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Taylor Osborne Date

Characterizing Determinants of Hand Contamination Across Public and Private Domains in Low-Income Neighborhoods of Accra, Ghana

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Master of Public Health

Global Epidemiology

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Bachelor of Arts
Gettysburg College
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Abstract

Characterizing Determinants of Hand Contamination Across Public and Private Domains in Low-Income Neighborhoods of Accra, Ghana

By Taylor Osborne

An estimated 72% of the urban population of Africa lives in slums, which are often characterized by poor access to sanitation and hygiene services and high rates of diarrheal disease. Limited prior data exists on the relationship between fecal contamination in the environment and fecal contamination of hands in low-income, urban settings. This study characterized hand contamination across four neighborhoods in Accra, Ghana and assessed the relationship between environmental fecal contamination and hand fecal contamination in these study neighborhoods. Handrinse samples and other environmental samples (swabs, soil, and stored drinking water) were collected from children and adults in four settings: households, public latrines, schools, and nurseries, and analyzed for fecal indicator organism concentrations (*Escherichia coli* and enterococci). Hand contamination levels were characterized by neighborhood and demographic factors using analysis of variance (ANOVA), and linear regression models were constructed to predict hand contamination from fecal contamination of environmental samples. Handrinse samples had a mean *E. coli* concentration of 2.53 $\log_{10}$ colony forming units (cfu)/ pair of hands (range: no detectable *E. coli* to 5.19 $\log_{10}$ cfu/ pair of hands) and a mean enterococci concentration of 3.08 $\log_{10}$ cfu/ pair of hands (range: no detectable enterococci to 5.85 $\log_{10}$ cfu/ pair of hands). Handrinse samples from public latrines had the highest concentrations of *E. coli*, while handrinse samples from nurseries had the highest concentrations of enterococci. There was a moderate, positive correlation between *E. coli* and enterococci handrinse concentrations (r=0.33), with higher enterococci concentrations across all settings. *E. coli* concentrations on swabs of surfaces were a significant predictor of handrinse *E. coli* concentrations (p-value<0.0001). Future studies should focus on determining the strength of the association between fecal contamination of environmental surfaces and hand contamination in different settings and the implications for pathogen transmission.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I. BACKGROUND

A. The Global Burden of Diarrheal Disease

Diarrheal diseases are leading causes of morbidity and mortality for all ages worldwide and represent the fourth highest contributor to years of life lost (1, 2). The World Health Organization (WHO) estimates that there are four billion cases of diarrhea annually, representing 5.7% of the global disease burden (3). Of the estimated 8.8 million deaths in children under five years old globally in 2008, greater than 1.3 million (18%) were attributed to diarrhea. In sub-Saharan Africa, diarrheal diseases play an even larger role in childhood mortality, causing over half of all deaths in children under five (4).

Unsafe water and inadequate sanitation account for 88% of diarrheal disease worldwide (5). As of 2014, 2.5 billion people lack access to improved sanitation, and 748 million people have no access to improved drinking water sources (6). Almost half of the world's population rely on non-networked water supply services, which makes it necessary to use in-home water storage (7). Furthermore, 783 million people use either public or shared sanitation facilities. The Joint Monitoring Programme for Water Supply and Sanitation of WHO and the United Nations Children's Fund (UNICEF) defines an improved drinking water source as a water source that, due to its construction and usage, adequately protects the source from outside contamination (6, 8). Examples of improved drinking water sources include a public tap or standpipe, a borehole, piped water into a dwelling, or a protected well. An improved sanitation facility is defined as a facility that hygienically separates human excreta from human contact (6, 8). Examples of an improved sanitation facility include a flush toilet, a ventilated improved pit latrine, or a pit latrine with a slab.

B. Urbanization and Informal Settlements

Lack of access to improved drinking water and sanitation facilities is especially common in informal settlements in urban areas, where rapid urbanization occurs more quickly than the development of urban infrastructure (9). By 2007, more people globally were living in urban areas than rural areas. According to the 2003 United Nations Human Settlement Programme's (UN-HABITAT) report, an estimated 72% of the urban population of Africa lives in low-income areas (10). Urban informal settlements, or slums, describe parts of the city where living conditions are at their poorest. Poverty is considered a central characteristic of informal settlements. Rather than being viewed as an inherent characteristic of slums, economic poverty is seen as a cause of the sub-standard living conditions found in slums. Informal settlements create barriers to human and social development, both through physical isolation and through the daily living conditions present in these settings (9).

Slums are characterized by a lack of basic services, such as electricity, poor rainwater drainage systems, and an absence of surfaced roads. Overcrowding is a common feature of slum settings, with residents living in extremely limited space, often on illegally settled land. Slums are associated with large numbers of substandard housing structures and are often built illegally or with non-permanent materials unsuitable for the location and climate (9). These sub-standard living conditions contribute to high rates of illnesses like diarrhea, cholera, malaria, and respiratory infections (11).

Lack of access to improved sanitation facilities and water sources, high population density, and poor infrastructure create a web of fecal exposure pathways in low-income, urban environments. For residents of urban informal settlements, exposure to fecal pathogens occurs through a number of pathways and settings, such as recreational water, drinking water, food, flies, open drains, public latrines, schools, nurseries, and households. These pathways can exist in either the public domain, in locations outside of the home, or in the private domain, within households. Direct exposure to fecal pathogens occurs at the end of these pathways, through contact with skin or ingestion of fecal pathogens through food and drinking water. Fecal contamination of human hands is an important intermediate step between exposure to microbial pathogens in the environment and ingestion of these microbes and has been implicated in the transmission of diarrheal diseases (7, 12).

C. Measuring Hand Contamination

i. Microbiological Measures of Hand Contamination

Microbiological hand contamination is commonly assessed through measurement of fecal indicator bacteria on hands. Fecal indicator organisms are used globally to monitor water quality and assess the safety of drinking water and recreational waters (13). In developing countries, handwashing with soap has been found to reduce fecal indicator bacteria on hands, which are often used a measure to evaluate the success of hand hygiene interventions (7). Fecal coliforms are bacteria that reside in the gastrointestinal tract of humans and animals, and occur in high concentrations in feces. These organisms are used to indicate the presence of feces in water and on hands, signifying risk of fecal-oral

disease transmission (7). Coliform bacteria are identified in water as either total coliforms or *Escherichia coli* (*E. coli*). Enterococci are a different type of fecal indicator bacteria, and are found in high concentrations in human feces, usually between 10^4 and 10^6 bacteria per gram wet weight (14). Due to their persistence in the environment and presence in human feces, enterococci have been adopted as an indicator organism for the presence of human fecal contamination.

Several studies using both *E. coli* and enterococci as measures of hand contamination in developing countries have found that enterococci may be the superior fecal indicator organism, due to longer survival times (15-18). Enterococci's superior performance over *E. coli* has been attributed to the prolonged survival of enterococci on inoculated clean hands and surfaces (18). *E. coli* has been found to persist on the hands for shorter periods of time than fecal streptococci (17, 19). Pickering et al. (16) has suggested that measuring *E. coli* on hands is a poor indicator of daily activities and hand hygiene behavior, as differential exposures to fecal contamination and time elapsed since handwashing with soap were not found to be associated with increasing and decreasing levels of *E. coli* on hands. In contrast, enterococci accumulation on hands may be traceable to specific activities and may be more strongly correlated with other hygiene indicators (14).

ii. Handrinse Samples

Handrinse samples are the most common method used to quantify the presence and level of fecal indicator organisms on human hands. Handrinse samples are typically obtained

by having study participants insert their hands, one at a time, into Whirl-Pak bags (Nasco, Fort Atkinson, WI) or other containers filled with sterile water (7, 20).

