.92 (1.92-1.92)	Lusaka
Stew	10 (3.3)	2.92 (0.32)	2.83 (2.70-3.83)	Dakar
Tuna sandwich	10 (3.3)	2.79 (0.29)	2.70 (2.70-3.61)	Dakar
Waakye	23 (7.6)	3.03 (1.67)	1.98 (1.32-6.55)	Accra, Kumasi
Total	303			

[a] Includes samples where *E. coli* concentration was not determined.

[b] Concentration in $\log_{10}$ colony forming units ($\log_{10}$ CFU) of *E. coli* per serving

[c] Food items with only one sample did not have a standard deviation

Appendix C. Summary of household survey results.

	Location of Deployment (Year)									
	Accra (2016/18)	**Atlanta (2016)**	**Dakar (2019)**	**Dhaka (2017)**	**Kampala (2018)**	**Kumasi (2018)**	**Lusaka (2018/19)**	**Maputo (2016)**	**Siem Reap (2016)**	**Vellore (2014)**
	N (%)	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**
Raw Produce Consumption per Week (Adults)										
>10 times	266 (26.05)	8 (36.36)	52 (10.40)	106 (12.88)	22 (4.01)	73 (18.25)	16 (4.00)	2 (1.47)	2 (0.49)	4 (2.00)
6-10 times	245 (24.00)	4 (18.18)	78 (15.60)	209 (25.39)	81 (14.78)	66 (16.50)	38 (9.50)	6 (4.41)	33 (8.05)	9 (4.50)
≤ 5 times	453 (44.37)	10 (45.45)	356 (71.20)	407 (49.45)	260 (47.45)	163 (40.75)	124 (31.00)	120 (88.24)	361 (88.05)	99 (49.50)
Never	54 (5.29)	0 (0)	14 (2.80)	54 (6.56)	180 (32.85)	97 (24.25)	215 (53.75)	5 (3.68)	14 (3.41)	88 (44.00)
Don't know	3 (0.29)	0 (0)	0 (0)	12 (1.46)	0 (0)	0 (0)	7 (1.75)	3 (2.21)	0 (0)	0 (0)
N/A	0 (0)	0 (0)	0 (0)	35 (4.25)	5 (0.91)	1 (0.25)	0 (0)	0 (0)	0 (0)	0 (0)
Total	1021	22	500	823	548	400	400	136	410	200
Raw Produce Consumption per Week (Children)										
>10 times	198 (29.69)	6 (31.58)	58 (11.60)	96 (11.97)	21 (3.83)	100 (25.00)	16 (4.01)	0 (0.00)	2 (0.62)	23 (11.50)
6-10 times	160 (23.99)	1 (5.26)	73 (14.60)	220 (27.43)	76 (13.87)	92 (23.00)	31 (7.77)	2 (2.13)	10 (3.12)	15 (7.50)
≤ 5 times	261 (39.13)	11 (57.89)	349 (69.80)	386 (48.13)	234 (42.70)	107 (26.75)	109 (27.32)	89 (94.68)	260 (81.00)	79 (39.50)
Never	35 (5.25)	0 (0.00)	19 (3.80)	60 (7.48)	205 (37.41)	64 (16.00)	196 (49.12)	3 (3.19)	49 (15.26)	82 (41.00)
Don't know	13 (1.95)	1 (5.26)	1 (0.20)	15 (1.87)	8 (1.46)	36 (9.00)	47 (11.78)	0 (0)	0 (0)	1 (0.50)
N/A	0 (0)	0 (0)	0 (0)	25 (3.12)	4 (0.73)	1 (0.25)	0 (0)	0 (0)	0 (0)	0 (0)
Total	667	19	500	802	548	400	399	94	321	200
Washing Raw Produce before Consumption										
Yes	183 (91.50)	-	499 (99.80)	722 (89.80)	461 (84.12)	359 (89.75)	383 (95.75)	-	-	-
No	16 (8.00)	-	1 (0.20)	40 (4.98)	36 (6.57)	19 (4.75)	16 (4.00)	-	-	-
Don't know	1 (0.50)	-	0 (0)	10 (1.24)	8 (1.46)	3 (0.75)	0 (0)	-	-	-
N/A	0 (0)	-	0 (0)	32 (3.98)	43 (7.85)	19 (4.75)	1 (0.25)	-	-	-
Total	200	-	500	804	548	400	400	-	-	-