Despite the frequent use of this method, measuring hand contamination at critical times can be challenging, and the use of handrinse samples has its limitations. In a study from north-east Thailand, *E. coli* contamination of fingertip rinses was strongly associated with the activity done prior to testing, highlighting the potential for variations in hand contamination throughout the day (17). Multiple studies have found that microbiological hand contamination can vary substantially from one sampling time to another (21, 22). One study was designed to assess whether measuring hand contamination at convenient times (as is often done by researchers) can predict hand contamination at times critical to pathogen transmission (before eating, before drinking, before feeding a child, and before storing water) (21). Hand contamination measured at a convenient time, such as when a researcher first arrives at a home, was not well-correlated to hand contamination measured at critical times. Further, single handrinses were not a valid proxy for handwashing behavior. The authors concluded that further research is needed to better understand the factors contributing to variability in handrinse sample measurements (21).

D. Characterizing Hand Contamination in Low-Income, Urban Settings

i. Determinants of Hand Contamination

As fecal contamination of hands is an established pathway in the transmission of diarrheal diseases, it is important to understand how hand contamination levels vary across different settings and populations. Several studies have assessed household factors

associated with hand contamination in low-income settings (7, 17, 23). In a study in peri-urban Tanzania, Pickering et al. (7) found that household factors associated with hand contamination included mother's educational level, use of an improved toilet, an infant living in the household, and dissatisfaction with the quantity of water available for hygiene. Completion of primary education by the mother was associated with lower fecal streptococci and *E. coli* hand contamination, while a mother's dissatisfaction with available water for hygiene purposes was associated with higher fecal streptococci contamination. Household income (measured by the proxy indicator regular weekly household expenditures per person) was not significantly associated with fecal streptococci hand contamination (7).

A separate household study found that hand contamination with enterococci was associated with childcare activities, while both *E. coli* and enterococci hand contamination were associated with food preparation (23). In this study, the authors were not able to find any basic household characteristics (education, water access, latrine type) which accounted for differences in hand contamination levels. An earlier study from a village in Thailand also found that childcare activities were associated with hand contamination (17). In this study, childcare, food, and water-related activities produced much higher levels of fingertip contamination than other activities. These studies highlight the important role that women, in particular, play in the spread of fecal contamination within the household. As women are primarily responsible for household activities and child care, they have the potential for frequent exposures to fecal contamination. If hands are not washed at key times, fecal pathogens are likely to be

transmitted to food, water, clothing, and household objects before ultimately make their way into the mouths of household residents (24).

Schools and nurseries represent the public domain and serve as a setting for exposure to fecal contamination by children. In a cluster-randomized trial assessing the impact of a handwashing intervention among primary school students in Kenya, detectable levels of *E. coli* were present on the hands of roughly 40% of schoolchildren at baseline (25, 26). The frequency of children with high levels of hand contamination, defined as greater than or equal to 100 colony forming units (cfu) per hand, ranged from 26% at baseline to 57% at follow-up (25). Being in a school in either intervention group did not affect whether a student was likely to have *E. coli* contamination present on his/ her hands (26). However, students in one geographic stratum were significantly more likely to have *E. coli* contamination on their hands than students in the other geographic stratum, regardless of intervention status (26). The authors suggest these differences could be attributable to differential socioeconomic status (SES), water availability at schools, and more rural versus less rural areas included in the study. These findings suggest that SES and geographic location could be important determinants of fecal hand contamination among children.

ii. Relationship between Hand Contamination and Environmental Contamination

In addition to understanding which demographic and structural factors are associated with hand contamination in low-income settings, examining the relationship between fecal contamination in the environment and contamination of hands is vital to fully

capturing the fecal exposure pathways in these settings. Several prior studies have focused on the relationship between fecal contamination of hands and contamination of environmental media, such as water, soil, and indoor surfaces (7, 22, 27-29). One such study found that enterococci contamination levels on hands of mothers and children were positively correlated with enterococci contamination in stored drinking water within households in peri-urban Tanzania (7). The authors suggest that in areas with low levels of sanitation, contamination of stored water following collection could be facilitated by hands contaminated during defecation or other activities.

Another media for measuring fecal contamination in the environment is particulates, such as sand and soil. Multiple studies have suggested that particulate contamination may be an important media for microbial exposure to fecal pathogens (27, 28). In a survey of fecal contamination and diarrheal pathogens in soil among households using private pit latrines in peri-urban Tanzania, Pickering et al. (27) found that soil collected from the house floor had significantly higher concentrations of both *E. coli* and enterococci than soil collected from the latrine floor. This study suggests that particulate fecal contamination varies within different areas of the household environment and may represent differential risk of exposure to fecal contamination. In another study, Whitman et al. (28) examined the potential for hand-mouth transfer of fecal contamination through an assessment of the transferability of *E. coli* from beach sand to hands. Handrinse samples were collected following manipulation of beach sand and were analyzed for *E. coli* and coliphage. *E. coli* densities transferred from sand to hands were correlated with density in sand rather than surface area of an individual's hand (28).

Limited data exists on the relationship between fecal contamination of surfaces and hand contamination in low-income, urban settings. One study has suggested the potential for transmission of fecal contamination from surfaces to hands among street food vendors in Guatemala (22). In a randomized, controlled intervention trial assessing recontamination of hands following handwashing, there was no difference in fecal coliforms or *E. coli* contamination of handrinse samples between vendors who received the handwashing intervention and those who did not (22). Only 8% of handrinse samples collected immediately after handwashing contained detectable levels of fecal coliform and *E. coli*. One hour later, however, fecal coliform bacteria were detected in 46% of handrinse samples and *E. coli* were detected in 23% of handrinse samples from the vendors (22). These findings suggests that the hands of street vendors were quickly recontaminated through contact with surfaces or other items following handwashing. This study also highlights the potential for high fluctuations in hand contamination levels in a short period of time.

Despite the limited data available from low-income settings, two studies from the United States support surface-mediated transmission as a potential route for the spread of infectious diseases, and highlight the importance of fecal indicator organisms as a metric for fecal contamination of hands and fomites (12, 30). In a study which examined the link between respiratory diseases and bacteria contamination at child care centers in California, symptomatic respiratory illness was positively associated with enterococci contamination on hands and fomites (30). In a separate study of bacterial communities found on surfaces in public restrooms in Colorado, Flores et al. (12) found that bacterial

communities were clustered into three categories: those found on surfaces associated with toilets, those on the restroom floors, and those found on surfaces commonly touched by human hands. On toilet surfaces, gut-associated bacteria were most prevalent, indicating fecal contamination of these surfaces, while surfaces touched frequently by human hands were contaminated with skin-associated bacteria. Floor surfaces were the most bacteriologically diverse of the three categories. These findings show that human-associated bacteria are commonly found on restroom surfaces and suggest that bacterial pathogens could be easily transmitted between individuals touching the same surfaces (12). Although these studies were not conducted in a low-income, urban setting, their findings highlight the importance of understanding the transmission of fecal contamination between surfaces and human hands.

Though several studies have examined the relationship between environmental contamination and hand contamination, one study has focused on ingestion of fecal contamination from environmental exposures (31). The authors modeled the amount of human feces ingested by children under five years old from hand-to-mouth contacts and stored drinking water in Bagamoyo, Tanzania. The model showed that children ingest a significantly greater amount of feces each day from hand-to-mouth contacts than from drinking water (31). This finding highlights the importance of understanding the role of different environmental fecal transmission routes linked to hands when assessing exposures for young children.

While several studies have used handrinse samples as a measure of fecal contamination, most have assessed the efficacy of handwashing interventions in low-income settings. These studies have found that rapid recontamination of hands after handwashing is likely in areas with poor access to water and sanitation facilities, but have not directly examined the relationship between environmental fecal contamination and hand contamination (22, 23, 32). Additionally, of the studies that have examined the relationship between environmental and hand fecal contamination levels, most have not considered how contamination of different environmental media impacts the level of fecal contamination on hands (7, 12, 30).

E. Description of Accra, Ghana

Ghana has a population of over 25 million and an estimated annual growth rate of 2.2%, with much of this growth occurring in urban areas (33, 34). In Accra, Ghana, a coastal city with a population of 3.9 million, the average annual population growth rate is 4.4%, and up to 7% in some informal settlements (35-37).

i. Sanitation and Hygiene in Accra

In Ghana, 5,193 deaths in children under five were attributable to diarrheal diseases in 2008 alone (38). Ninety-three percent of the urban population in Ghana has some access to an improved water source, with 34% having access to piped water (39). However, handwashing rates are extremely low, and much of the urban population does not have access to improved sanitation facilities. A 2007 national survey found that only about 4% of mothers in Ghana washed their hands with soap following defecation, and only 2%

washed their hands with soap after cleaning a child's bottom (40). In urban areas, only 19% of the population has access to improved sanitation facilities, while 73% use shared facilities, 2% use other types of unimproved facilities, and 6% of residents practice open defecation (41).

In the Accra Metropolitan Area (AMA), 41.3% of residents do not have sanitation facilities in their homes, and instead rely on the use of public latrines (42). It is estimated that only 4.3% of Accra residents practice open defecation or use bucket or pan latrines, but observations of "flying toilets" (excreta in plastic bags) and open defecation at beaches suggest this is an underestimation (42, 43). In a more recent survey, almost one-third of Accra residents reported using a bucket or pan latrine (44). Low rates of handwashing, poor access to improved sanitation facilities, and heavy use of shared facilities and public latrines contribute to exposure to fecal contamination in both public and private domains in Accra.

ii. Description of the Study Neighborhoods

Earlier reports have described the demographics of Bukom, Alajo, Old Fadama, and Shiabu, the four neighborhoods in Accra, Ghana where data was collected for Phase 1 of the SaniPath study (43, 45). Alajo and Bukom are formal settlements, Old Fadama is an informal settlement, and Shiabu is a mixed settlement. Old Fadama is the poorest of the four neighborhoods, with an estimated average per capita daily income of less than $2 per day (45). The dominant religion in Alajo, Bukom and Shiabu is Christianity, while Islam is the dominant religion in Old Fadama. Old Fadama has the lowest levels of education,

with 40% of respondents reporting no formal education. Alajo and Shiabu report the highest levels of education, with 40% of residents having completed secondary school or higher (43).

In all four neighborhoods, at least 10% of the children in surveyed households had diarrhea within the past two weeks (43). Old Fadama reported the highest rates of diarrhea, with 25% of children in surveyed households having had diarrhea in the past two weeks. Less than 10% of residents in Bukom or Old Fadama have access to sanitation facilities within their household compound, and nearly all residents rely on public latrines to some extent (43, 45). In comparison, over half of residents living in Alajo and just over 40% of residents in Shiabu have access to an improved toilet in their household compound. Piped water is intermittent, so household and school storage of drinking water is common in the study neighborhoods.

F. Study Objectives

Previous studies have discussed the importance of hand contamination as a fecal exposure route in low-income, urban settings and have identified structural and environmental factors associated with hand contamination in these settings. Limitations in understanding variations in hand contamination in public and private domains and the influence of environmental fecal contamination on hand contamination in these settings motivated this study to examine hand contamination as a fecal exposure pathway in further depth. The research questions for this study were threefold:

1) How do *E. coli* and enterococci contamination of hands in Accra, Ghana differ across neighborhoods, settings, and demographic characteristics?

2) What is the correlation between *E. coli* and enterococci handrinse concentrations overall and within different neighborhoods and settings?

3) Is environmental fecal contamination, as measured by surface swabs, soil, and stored drinking water, associated with *E. coli* contamination of hands in the study areas?

The results of this study can be used to raise awareness about the link between fecal contamination in the environment and fecal contamination of hands. These results can also be used by stakeholders to inform well-designed interventions which aim to reduce human exposure to fecal contamination by identifying settings and sub-populations with the potential for high levels of hand contamination.

II. MANUSCRIPT

A. Introduction

The World Health Organization estimates that there are four billion cases of diarrhea annually, representing 5.7% of the global disease burden (3). In sub-Saharan Africa, diarrheal diseases play a large role in childhood mortality, causing over half of all deaths in children under five (4). Unsafe water and inadequate sanitation account for 88% of diarrheal disease worldwide (5). As of 2014, 2.5 billion people lack access to improved sanitation, and 748 million people have no access to improved drinking water sources (6). Furthermore, 783 million people use either public or shared sanitation facilities. Low access to improved drinking water and sanitation facilities is especially common in informal settlements, or slums, in urban areas, where rapid urbanization occurs more quickly than the development of urban infrastructure (9). An estimated 72% of the urban population of Africa lives in low-income areas which are rapidly growing in size (10). In Accra, Ghana, a coastal city with a population of 3.9 million, the average annual population growth rate is 4.4%, and up to 7% in some informal settlements (35-37). In urban areas of Ghana, only 19% of the population has access to improved sanitation facilities (41).

Lack of access to improved sanitation facilities, high population density, and poor infrastructure create a web of fecal exposure pathways in low-income, urban environments. For residents of urban informal settlements, exposure to fecal pathogens occurs through a number of pathways and settings, such as recreational water, drinking water, food, flies, open drains, public latrines, schools, and households. Fecal

contamination of human hands is an important intermediate step between exposure to microbial pathogens in the environment and ingestion of these microbes and has been implicated in the transmission of diarrheal diseases (7, 12).

Microbiological hand contamination is commonly assessed through measurement of fecal indicator bacteria on hands. Fecal indicator organisms are bacteria that reside in the gastrointestinal tract of humans and animals, and occur in high concentrations in feces. These organisms are used to indicate the presence of feces in water and on hands, signifying risk of fecal-oral transmission of disease (7). Several studies using both *Escherichia coli* (*E. coli*) and enterococci as measures of hand contamination in developing countries have suggested that enterococci may be the superior fecal indicator organism, due to longer survival times (15-18).

As fecal contamination of hands is an established exposure pathway in the transmission of diarrheal diseases, it is important to understand how hand contamination levels vary across different settings and populations. While several prior studies have used handrinse samples as a measure of fecal contamination, most have focused on the efficacy of handwashing interventions in low-income settings. These studies have found that rapid recontamination of hands after handwashing is likely in areas with poor access to water and sanitation facilities, but have not directly examined the relationship between environmental fecal contamination and hand contamination (22, 23, 32). Additionally, of the studies that have examined the relationship between environmental and hand fecal contamination levels, most have not considered how contamination of different

environmental media (stored drinking water, soil, and indoor surfaces) impacts the level of fecal contamination on hands (7, 12, 30). Limitations in understanding variations in hand contamination and the influence of environmental fecal contamination on hand contamination in low-income, urban settings motivated this study to examine hand contamination as a fecal exposure pathway in further depth. The research questions for this study were threefold:

1) How do *E. coli* and enterococci contamination of hands in Accra, Ghana differ across neighborhoods, settings, and demographic characteristics?

2) What is the correlation between *E. coli* and enterococci handrinse concentrations overall and within different neighborhoods and settings?

3) Is environmental fecal contamination, as measured by surface swabs, soil, and stored drinking water, associated with *E. coli* contamination of hands in the study areas?