Street Food Consumption per Week (Adults)										
>10 times	21 (10.50)	-	105 (21.00)	60 (7.48)	109 (19.89)	98 (24.50)	32 (8.00)	-	-	-
6-10 times	26 (13.00)	-	84 (16.80)	101 (12.59)	241 (43.98)	82 (20.50)	49 (12.25)	-	-	-
≤ 5 times	106 (53.00)	-	150 (30.00)	378 (47.13)	144 (26.28)	149 (37.25)	125 (31.25)	-	-	-
Never	47 (23.50)	-	135 (27.00)	236 (29.43)	54 (9.85)	71 (17.75)	190 (47.50)	-	-	-
Don't know	0 (0)	-	2 (0.40)	6 (0.75)	0 (0)	0 (0)	4 (1.00)	-	-	-
N/A	0 (0)	-	24 (4.80)	40 (4.99)	0 (0)	0 (0)	0 (0)	-	-	-
Total	200	-	500	821	548	400	400	-	-	-
Street Food Consumption per Week (Children)										
>10 times	19 (9.50)	-	151 (30.20)	111 (13.89)	163 (29.74)	155 (38.75)	29 (7.27)	-	-	-
6-10 times	33 (16.50)	-	84 (16.80)	207 (25.91)	236 (43.07)	153 (38.25)	54 (13.53)	-	-	-
≤ 5 times	106 (53.00)	-	134 (26.80)	361 (45.18)	106 (19.34)	68 (17.00)	98 (24.56)	-	-	-
Never	40 (20.00)	-	101 (20.20)	70 (8.76)	34 (6.20)	13 (3.25)	156 (39.10)	-	-	-
Don't know	2 (1.00)	-	7 (1.40)	20 (2.50)	9 (1.64)	11 (2.75)	62 (15.54)	-	-	-
N/A	0 (0)	-	23 (4.60)	30 (3.75)	0 (0)	0 (0)	0 (0)	-	-	-
Total	200	-	500	799	548	400	399	-	-	-

Appendix D. SaniPath Environmental Sampling Protocol for produce and street food samples.

SaniPath | Environmental Sampling Protocols

Collecting and analyzing environmental samples

The assessment team will collect environmental samples. The purpose of the environmental sampling is to identify which areas in the urban environment have fecal contamination and determine the magnitude of that contamination. The focus of the sampling is the public domain that would be affected by sanitation infrastructure changes. If the team has enough staff, this can be done at the same time as the surveys. Another option is to have a laboratory collect and analyze the samples. When planning logistics for sampling, the assessment team needs to consider traffic patterns, typical working days, safety, when the laboratories are open, etc. Sampling can also be used as an opportunity to collect GPS data and photos at the site of each sampling to use in conjunction with the environmental samples to develop a contamination map.

Figure 1: The rapid assessment team in Vellore, India collects samples from an open drain.

Sites for environmental sampling should be identified based primarily on prior transect walk visits in a neighborhood and information culled from key informant interviews.

Table 1 shows the types of samples to be collected from the selected target neighborhoods based on the pathways selected for this assessment.

Table 1 – Types of samples to be collected

Pathways	Type of Sample(s) to be Collected
Surface Water (River, lake, or pond water)	Surface water
Ocean Water	Ocean Water (coastal or inland)
Produce	Produce (vegetables) that might be irrigated by wastewater and eaten raw
Street Food	Food from street vendors
Soil	Soil in public spaces
Drinking Water	Piped (City) drinking water, bottled water, well water (groundwater)
Bathing water	Bathing water (if other than from public taps or surface water)
Open Drains	Wastewater in open drains
Public/community*/shared* Latrine Surfaces	Swab from surfaces of public/community/shared latrines

After identification of these sites, a daily sampling plan should be designed to enable the field team to efficiently collect samples for laboratory processing. The sampling plan should be based on the capacity of the laboratory to process samples within the workweek. Typically a laboratory may be able to process samples for only 4 out of a 5 day work week due to laboratory assay processing time. 40 samples per week/10 samples per day is advised for the SaniPath Exposure Assessment Tool. If two technicians are processing laboratory samples the process can be expedited (80-100 samples per week/20-25 samples per day). **Table 2** shows an example daily sampling plan.

Table 2 - Example Daily Sampling Plan for Neighborhood X for Week 1 (with one lab technician)

Monday	Tuesday	Wednesday	Thursday
Produce rinse = 4	Drain Water= 3	Drain water = 4	Drinking water= 5
Drinking water = 3	Surface water = 3	Soil = 2	Produce rinse =5
Flood Water = 3	Soil = 4	Flood Water = 4	

Standard Operating Procedures for Environmental Sampling

Summary

These protocols provide instruction for sterile collection of drinking water, surface water, ocean water, open drains, flooded areas, street food from vendors, produce (vegetables) from markets that are eaten raw, swabs of latrine surfaces, and soil. Methods are designed to collect samples for microbial analysis to detect possible fecal contamination in these environments.