B. Methods

The SaniPath study was a cross-sectional study which aimed to characterize fecal contamination pathways in urban, low-income settings. Phase 1 of this study was conducted from July 2011 to November 2012 in four low-income neighborhoods in Accra, Ghana. Study components pertinent to this analysis are: 1) environmental sampling and testing of handrinse samples collected from children and adults in four settings: households, public latrines, schools, and nurseries, and 2) environmental sampling and testing of surface swabs, soil, and stored drinking water samples from these

settings. Exemption status was obtained for this analysis from Emory University's Institutional Review Board.

i. Study Setting and Context

Neighborhood selection

Four low-resource neighborhoods in the Accra Metropolitan Area (AMA) were selected for this study: Alajo, Bukom, Old Fadama, and Shiabu. Neighborhood selection was based on a set of demographic and physical characteristics described in Table 1. Secondary selection criteria considered were logistics, receptiveness of community members, and the safety of the study team. All neighborhoods were known to have public latrines, schools, and varying levels of latrine coverage.

Neighborhood Characteristics

Primarily, the study neighborhoods differed in the following characteristics: proximity to canals/low elevations, settlement type (planned/unplanned), proximity to ocean/open water, and proximity to a major market. Some of these neighborhoods are acknowledged by the Ghanaian government, while some are considered illegal settlements. The neighborhoods also differed from each other in terms of demographics and sanitation characteristics.

ii. Data Collection

All data collection was conducted by trained staff who were accompanied by community liaisons. Community liaisons were familiar with the study neighborhoods and helped

guide the selection of sampling sites. Environmental samples were collected from March to December 2012 by study staff at the Water Research Institute (WRI).

Handrinse Samples

Samples were collected from participants in four different settings: households, schools, nurseries, and public latrines. After providing oral consent, each participant underwent a quick visual inspection of their hands, in which the enumerators recorded whether dirt was visible under the subject's nails, on the subject's finger pads, or on the subject's palms. Enumerators then asked each subject to place his/ her right hand in a 500mL Whirl-Pak bag (Nasco, Fort Atkinson, WI) containing 250mL of sterile PBS (phosphate-buffered saline) solution. Enumerators grasped the bag around the participant's wrist to secure it and gently massaged the fingers and the palm of the hand from the outside of the Whirl-Pak bag for 30 seconds. The participant was asked to carefully remove the right hand and to insert his/ her left hand into the bag, repeating the procedure. Samples were sealed, stored on ice, and returned to WRI within six hours of collection (46). A sample collection form was also completed at the time of sampling to record participant demographics and visual inspection results (Appendix A).

Stored Drinking Water Samples

Stored drinking water samples were collected from households, schools, and nurseries. Participants were asked to fill a 500mL Whirl-Pak bag from the stored water reservoir in the same manner by which they normally collect water for drinking. Samples were sealed, stored on ice, and returned to WRI within six hours of collection (46). A sample

collection form was also completed at the time of sampling to record storage container characteristics and water collection methods (Appendix B).

Swab Samples

Items selected for swab sampling were chosen after enumerators observed children touching these objects. If the item to be swabbed was flat, enumerators held a framing square against the surface and, moving right to left horizontally, swabbed an area of at least $5cm^2$ or 2.5cm by 10cm on each arm of the framing square with an EnviroMax Plus Sterile Environmental Sampling Swab (Puritan Medical Products, Guilford, ME). At the midway point, they turned the swab over and used the opposite side to finish swabbing the other half of the object. If the item was small and round, or measurement on a flat surface was not possible, the object was held between two fingertips and the entire surface of the object was swabbed. If the item was not flat, but was larger than a soccer ball, the framing square was used to estimate an area of approximately 10cm by 10cm and swabbed as described above. Swabs were placed into the tube rack in a cooler and returned to WRI within six hours of collection (46). A sample collection form was also completed at the time of sampling to record the type and size of object swabbed (Appendix C).

Soil Samples

For each soil sample, enumerators grasped the end of a sterile spatula and inserted the scoop into the soil at a 45° angle to a depth of 5cm. Three scoops of soil were placed into a 250mL Whirl-Pak bag. Enumerators attempted to collect samples from a variety of

locations, including wet soil and dry soil. At public latrines, a composite was collected from an area of approximately $3m^2$. At households and schools, composite samples were collected from up to seven areas at one location, including the compound/ structure entrance, near a latrine, near a cooking/ food preparation area, near an area where water and/ or trash is disposed, and near an area where children play, and placed into one 250mL Whirl-Pak bag. Samples were stored on ice and returned to WRI within six hours of collection (46). A sample collection form was also completed at the time of sampling to record collection location details (Appendix D).

iii. Data Management

All paper forms completed in the field were entered into a central Microsoft Access database (Microsoft Corporation, Redmond, WA) managed by the study team. Double-data entry was completed on 25% of all forms to ensure data quality.

iv. Laboratory Methods

For the handrinse and stored drinking water samples, Whirl-Pak bags were removed from cold storage, and the contents of each bag were mixed by turning it end over end five times. Swab samples were eluted in 8mL PBST (phosphate-buffered saline, pH 7.2 with 0.04% Tween-80). Whirl-Pak bags containing the soil samples were rotated five times to mix the sample. Ten grams of the soil sample were measured and added to 20mL of sterile PBS, before adjusting the pH to 9.0 with 0.1N NaOH.

All four sample types were analyzed for *E. coli* by membrane filtration, according to EPA Method 1604 using MI agar (47). Handrinse samples were also analyzed for enterococci by membrane filtration, according to EPA Method 1600 using MEI agar (48). Three volumes were filtered per sample: 1mL, 10mL, and 100mL for handrinse and stored drinking water samples, and 0.01mL, 0.1 mL, and 1mL for swab and soil samples. *E. coli* and enterococci concentrations were assessed as colony forming units (cfu) per pair of hands for handrinse samples, cfu per 100mL for stored drinking water, cfu per swab for swabs, and cfu per gram for soil. Following 20 to 24 hours of incubation at 37°C, samples were examined under ambient light, and the number of blue colonies were counted, beginning with plates showing the lowest dilution/ highest concentration (46).

Samples in which individual colonies could not be clearly distinguished from each other and from background growth and dirt on the filter were not included in the analysis. If the counts on all three dilutions were zero, half the theoretical lower limit of detection was used to estimate the concentration, with imputed values of 2.5 cfu per pair of hands, 0.5 cfu per 100mL of drinking water, 4 cfu per swab, and 1 cfu per gram of soil. Plates exceeding 200 cfu were considered "too numerous to count" (TNTC) and above the upper limit of detection. If counts of all three plates were TNTC, an imputed value of 200 cfu was used for the plate with the highest dilution. If only one or two plates were in countable range, only the plates in the countable range were selected when estimating the indicator concentration. Each MI Agar plate was exposed to long-wave ultraviolet light (366 nm), to determine whether any colonies fluoresced, and the presence of total coliforms was recorded.

v. Statistical Methods

Statistical analyses were conducted in SAS 9.4 (Cary, NC). All statistical tests were evaluated at an alpha level of 0.05. From the three environmental sample dilutions, all plates in the countable range described above were used to determine the final bacteria indicator concentration in each sample. Since data for these indicators was not normally distributed, the final *E. coli* and enterococci concentrations were log-transformed. For both *E. coli* and enterococci, the mean, standard deviation, minimum, and maximum were calculated for handrinse samples overall and by neighborhood, setting, sex, age category (under 5 years, 5-12 years, and adults), and presence of visible dirt on the finger pads, on the palms of hands, and under the fingernails. Mean *E. coli* and enterococci concentrations in the handrinse samples were normalized by average hand surface area for the different age categories, using values from the "Recommended Values for Surface Area of Body Parts" table from the Exposure Factors Handbook (Appendix E) (49).

E. coli and enterococci concentrations were analyzed with analysis of variance (ANOVA) to determine if significant differences existed between neighborhoods, setting, and age categories. To further understand these differences, all pair-wise comparisons were conducted for variables in which significant differences were detected between levels of *E. coli* and/ or enterococci concentrations. Fecal indicator bacteria concentrations were also analyzed using two-sample t-tests to determine if there were significant differences by sex and the presence of visible dirt on hands. Histograms of both *E. coli* and enterococci concentrations were constructed for each of the above characteristics. To further understand differences in *E. coli* and enterococci concentrations in different

settings, mean, standard deviation, minimum, and maximum were calculated for all handrinse samples across age categories and neighborhoods by setting, and *E. coli* and enterococci concentrations were analyzed with two-sample t-tests or ANOVA, as appropriate.

Correlation analysis was conducted to examine the relationship between *E. coli* and enterococci concentrations. Since data for these indicators was log-transformed, Pearson correlation coefficients were calculated to assess the significance and strength of the linear relationship between *E. coli* and enterococci concentrations. This analysis was repeated to assess the correlation between *E. coli* and enterococci concentrations for handrinse samples by neighborhood, setting, sex, age category, and presence of visible dirt on hands. Scatterplots were constructed to graphically display the linear relationship between *E. coli* and enterococci for the demographic variables.

A subset of the environmental samples data was constructed, which included only those swab, soil, and stored drinking water samples collected from the same locations where handrinse samples were collected. For locations where multiple samples of the same type were tested, the *E. coli* concentrations of individual samples were averaged for non-handrinse samples to create a mean *E. coli* concentration for each sample type at each location. Mean, standard deviation, minimum, and maximum *E. coli* concentrations were calculated for the subset of swab, soil, and stored drinking water samples, and histograms of *E. coli* concentrations were constructed for each sample type. Simple linear regression models were constructed to assess the individual influence of *E. coli* concentrations from

swab, soil, and stored drinking water samples, as well as select demographic characteristics, on *E. coli* concentrations from handrinse samples. Multiple linear regression was conducted to examine combined effects of the environmental samples and demographic characteristics on *E. coli* concentrations of handrinse samples.

Predictive linear regression modeling was performed using four approaches: All Possible/ Best Subsets, Backwards Elimination, Forward Selection, and Stepwise Selection. The full model included the three predictors of interest (swab, stored drinking water, and soil samples) and the following covariates: neighborhood, setting, sex, and age category; the covariates were determined *a priori* to model fitting. This produced potential reduced models, from which a final 'best' predictive model was selected.

C. Results

i. Microbiological Contamination of Handrinse Samples

There were 276 handrinse samples tested for *E. coli* and 278 handrinse samples tested for enterococci. The concentrations of *E. coli* in the handrinse samples were consistently lower than the enterococci concentrations across all categories (Tables 2, 3). The overall mean *E. coli* concentration in the handrinse samples was 2.53 $\log_{10}$ cfu/ pair of hands, ranging from no detectable *E. coli* (<1 cfu/ pair of hands) to a maximum of 5.19 $\log_{10}$ cfu/ pair of hands (Figure 1). The overall mean enterococci concentration was 3.08 $\log_{10}$ cfu/ pair of hands, ranging from no detectable enterococci (<1 cfu/ pair of hands) to a maximum of 5.85 $\log_{10}$ cfu/ pair of hands (Figure 2). No significant differences were observed in *E. coli* concentrations between handrinse samples taken from males and

females (Figure 3), but enterococci concentrations in the handrinse samples were significantly higher for females (mean = 3.19 $\log_{10}$ cfu/ pair of hands) than for males (mean = 2.92 $\log_{10}$ cfu/ pair of hands) (p-value=0.0181) (Figure 4).

There were significant differences in both *E. coli* and enterococci concentrations in handrinse samples across different age categories (p-value=0.0006, p-value=0.0350, respectively) (Figures 5, 6). Adults had significantly higher *E. coli* concentrations on their hands (mean = 2.84 $\log_{10}$ cfu/ pair of hands) than both children under five (mean = 2.43 $\log_{10}$ cfu/ pair of hands) and children 5-12 years old (mean = 2.12 $\log_{10}$ cfu/ pair of hands) (Table 4). Children under five had significantly higher enterococci concentrations on their hands (mean = 3.25 $\log_{10}$ cfu/ pair of hands) than children 5-12 years old (mean = 2.88 $\log_{10}$ cfu/ pair of hands). Handrinse samples from adults had a mean enterococci concentration of 3.03 $\log_{10}$ cfu/ pair of hands (Table 3).

However, when we normalized these concentrations by average hand surface areas, handrinse samples from children under five had the highest mean concentrations of *E. coli* and enterococci, while handrinse samples from adults had the lowest mean concentrations of *E. coli* and enterococci, per cm^2 of hand surface (Appendix E). Children under five had a mean *E. coli* concentration of 0.77 $\log_{10}$ cfu/ cm^2 of hand surface, compared to 0.42 $\log_{10}$ cfu/ cm^2 of hand surface for children 5-12 years old, and 0.29 $\log_{10}$ cfu/ cm^2 of hand surface for adults. Children under five had a mean enterococci concentration of 1.02 $\log_{10}$ cfu/ cm^2 of hand surface, compared to 0.57 $\log_{10}$ cfu/ cm^2 of

hand surface for children 5-12 years old, and 0.31 $\log_{10}$ cfu/ cm^2 of hand surface for adults.

While there were no significant differences in handrinse *E. coli* concentrations by neighborhood (Table 2, Figure 7), there were significant differences in enterococci concentrations in the handrinse samples by neighborhood (p-value<0.0001) (Table 3, Figure 8). Enterococci concentration in these samples ranged from a mean of 2.78 $\log_{10}$ cfu/ pair of hands in Shiabu to 3.50 $\log_{10}$ cfu/ pair of hands in Old Fadama. Handrinse samples from Old Fadama and Bukom (mean = 3.23 $\log_{10}$ cfu/ pair of hands) had significantly higher enterococci concentrations that handrinse samples from Alajo (mean = 2.83 $\log_{10}$ cfu/ pair of hands) and Shiabu (Table 5).

There were significant differences in both *E. coli* and enterococci concentrations in the handrinse samples by setting (p-value=0.0015, p-value=0.0154, respectively) (Tables 2, 3). *E. coli* concentrations in the handrinse samples ranged from no detectable *E. coli* (<1 cfu/ pair of hands) to a maximum of 5.19 $\log_{10}$ cfu/ pair of hands in public latrines (Table 2, Figure 9). Handrinse samples from public latrines (mean = 2.95 $\log_{10}$ cfu/ pair of hands) had significantly higher *E. coli* concentrations than handrinse samples from households (mean = 2.46 $\log_{10}$ cfu/ pair of hands) and schools (mean = 2.15 $\log_{10}$ cfu/ pair of hands) (Table 4). Enterococci concentrations in the handrinse samples were also lowest in schools (mean = 2.78 $\log_{10}$ cfu/ pair of hands), and were highest in nurseries (mean = 3.27 $\log_{10}$ cfu/ pair of hands) (Table 3, Figure 10). Handrinse samples from

schools had significantly lower enterococci concentrations than handrinse samples from nurseries and households (mean = 3.20 $\log_{10}$ cfu/ pair of hands) (Table 5).