Sample Collection of Produce (Raw Vegetables)

The materials and equipment needed to collect produce samples are below:

1. Gloves
2. Ice chest with frozen ice packs
3. 70% ethanol
4. Sterile 2 liter Whirl-Pak bags
5. Android device
6. Pen
7. Permanent Marker
8. Extra Paper Forms
9. Money for purchasing produce

Preparation for field work

The day before fieldwork, make sure all sampling materials are clean, sterile and of adequate quantity and quality. Generate unique sample identification codes (IDs) for labeling Whirl-Pak bags of samples collected.

Sampling protocol for produce (fruits and vegetables)

1. Take a photo of the sampling location using the ONA Sample Picture form.
2. Using a permanent marker, label clean, sterile 2L Whirl-Pak bag with the Sample ID.
3. Put on gloves and spray your hands with 70% ethanol.
4. Open the labeled Whirl-Pak bag by gently pulling out the tabs of the side of the bag without touching the mouth or inside of the bag.
5. If you are selecting produce at a market vendor's stall, carefully open the Whirl-Pak bags without touching the mouth of the bag. Ask the vendor to place the produce in the bag. Only collect as much produce as you think one person may consume during a single meal (i.e. one serving). Quickly close bag.
6. Confirm that the label is still affixed to the bag. Place the Whirl-Pak bag in the ice chest with ice packs.
7. Transport samples to the lab within 6 hours of collection and deliver samples to one of the designated laboratory personnel.

8. Immediately transfer the sample(s) into a 4°C refrigerator until they are ready to be analyzed. Samples should be stored no longer than 6 hours before analysis.

Sample Collection of Street Food (Commonly Eaten In Neighborhood)*

*Protocol adapted from WASH Benefits study

The materials and equipment needed to collect street food samples are below:

1. Gloves
2. Ice chest with frozen ice packs
3. 70% ethanol
4. Sterile 2 liter Whirl-Pak bags
5. Android device
6. Pen
7. Permanent Marker
8. Extra Paper Forms
9. Money for purchasing street food

Preparation for field work

The day before fieldwork, make sure all sampling materials are clean, sterile and of adequate quantity and quality. Generate unique sample identification codes (IDs) for labeling Whirl-Pak bags of samples collected.

Sampling protocol for street food

9. Using a permanent marker, label clean, sterile 2L Whirl-Pak bag with the Sample ID.
10. Put on gloves and spray your hands with 70% ethanol.
11. Open the labeled Whirl-Pak bag by gently pulling out the tabs of the side of the bag without touching the mouth or inside of the bag.
12. If you are selecting street food at a market vendor's stall, carefully open the Whirl- Pak bags without touching the mouth of the bag. Ask the vendor to place the food in the bag. Only collect as much food as you think one person may consume during a single meal (i.e. one serving). Quickly close bag.
13. Confirm that the label is still affixed to the bag. Place the Whirl-Pak bag in the ice chest with ice packs.
14. Transport samples to the lab within 6 hours of collection and deliver samples to one of the designated laboratory personnel.
15. Immediately transfer the sample(s) into a 4°C refrigerator until they are ready to be analyzed. Samples should be stored no longer than 6 hours before analysis.

Appendix E. Procedures for processing produce and street food samples.

Procedures for Sample Processing

This document consists of the protocols for laboratory processing of the various sample types collected as a part of the SaniPath Exposure Assessment Tool. We recommend that sample collection occur in the mornings so that samples can be transported to the laboratory by lunch time to allow for adequate time for the laboratory to process and analyze the samples. Due to the time and resource constraints of conducting a rapid assessment we do not recommend processing replicates of samples.

Processing of raw produce samples

We measure contamination on produce by rinsing whole pieces of produce and testing the rinse solution for *E. coli*. Produce samples tend to have a wide range of contamination levels. We recommend testing 10 ml and 1 ml volumes of the undiluted, rinse solution, as well as 1 ml of a 1:10 dilution. This will allow you to accurately measure a wide range of contamination levels. See the Dilution Protocols on page 7. Follow the directions below to prepare the produce rinses.