Comparison of Hand Contamination by Age, Sex, and Setting

E. coli and enterococci concentrations in the handrinse samples were compared across sex and age categories within specific settings. About 40% of the handrinse samples from males were from public latrines, about 45% of handrinse samples from males were split evenly between schools and nurseries, and the remaining 16% of male handrinse samples were from households (Figure 11). Forty-four percent of the handrinse samples from females were from households, about 40% of handrinse samples from females were split evenly between public latrines and schools, and the remaining 16% of female handrinse samples were from nurseries (Figure 11). For the handrinse samples from children under five, about 50% were from households, while just under 50% were from nurseries (Figure 12). For the handrinse samples from children 5-12 years old, nearly 85% were from schools, 11% were from nurseries, and the remaining 4% of handrinse samples were from households and public latrines (Figure 12). For the handrinse samples from adults, nearly two-thirds were from public latrines, while one-third were from households (Figure 12).

Examining the number of handrinse samples collected in each age and sex category by setting, there were a sufficient number of handrinse samples to compare fecal indicator bacteria concentrations from male and female 5-12 year olds at schools, male and female adults at public latrines, and female adults and children under five at households. In schools, handrinse samples from female 5-12 year olds had significantly higher mean

enterococci concentrations (mean = 2.97 $\log_{10}$ cfu/ pair of hands, n=30) than handrinse samples from male 5-12 year olds (mean=2.54 $\log_{10}$ cfu/ pair of hands, n=23) (p-value=0.0447). There was not a significant difference in *E. coli* concentrations of school handrinse samples from male and female 5-12 year olds. No significant differences were observed between adult males and females for *E. coli* or enterococci concentrations in the handrinse samples collected at public latrines. There were also no significant differences observed between female adults and children under five for *E. coli* or enterococci concentrations in the handrinse samples collected at households. However, when we normalized these concentrations by average hand surface areas, handrinse samples from children under five had higher mean concentrations of *E. coli* and enterococci (0.75 $\log_{10}$ cfu/ cm^2 of hand surface, 1.03 $\log_{10}$ cfu/ cm^2 of hand surface, respectively), compared to handrinse samples from female adults (0.28 $\log_{10}$ cfu/ cm^2 of hand surface, 0.34 $\log_{10}$ cfu/ cm^2 of hand surface, respectively) collected at households (Appendix E).

Comparison of Hand Contamination by Neighborhood and Setting

E. coli and enterococci handrinse concentrations were also compared across neighborhoods for specific settings. There were significant differences in *E. coli* concentrations in all handrinse samples from households by neighborhood (p-value=0.0381) (Table 6), but no significant differences were observed in enterococci concentrations in the handrinse samples from households by neighborhood (Table 7). *E. coli* concentrations in the household handrinse samples ranged from a mean of 2.11 $\log_{10}$ cfu/ pair of hands in Shiabu to 2.99 $\log_{10}$ cfu/ pair of hands in Bukom. However, all pair-wise comparisons tests revealed no significant differences in household handrinse *E. coli*

concentration between individual neighborhoods. There were significant differences in enterococci concentrations in the handrinse samples from schools by neighborhood (p-value=0.0003) (Table 7). Handrinse samples from schools in Old Fadama (mean = 3.33 $\log_{10}$ cfu/ pair of hands) had significantly higher enterococci concentrations than handrinse samples from schools in Alajo (mean = 2.60 $\log_{10}$ cfu/ pair of hands) and Shiabu (mean = 2.31 $\log_{10}$ cfu/ pair of hands) (Table 9). There were no significant differences in *E. coli* concentrations in the handrinse samples from schools by neighborhood.

Significant differences were observed in enterococci concentrations in the handrinse samples from nurseries by neighborhood (p-value=0.0100) (Table 7). Enterococci concentrations in the nursery handrinse samples were significantly lower in Shiabu (mean = 2.83 $\log_{10}$ cfu/ pair of hands) than Old Fadama (mean = 3.79 $\log_{10}$ cfu/ pair of hands) (Tables 7, 9). No significant differences were observed in *E. coli* concentrations in the nursery handrinse samples by neighborhood (Table 6). There were significant differences in both *E. coli* and enterococci concentrations in the handrinse samples from public latrines by neighborhood (p-values=0.0495, p-value = 0.0174, respectively) (Tables 6, 7). *E. coli* concentrations in the handrinse samples from public latrines were significantly lower in Bukom (mean = 2.52 $\log_{10}$ cfu/ pair of hands) than Old Fadama (mean = 3.55 $\log_{10}$ cfu/ pair of hands) (Tables 6, 8). Enterococci concentrations in the handrinse samples from public latrine were significantly higher in Old Fadama (mean = 3.56 $\log_{10}$ cfu/ pair of hands) than Alajo (mean = 2.66 $\log_{10}$ cfu/ pair of hands) (Tables 7, 9).

Comparison of Hand Contamination by Presence of Visible Dirt on Hands

During collection of handrinse samples, study staff recorded whether visible dirt was present on the finger pads, on the palms of hands, and under the fingernails of all participants. Visible dirt was observed on the finger pads of about 25% of all participants, on the palms of hands of about 30% of all participants, and under the fingernails of about 77% of all participants (Tables 4, 5). There were significantly higher enterococci concentrations in handrinse samples taken from participants with visible dirt on the finger pads (mean = 3.48 $\log_{10}$ cfu/ pair of hands), compared to those without visible dirt on the finger pads (mean = 2.92 $\log_{10}$ cfu/ pair of hands) (p-value<0.0001) (Table 5). There were also significantly higher enterococci concentrations in handrinse samples taken from participants with visible dirt on the palms (mean = 3.38 $\log_{10}$ cfu/ pair of hands), compared with without visible dirt on the palms (mean = 2.94 $\log_{10}$ cfu/ pair of hands) (p-value=0.0002). There was no significant difference in mean handrinse enterococci concentrations between those with and without visible dirt under their fingernails. There was also no significant difference in mean handrinse *E. coli* concentrations between those with and without visible dirt on the finger pads, on the palms of hands, or under the fingernails (Table 4).

ii. Relationship between *E. coli* and Enterococci Hand Contamination

To assess the relationship between *E. coli* and enterococci concentrations in the handrinse samples, the correlation between these two measures was calculated overall and for different demographic populations, settings, and neighborhoods. Overall, as the concentration ($\log_{10}$ cfu/ pair of hands) of *E. coli* in the handrinse samples increased, the

enterococci concentration also increased (Figure 13). There was a moderate, positive and statistically significant linear relationship between *E. coli* concentrations and enterococci concentrations in the handrinse samples (r=0.33, p-value<0.0001) (Table 10). There were moderate, positive and statistically significant linear relationships between *E. coli* and enterococci concentrations in the handrinse samples for both males and females (r=0.38, p-value<0.0001; r=0.34, p-value<0.0001, respectively) (Figure 14).

By age, there was a strong, positive and statistically significant linear relationship between *E. coli* and enterococci concentrations in the handrinse samples from participants under five years old (r=0.57, p-value<0.0001) (Table 10, Figure 15). There was a weak, positive and statistically significant linear relationship between *E. coli* and enterococci concentrations in the handrinse samples from adult participants (r=0.27, p-value=0.0042) (Table 10, Figure 15). There was not a statistically significant linear relationship between *E. coli* and enterococci concentrations in the handrinse samples from 5-12 year olds.

By neighborhood, there was a strong, positive and statistically significant linear relationship between *E. coli* and enterococci concentrations in the handrinse samples from Bukom and Shiabu (r=0.47, p-value=0.0003; r=0.48, p-value<0.0001, respectively) (Table 10, Figure 16). There was a weak, positive and statistically significant linear relationship between *E. coli* and enterococci concentrations in the handrinse samples from Alajo (r=0.25, p-value=0.0239) (Table 10, Figure 16). There was not a statistically

significant linear relationship between *E. coli* and enterococci concentrations in the handrinse samples from Old Fadama.

By setting, there was a strong, positive and statistically significant linear relationship between *E. coli* and enterococci concentrations in the handrinse samples from nurseries ($r=0.69$, p-value<0.0001) (Table 10, Figure 17). There was a moderate/weak, positive and statistically significant linear relationship between *E. coli* and enterococci concentrations in the handrinse samples from households and public latrines ($r=0.33$, p-value$=0.0013$; $r=0.25$, p-value$=0.0331$, respectively) (Table 10, Figure 17). There was not a statistically significant linear relationship between *E. coli* and enterococci concentrations in the handrinse samples from schools.

As all linear relationships were statistically significant, there were no clear differences in the correlations between *E. coli* and enterococci concentrations for participants who had visible dirt on their hands and participants who did not (Table 10). There were moderate/ weak, positive and statistically significant linear relationships between *E. coli* and enterococci concentrations in the handrinse samples from participants who had visible dirt on their finger pads, on the palms of their hands, and under their fingernails ($r=0.34$, p-value$=0.0038$; $r=0.28$, p-value$=0.0122$; $r=0.34$, p-value<0.0001, respectively), as well as in the handrinse samples from participants who had no visible dirt on their finger pads, on the palms of their hands, and under their fingernails ($r=0.35$, p-value<0.0001; $r=0.37$, p-value<0.0001; $r=0.33$, p-value$=0.0081$, respectively) (Table 10).

iii. Determinants of *E. coli* Hand Contamination

For the subset of environmental samples (stored drinking water, swabs, and soil) used in predictive modeling (those collected from the same locations where handrinse samples were collected), the distributions of *E. coli* concentrations were assessed (Figures 18-20). For the subset of stored drinking water samples, *E. coli* concentrations ranged from no detectable *E. coli* (<1 cfu/ 100mL) to 4.30 $\log_{10}$ cfu/ 100mL (mean = 1.60 $\log_{10}$ cfu/ 100mL, n=121). For the subset of swab samples, *E. coli* concentrations ranged from no detectable *E. coli* (<8 cfu/ swab) to 4.52 $\log_{10}$ cfu/ swab (mean = 1.84 $\log_{10}$ cfu/ swab, n=203). For the subset of soil samples, *E. coli* concentrations ranged from no detectable *E. coli* (<2 cfu/ gram) to 4.90 $\log_{10}$ cfu/ gram (mean = 2.54 $\log_{10}$ cfu/ gram, n=149).

E. coli concentrations in swabs ($\log_{10}$ cfu/ swab) were significantly associated with *E. coli* concentrations in the handrinse samples ($\log_{10}$ cfu/ pair of hands) (p-value<0.0001) (Table 11). *E. coli* concentrations in swabs accounted for approximately 26% of the variation in *E. coli* concentrations in the handrinse samples (R^2=0.2625). *E. coli* concentrations in the handrinse samples were not significantly associated with *E. coli* concentrations in soil ($\log_{10}$ cfu/ gram) or stored drinking water ($\log_{10}$ cfu/ 100 mL), or with neighborhood, setting, sex, or age category. In the full model containing *E. coli* concentrations in swabs, soil, and stored drinking water samples, as well as neighborhood, setting, sex, and age category, at least one of the independent variables was a significant predictor of the variation in *E. coli* concentrations in the handrinse samples (p-value=0.0054) (Table 11).

From the model selection methods, three possible 'best' models were chosen, with the following variables: 1) swab *E. coli* concentration, neighborhood, and setting (p-value<0.0001), selected by the All Possible/ Best Subsets method, 2) swab *E. coli* concentration and setting (p-value<0.0001), selected by the All Possible/ Best Subsets method, and 3) only swab *E. coli* concentration (p-value<0.0001), selected by Backwards Elimination, Forwards Selection, and Stepwise Selection methods (Table 11). Although sex and age category are of sectoral interest, these variables were largely related to setting for the majority of handrinse samples, and were not deemed necessary for individual inclusion in the final model. The final 'best' model chosen for predicting *E. coli* concentrations in the handrinse samples included swab *E. coli* concentration, neighborhood, and setting (F=27.21, p-value<0.0001) (Table 11).

D. Discussion

This study found that people living in four low-income neighborhoods of Accra, Ghana had high levels of *E. coli* and enterococci hand contamination, which varied by neighborhood, setting, sex, age category, and the presence of visible dirt on the hands. For this study population, the overall mean handrinse *E. coli* concentration was 2.53 $\log_{10}$ cfu/ pair of hands and the overall mean handrinse enterococci concentration was 3.08 $\log_{10}$ cfu/ pair of hands. Handrinse samples from Old Fadama and Bukom had significantly higher overall mean enterococci concentrations than handrinse samples from Alajo or Shiabu. Handrinse samples collected at public latrines in Old Fadama had the highest mean *E. coli* concentrations. Old Fadama also had significantly higher mean

handrinse enterococci concentrations than Alajo in schools and public latrines and Shiabu in schools and nurseries.

Differential hand contamination levels may be explained by variations in socioeconomic status. A previous study on handwashing among primary school children in Kenya also found differential hand contamination by study neighborhood and suggested socioeconomic status as a potential explanation (26). Our study findings are consistent with this explanation. Old Fadama, with the highest hand contamination levels, was the poorest of the study neighborhoods and had the greatest proportion of people with no formal education. Another explanation for differential hand contamination levels is disparities in sanitation and hygiene coverage between neighborhoods. An earlier SaniPath report found that 93% and 98% of homes in Bukom and Old Fadama reported having no sanitation facilities, compared to 42% and 54% of households in Alajo and Shiabu, respectively (45). Overall, public latrines in Bukom and Old Fadama appeared to be less clean than toilets in Alajo and Shiabu, and handwashing stations were less common. At public latrines, 80% of facilities in Alajo had handwashing stations, while only 16% of facilities in Old Fadama had handwashing stations (45). Heavy reliance on public latrines, paired with poor or no hand hygiene facilities at public toilets, could explain the higher mean handrinse contamination found at public latrines in Old Fadama, but does not support the lower mean handrinse contamination found at public latrines in Bukom.

Handrinse samples from households and nurseries had the second highest mean *E. coli* concentrations of the four settings (2.46 $\log_{10}$ cfu/ pair of hands), after public latrines, and had the second highest mean enterococci concentrations (3.20 $\log_{10}$ cfu/ pair of hands), after nurseries. Two earlier studies have assessed hand contamination levels within households in peri-urban Tanzania (7, 50). The first study found that the mean *E. coli* and enterococci concentrations in handrinse samples were 2.5 and 2.7 $\log_{10}$ cfu/ pair of hands (N = 223), respectively (50). This is consistent with the mean *E. coli* concentration found on hands in households in our study, but is a lower mean enterococci concentration than found in our study.

These contamination levels are also lower than those found in the second study of hand contamination among peri-urban households in Tanzania (7). In the second study, the mean concentration of *E. coli* on hands was 3.10 $\log_{10}$ cfu/ pair of hands (N = 2,027), while the mean concentration of fecal streptococci was 4.5 $\log_{10}$ cfu/ pair of hands (N = 2,032). Enumerators asked mothers how much time had elapsed since she and each participating child had last washed their hands, but did not record what participants were doing just prior to sample collection (7). As these were single handrinse measurements, variations in household hand contamination in these studies may be explained by differences in the activities study participants were engaged in prior to sample collection.