Materials and Equipment:

- Sterile (autoclaved or sterile filtered) water or PBS
- PBST (1L phosphate buffered saline, pH 7.2 with 0.05mL Tween-80)
- 15mL conical tube (for dilution)
- Sterile graduated cylinder that can measure 500 mls
- Gloves
- 70% ethanol
- Felt tip pen
- Produce sample in bag
- 37°C incubator
- 4°C refrigerator
- Produce Laboratory Form

1. Put on gloves and spray hands with 70% ethanol. Rub hands together to sanitize all surfaces of the gloves.

2. Check that the sample ID on the Produce Laboratory Form and the sample are the same and enter the sample ID into the form. Record the date and time of sample processing on the Produce Laboratory Form.
3. Prepare your work surface by cleaning it with 70% ethanol.
4. Samples should arrive at the lab in a large Whirl-Pak bag. Spray the outside of the bag with 70% ethanol and rub it well. Inspect the bag to determine whether liquid can be added without overflowing. The bag should be no more than 2/3rds full with produce. You should be able to add 500 mL of PBST and still close the bag without it overflowing. If you suspect that the bag is too full, open the bag by untwisting the ties and pulling them gently outwards until the mouth of the bag opens. Remove the extra produce items one at a time by pressing upward underneath them on the outside of the bag, and move them to the top, leaving the bag about 2/3rds full of produce. **Never stick your hands into the bag.** Discard the removed produce. Only record the produce remaining in the bag on the Data Recording Form.
5. Add 500 mL of PBST to the bag. Seal the bag, trapping minimal amounts of air inside. Incubate for 10 minutes at 37°C.
6. Vigorously shake the bag with the produce for 30 seconds. Next gently massage the surface of each piece of produce item through the bag for 60 seconds. For delicate items like lettuce or onions, try to rub at least the outer leaves. Try not to break open any items. Shake the bag again for 30 seconds.
7. To remove the produce, open the Whirl-Pak bag, gently press upwards underneath the item and move it to the top. Take care not to lose any water or smash any produce. Remove the produce at the top of the bag and set aside
8. Close the Whirl-Pak bag.
9. Weigh the produce (using aluminum foil) and record the weight on a Produce Laboratory Form.
10. Store all samples at 4°C until they are ready for processing.

Processing of street food samples

We measure contamination on street food according to the protocol for food processing using a modification of the protocol from the WASH Benefits Study—Bangladesh, World Bank Add-on (refer to LabSOP_20August.doc). Street food is processed by homogenizing the street food and testing the solution for *E. coli*. Street food samples may have a wide range of contamination levels. Our preliminary recommendation for dilutions is to measure 10 ml and 1 ml volumes of the homogenized solution, as well as 1 ml of a 1:10 dilution. This will allow you to accurately measure a wide range of contamination levels.

See the Dilution Protocols on page 7. Follow the directions below to prepare the produce rinses.

Materials and Equipment:

- Sterile (autoclaved or sterile filtered) water or PBST (phosphate buffered saline, pH 7.2 with 0.04% Tween-80)
- Gloves
- 70% ethanol
- Felt tip pen
- Street food sample in bag
- 37°C incubator
- 4°C refrigerator
- Street food Laboratory Form

1. Shake the food sample to mix it well. Weigh the entire street food sample and record the weight on a lab processing form.
2. On aluminum foil, weigh out 10 g of food (acceptable range: 9.50 to 10.50 g).
3. Add 10 g of food into a sterile Whirl-Pak bag.
4. Using a sterile graduated cylinder, add 100 mL of distilled water into Whirl-Pakbag.
5. Homogenize and thoroughly mix for 1 minute by hand.
6. Using a sterile disposable pipette, immediately remove homogenized solution from the bottom of the bag (do not allow particles to settle) and add to dilutions outlined in the Dilution Protocols table.

Dilution Protocols

Below, we have provided the recommended dilutions for the SaniPath Exposure Assessment Lab Standard Operating Procedures. Depending on the sample type and the expected contamination level, we recommend between one and four dilutions. While these are our recommendations based on previous experience with the tool, they may not be the best dilutions for your setting. Therefore, we encourage users of this protocol to adapt the dilution methods and use any two to three dilutions on the lab data entry form for their work.

Recommended dilutions by sample type*:

Sample Type	Dilution 1	Dilution 2	Dilution 3	Dilution 4
Produce	1:10	-	-	-
Street Food	1:10			

*Piped drinking water, bathing water, and surface water require no dilution prior to testing.

Materials and Equipment:

- Autoclaved or sterile filtered water/distilled water
- Up to 4 sterile 15 ml conical tubes, depending on number of dilutions to be prepared. Gloves
- 70% ethanol
- Felt tip pen
- Pipet with 1 ml tips (preferred but not required)
- Pipet Aid
- 10 ml serological pipettes
- 4°C refrigerator

1. Prepare your work surface by cleaning it with 70% ethanol.
2. Put on gloves and spray hands with 70% ethanol. Rub hands together to sanitize all surfaces of the gloves.
3. Prepare your dilution containers.
    a. For each sample dilution, label a 15 ml conical tube with the sample ID and the dilution.
    b. Using a 10 ml serological pipet, add 9 ml volume of distilled water to each of the 15 ml tubes.
4. Samples should have been collected in a Whirl-Pak bag. Spray the outside of the packaging/bag with 70% ethanol and rub it well.
5. Mix the contents of the bag by turning it end over end 5 times.
6. Open your bag of sample water, and transfer 1 ml volume of sample to the first dilution tube. This will make a 1:10 dilution.
7. Mix the diluted sample by swirling gently.
8. Transfer 1 ml of the diluted sample to the next dilution tube to prepare 1:100 dilution.
9. Repeat steps 7 and 8 for each subsequent dilution, as shown in the table below.

Intended Dilution	Volume to Add
1:10	1 ml of original sample
1:100	1 ml of 1:10 dilution
1:1000	1 ml of 1:100 dilution
1:10,000	1 ml of 1:1000 dilution

10. If the samples will not be processed immediately, store all samples at 4°C until they are ready for processing. For all diluted samples, 1 ml of sample will be mixed with 99 ml volume of sterile distilled water for processing by IDEXX.

Recommended tray volumes by sample type*:

Sample Type	Undiluted (1:1)	1:10	1:100	1:1000	1:10,000
Produce	10 ml, 1 ml	1 ml			
Street Food	10 ml, 1 ml	1 ml			

Appendix F. Membrane filtration procedures for produce and street food samples.

Procedures for Quantifying E. coli Contamination by Membrane Filtration

Membrane Filtration Laboratory

Analysis of Environmental Samples for *E. coli*

Recommended methods for measuring fecal *E. coli* are:

1. Membrane filtration and plating on BBL MI agar, mColiBlue, BioRad Rapid *E. coli 2* media, or Chromocult
   - Works well for all sample types
   - See the manufacturer's instructions for use and correct identification of *E. coli* colonies
2. IDEXX Quantitray and Colilert media
   - Not recommended for swabs

Important note on microbiological assays:
Be sure to use 100 mls of the sterile water or PBS used for washing items or making dilutions as a negative control in each assay. This is especially important if you are purchasing bottled water from a vendor, rather than using lab-prepared filtered or autoclaved water, as bottled water is not always sterile.

Membrane filtration protocol

A. Preparation

1. Wipe down bench or hood with 10% bleach followed by 70% ethanol.
2. Assemble the filter equipment, making sure all filter membrane holders have been autoclaved
3. Attach vacuum tubing to side arm of 1 liter flask and the vacuum source.
4. Attach tubing from output on side of manifold or vacuum flask to mouth of filter flask.
5. Insert filter base into mouth of manifold.
6. Prepare alcohol burner with lighter.

7. Prepare a small beaker with 100% ethanol for sterilizing the tips of the forceps. The ethanol should be 2-3 cm deep, just enough to cover the tips of the forceps when they are resting in the beaker. NOTE: The alcohol burner and ethanol should be on the same side as your dominant hand for easy forceps sterilization.
8. If you are using mColiBlue, prepare the plates then label the bottom of the plates with the date, dilution, sample ID, and initials. Be sure to include a plate for the negative control.

B. Sample Processing:

Materials and Equipment:

- Petri dishes, purchased or prepared
- 10-20 ml 100% ethanol in a small beaker (50-100 ml)
- Distilled or deionized water
- Sterile 1 x PBS
- 70% ethanol
- 10% bleach
- 10 ml serological pipets
- Pipet Aid or bulb
- Vacuum manifold or vacuum flask and tubing
- Vacuum pump
- Autoclaved membrane filter holder and vacuum funnel
- Sterile filters, mixed cellulose esters, 0.45um pore size, white gridded, 47mm diameter
- Flat blade forceps
- 1 liter side-arm flask
- Alcohol burner
- Lighter

1. Flame forceps for ~5 seconds to sterilize. Take care to hold the forceps horizontally to avoid burning your hand.
2. Remove a sterile filter from the packaging with sterile forceps.
3. Remove the filter holder and place the filter on the filter base, grid side up. Affix filter holder to the base.
4. Pour 10 mL sterile PBS on the filter.
5. Turn on vacuum, open the valve and close the manifold valves.
6. Pour another 10 mL of PBS on the filter and vacuum it through.
7. Carefully, remove membrane filter from filter base with sterile forceps, avoiding contact with the center of the membrane.

8. Place the filter, gridded side up, onto the plate labeled “Negative Control”. By rolling the filter onto the plate, you can avoid the formation of bubbles between the membrane and the agar surface, which can invalidate your results.
9. Replace the lid of the Petri plate.
10. Repeat steps 2 to 5
11. Add a minimum of 10 mL and up to 100 ml of liquid containing the highest dilution of the sample to the filter. Note: always start with highest dilution (lowest concentration) of sample to avoid introducing significant contamination from higher concentrations. If the test volume is 1ml, add 9mL PBS and spike the PBS with the 1mL sample aliquot (the total volume filtered is 10mL). This ensures that the solution is dispersed evenly around the filter surface.
12. Open the manifold valve and vacuum the liquid through the filter.
13. Use a 10 ml serological pipet to rinse the sides of the filter cup with 10 ml PBS.
14. Close valve on manifold and remove filter cup.
15. Flame forceps for ~5 seconds to sterilize.
16. Remove filter from base using sterile forceps, taking care not to disturb inner area of filter.
17. Place the filter onto a plate labeled with the Sample ID, date, dilution, and your initials. Take care to avoid the formation of bubbles between the filter and the agar.
18. Replace the plate lid.
19. Using the same filter holder, repeat steps 10 to 18 for the other dilutions of the sample being tested, going from most dilute to least dilute.
20. For each new sample you will need to re-sterilize gloves with alcohol and use a new filter holder.
21. Finish by processing the positive control (optional).
22. Invert the Petri plates, unless you are using mColiBlue, in which case plates should not be inverted to prevent the broth media from leaking and the plate drying.
23. Incubate the plates in a box at 37°C for 20 to 24 hours (according to media manufacturer’s guidelines). If using mColiBlue, incubate plates in box to avoid desiccation. Be sure not to close the lid tightly, it should just sit on top of the box so that oxygen can still circulate.
24. Record the date and time that the sample was placed in the incubator and your name (Lab Operator) on the laboratory form.

Recommended dilutions to be plated by Sample type:

Sample Type	1:1 (undiluted)	1:10	1:100	1:1000	1:10,000
Produce	10 ml, 1 ml	1 ml			
Street Food	10 ml, 1 ml	1 ml			

C. Counting and recording colonies:

1. Retrieve the laboratory form for your sample.
2. Check the box of the concentration of sample tested.
3. Retrieve the incubated samples and record the date and time the samples were removed from the incubator on the Laboratory.
4. Refer to the manufacturer's instructions for your media for proper identification of *E. coli* colonies.
    i. If there are > 200 colonies, record the results as 999 for "too numerous to count (TNTC)".
    ii. If individual *E. coli* colonies cannot be clearly distinguished from background growth or dirt on the filter, record the result as 998 "too dirty to count" (TDTC)).
    iii. If any *E. coli* colonies are found on the Negative Control plate, indicate the results next to "Negative Control". Record 0 for no colonies.

Appendix G. IDEXX procedures for produce and street food samples.

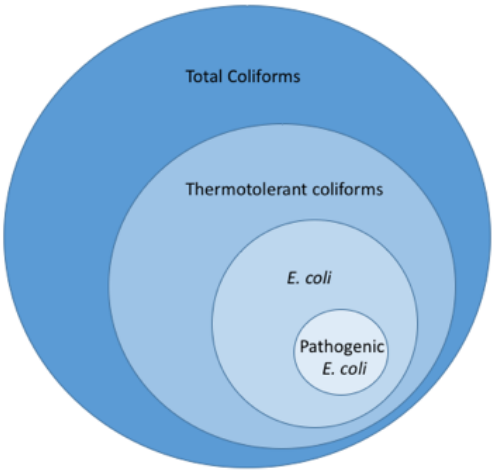
SaniPath

Quantifying E. coli contamination using

Important note on microbiological assays:

Be sure to use 100 mls of the sterile water or PBS used for washing items or making dilutions as a negative control in each assay. This is especially important if you are purchasing bottled water from a vendor, rather than using lab-prepared filtered or autoclaved water, as bottled water is not always sterile.

I. Introduction



Coliforms are a group of bacteria that are normal inhabitants of the intestinal tract of humans and animals. Historically, they were defined as the group of facultative anaerobic, gram-negative, non-spore-forming, rod-shaped bacteria that ferment lactose with gas and acid formation within 48 hours at 35°C. Fecal coliforms are a sub-group of the coliforms that grow at 44.5°C. It is actually more accurate to refer to them as "thermotolerant coliforms" because there are some non-fecal sources of these bacteria. One specific fecal coliform of interest is *E. coli* because it is almost exclusively fecal in origin. (There are some reports of *E. coli* detection in pristine tropical waters that suggest non-fecal sources.) While some strains of *E. coli* are harmless, other types of *E. coli*, such as the enteropathogenic and enterohemorrhagic strains, can cause severe disease in humans. The presence of fecal coliforms, and especially *E. coli,* in water is considered an indication of fecal contamination. However, the absence of fecal coliforms does not guarantee that the water is free of pathogens. Some pathogens, such as viruses, tend to survive longer than fecal coliforms and may still be present in water after the fecal coliforms have died off.

IDEXX Colilert Quanti-Tray system.

The IDEXX Quanti-Tray system is a testing technique that determines the most probable number (MPN) of coliforms in a water sample based on predetermined statistical parameters. One hundred mL of water (or, for highly contaminated samples, 100mL of a diluted water sample) are mixed with the Colilert reagent, poured into the Quanti-Tray and sealed. The compartments in the tray each contain a specific volume of water + reagent, similar to using a test tube. The tray is incubated (at 37.5 °C for total coliforms and 44.5 °C for fecal coliforms) for 18 to 24 hours. During this time, the target organisms will interact with the reagent, causing compartments that contain at least one coliform to turn yellow, while those that do not contain any coliforms will remain clear. Small and large cells are counted and the MPN is determined according to the IDEXX table

found at the end of this hand-out. Wells that contain at least one *E. coli* will turn yellow and fluoresce under UV light. We will also examine the Quanti-Tray under UV light to look for fluorescence in order to determine the MPN of *E. coli* in our samples.

The Colilert reagent in the Quanti-Tray system uses "patented Defined Substrate Technology® (DST®) to simultaneously detect total coliforms and *E. coli*. Two nutrient-indicators, ONPG and MUG, are the major sources of carbon in Colilert and can be metabolized by the coliform enzyme *β*-galactosidase and the *E. coli* enzyme *β*-glucuronidase, respectively. As coliforms grow in Colilert, they use *β*-galactosidase to metabolize ONPG and change it from colorless to yellow. *E. coli* use *β*-glucuronidase to metabolize MUG and create fluorescence. Since most non-coliforms do not have these enzymes, they are unable to grow and interfere. The few non-coliforms that do have these enzymes are selectively suppressed by Colilert's specifically formulated matrix." (Description from IDEXX website)

II. Equipment and Supplies

1. IDEXX Colilert

- WhirlPak bags containing samples or 100mL flasks/bottles
- PBS (Phosphate Buffered Saline)
- Sterile pipets
- Sterile graduated cylinders
- IDEXX Quanti-Tray
- Colilert-24 Reagent (blue and white box)
- IDEXX sealer
- Rubber tray (red one only)
- Sharpie Marker
- 125mL Erlenmeyer flasks (orange cap)
- Incubators at 37.5°C

III. Procedures

1. IDEXX QUANTI-TRAY METHOD FOR FECAL COLIFORMS AND *E. COLI*

A. Sample dilution

You will process multiple dilutions of your environmental samples. Follow the SaniPath dilution protocol. *We might adjust the dilutions after the first round of sampling*. **The total volume of water processed must be 100 ml!** That means, for example, for a 1 ml sample volume, you must mix the 1 ml of your sample water with 99 ml of PBS to give a total volume of 100 ml.

B. IDEXX Processing procedure

1. Turn on the IDEXX sealer. It will take 15 minutes or so to warm up. Using a Sharpie, label the backs of the IDEXX trays with your initials, sample ID, dilution, volume of sample per 100 ml, date, and time.

2. Open the reagent packet and tap into the Erlenmeyer flask with your final dilution to be tested. Close the cap to avoid contamination while you prepare your sample.

Note: adding less than 100ml to each tray will result in unfilled wells and an invalid test result. Adding more than 100ml will cause the liquid to overflow the tray during sealing, potentially damaging the sealer. It is important that you measure carefully!*

3. Swirl the flask with the sample until the reagent has dissolved. Allow foam to settle completely.

4. Without touching the inside of the tray, pull the tab to open the top of the tray, flexing the plastic to allow a gap between the plastic and the paper back. Be careful not to tear the paper back or tab. Have your partner pour in the sample. If there are bubbles in any of the cells, gently "flick" the back of the try with your finger so that the bubbles float to the top of the tray. Tiny bubbles are not a problem, but try to get rid of any large bubbles or foam.

5. Place the sample tray face down (paper side up) in the rubber tray so that the compartments align.

6. Gently push the tray into the IDEXX sealer. When the sealer senses the tray, it will automatically pull the tray in, and you can stop pushing. **Do not try to force the tray through the sealer.**

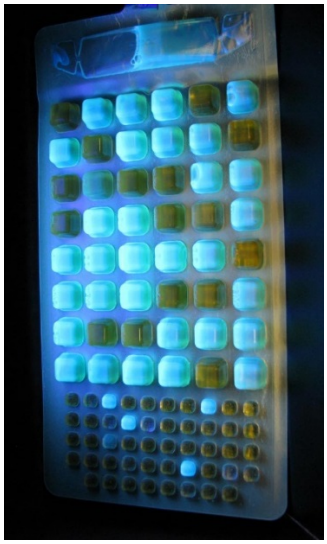
7. The tray will come out the back of the sealer. Do not pull it out until the sealer has stopped.

8. Place the tray in the incubator at 37°C.
9. Record the date and time that the sample was placed in the incubator and your name (Lab Operator) on the laboratory form.

C. Interpretation

1. You must return to the lab 24 hours after you have processed your sample to examine your trays.

2. Examine the Quanti-Tray **under UV light** and record the number of small and large cells that **fluoresce** (light blue). Use the MPN chart to determine the concentration of *E. coli* in your sample.



Count the number of small and large cells that fluoresce
Example: 32 large wells and 5 small wells (MPN count is 57.3)

Recommended SaniPath dilutions to be plated by Sample type:

Sample Type	Undiluted (1:1)	1:10	1:100	1:1000	1:10,000
Produce	10 ml, 1 ml	1 ml			
Street Food	10 ml, 1 ml	1 ml			

Appendix H. SaniPath Household Exposure Behavior Survey

Household Survey

Demographic Data	
Household ID	
Date of Survey	__ __ / __ __ / __ __ Month Day Year
Time at Start of Survey	__ __ : __ __ ____ Hour Minute AM/PM
Neighborhood	☐ Neighborhood A ☐ Neighborhood B ☐ Neighborhood C ☐ Neighborhood D ☐ Neighborhood E
Observe the type of home the respondent is living in.	☐ Single family home ☐ Compound
Did it rain in the past week?	☐ Yes ☐ No
Ask the respondent: How many people live in your household?	
Ask the respondent: Do you have children between the ages of 5-12?	☐ Yes ☐ No

Raw Produce	
If raw produce applies to this household, answer the following questions. *If not, skip to the street food section.*	
1. Think about whether you eat produce that is raw (uncooked). For this question, we are referring to any produce that does not grow on a tree, and that does not have a peel or shell. Think both about the produce you eat whole and produce you prepare but eat raw, such as tomato, cucumber, or lettuce. How many times within the past week did you eat raw produce? *If not applicable/unable to collect information, explain.*	☐ More than 10 times in the past week ☐ 6 to 10 times in the past week ☐ 1 to 5 times in the past week ☐ Never ☐ Do not know ☐ Not applicable/unable to collect information:_____
2. Think about whether your children eat produce that is raw (uncooked). For this question, we are referring to any produce that does not grow on a tree, and that does not have a peel or shell. Think both about the produce you eat whole and produce you prepare but eat raw, such as tomato, cucumber, or lettuce. How many times within the past week did your children eat raw produce? *If not applicable/unable to collect information, explain.*	☐ More than 10 times in the past week ☐ 6 to 10 times in the past week ☐ 1 to 5 times in the past week ☐ Never ☐ Do not know ☐ Not applicable/unable to collect information:_____
3. Think about whether anyone in your household washes the produce that your household eats raw before eating it. Does anyone in your household wash the produce that you eat before eating it? *If not applicable/unable to collect information, explain.*	☐ Yes ☐ No ☐ Do not know ☐ Not applicable/unable to collect information:_____

Street Food	
If street food applies to this household, answer the following questions. *If not, skip to the Public latrines section.*	
4. Think about whether you eat food that is prepared and sold on the street, such as fuska/chotpoti or puffed rice. How many times in the past week did you eat street food? *If not applicable/unable to collect information, explain.*	☐ More than 10 times in the past week ☐ 6 to 10 times in the past week ☐ 1 to 5 times in the past week ☐ Never ☐ Do not know ☐ Not applicable/unable to collect information:_____
5. Think about whether your children eat food that is prepared and sold on the street, such as fuska/chotpoti or puffed rice. How many times in the past week did your children eat street food? *If not applicable/unable to collect information, explain.*	☐ More than 10 times in the past week ☐ 6 to 10 times in the past week ☐ 1 to 5 times in the past week ☐ Never ☐ Do not know ☐ Not applicable/unable to collect information:_____