Pickering et al. (7) found no significant difference between the bacterial concentrations on the hands of mothers and children under five within households. Our study also detected no significant differences between adult females and children under five for *E.*

coli or enterococci concentrations in the handrinse samples collected at households, which is consistent with this study. However, our study found that mean *E. coli* concentrations on hands were significantly higher for adults than for children under five (p-value=0.0006), and that mean enterococci concentrations were higher for children under five than for adults, across all settings. This difference may be attributable to variations in the number of adult and child handrinse samples collected in each setting, or to differences in hand surface area between children and adults. Pickering et al. (7) did not adjust handrinse fecal indicator bacteria concentrations by hand surface area. In our study, after normalizing *E. coli* and enterococci concentrations by average hand surface area, children under five had the highest concentrations of *E. coli* and enterococci per cm^2 of hand surface, while adults had the lowest concentrations per cm^2 of hand surface.

A previous study of hygiene behaviors of mothers and infants in rural Zimbabwe found that one-third of mothers had visibly dirty hands (51). Our study reported that visible dirt was observed on the finger pads and the palms of hands for about 25% and 30% of all participants, respectively, which is consistent with the Zimbabwe study. In the current study, there were significantly higher enterococci concentrations in handrinse samples taken from participants with visible dirt on the finger pads and on the palms of hands, compared with enterococci concentrations from those who did not have visible dirt on their hands (p-value<0.0001, p-value=0.0002, respectively). Visible dirt was observed under the fingernails of about 78% of all participants, and no significant differences were found in handrinse *E. coli* or enterococci concentrations between those with and without dirt under their nails. The earlier study by Pickering et al. (7) found that hands with

palms, finger pads, and under fingernails classified as ‘very dirty’ had higher fecal indicator bacteria levels than hands classified as ‘somewhat dirty’ and hands with ‘no visible dirt’. These findings are consistent with our study for dirt on the finger pads and palms of hands, but not for dirt under the fingernails.

To our knowledge, this is the first study to assess the correlation between *E. coli* and enterococci concentrations in handrinse samples from low-income, urban settings. This study found that *E. coli* concentrations in the handrinse samples were consistently lower than enterococci concentrations. There was a moderate, positive and statistically significant linear relationship between *E. coli* and enterococci concentrations in all handrinse samples ($r=0.33$, p-value<0.0001). The correlation between *E. coli* and enterococci concentrations was highest in the handrinse samples from nurseries ($r=0.69$, p-value<0.0001) and from children under five years ($r=0.57$, p-value<0.0001).There was not a statistically significant linear relationship between *E. coli* and enterococci concentrations in the handrinse samples from schools or children 5-12 years old.

The exact reason for these observed variations in correlation cannot be explained within the scope of this study. However, one potential explanation for the weaker correlation between *E. coli* and enterococci handrinse concentrations in some settings and age categories is less frequent and less recent exposures to fecal contamination. Multiple studies have suggested that enterococci may be the superior indicator of fecal contamination of hands, due to its longer survival time (15-18). Among a cohort of participants measured for hand contamination levels after different activities in peri-urban

households in Tanzania, *E. coli* levels were observed to decrease in the 'sitting' (control) group during a two hour period preceding handrinse sample collection, while enterococci levels did not (16). This finding suggests that *E. coli* concentrations decline faster than enterococci concentrations on hands after exposure to fecal contamination.

Children 5-12 years of age, the predominant age category of the handrinse samples collected in schools, may be less likely to have frequent exposures to fecal contamination, as they are not directly handling human excreta, like adult caregivers, or typically visiting public latrines during the day (45). It is possible that *E. coli* concentrations on hands among this group would have more time to decline between exposures. If *E. coli* are less persistent indicators of fecal contamination on hands, it is reasonable to expect that there would be a greater disparity, and weaker correlation, between *E. coli* and enterococci concentrations in handrinse samples from this group. Accordingly, in the populations where there is likely more frequent exposure to fecal contamination (nurseries and among children under five), there was a stronger correlation between *E. coli* and enterococci concentrations in the handrinse samples. However, it is surprising that there was only a weak correlation (r=0.25) between *E. coli* and enterococci concentrations in the handrinse samples from public latrines, where samples would have been collected shortly after the time of exposure to fecal contamination.

This study found that *E. coli* concentrations in surface swabs were a significant predictor of *E. coli* concentrations in handrinse samples (p-value<0.0001). Neighborhood and setting were not individually significant predictors of handrinse *E. coli* concentrations,

but were important predictors selected for the final 'best' predictive model by the All Possible/ Best Subsets method. Although sex and age category are of sectoral interest, these variables were largely related to setting for the majority of the handrinse samples in this study. For example, the school setting was nearly all participants in the 5-12 years age category and households were predominantly adult females and children under five. As setting was included in the final model, sex and age category were deemed unnecessary for individual inclusion.

To our knowledge, this is the first study that found that *E. coli* concentrations in surface swabs were a significant predictor of *E. coli* concentrations in handrinse samples in a low-resource setting. A previous study found that fecal indicator bacteria contamination on hands of mothers and children was significantly associated with fecal indicator bacteria in household stored water (7). This is not consistent with the findings from our study, in which *E. coli* concentrations in stored drinking water were not significantly associated with handrinse *E. coli* concentrations. A different study found that *E. coli* contamination of finger-tip rinses was strongly associated with the activity done prior to testing, highlighting a potential limitation in our study (17).

This study is limited by the lack of information collected on the activity participants were engaged in just prior to handrinse sample collection. Setting was an important determinant of hand contamination, as activities conducted in public latrines, schools, nurseries, and households differ and involve varying levels of exposure to human excreta. However, without knowing what activity participants were engaged in prior to sample

collection, it is impossible to know when exposures to fecal contamination occurred and the handrinse contamination findings presented here are limited. Further, it is impossible to know whether the fecal indicator bacteria concentrations detected on participants' hands are reflective of hand contamination with their own excreta or with fecal matter from other people. Fecal exposure through hand contamination is an important pathway for disease transmission, but is not a major health risk if the participants' hands are contaminated with their own feces. Additionally, as fecal indicator organisms reside in the gastrointestinal tract of both humans and animals, it is also possible that the contamination on participants' hands is from animal feces. This would still indicate a health risk, but does not represent human-human transmission of excreta.

Handrinse sample collection at a single point in time serves as another potential limitation of his study. Ram et al. (21) found that hand contamination measured at a convenient time, such as when a researcher first arrives at a home, was not well-correlated to hand contamination measured at critical times for handwashing. The authors concluded that microbiological hand contamination varied substantially from one sampling time to another. However, from structured observations conducted in households in the study neighborhoods, we know that handwashing was infrequent. For children under five, the probability of handwashing before eating was less than 0.20 in all study neighborhoods. The probability of handwashing after defecation was also less than 0.20 for children under five in all neighborhoods, except for Alajo (52). As handwashing was uncommon for this age category, it is likely that hand contamination levels remained high throughout the day, and may have contributed to lower variability in daily hand

contamination levels. For this population, single handrinse sample collection may not present a major limitation to accurately characterizing hand contamination levels.

A third limitation of this study is the broad range of objects included in the swab samples for analysis. Items selected for swab sampling were chosen after enumerators observed children touching these objects, which is a strength of this study. However, items swabbed were a broad category that included walls, floors, and a range of other objects, such as toys and articles of clothing. The areas swabbed varied depending on the size of the object swabbed and the ability to collect the swab sample on a flat surface. This study found that *E. coli* concentrations in the swab samples were a significant predictor of *E. coli* concentrations in the handrinse samples. Since there was such a range of objects sampled and variations in sample collection methodology, there was limited ability to separate this sample type into meaningful categories for predictive modeling. The finding that environmental swab contamination can predict handrinse contamination is important, but only provides a broad, initial understanding of how environmental fecal contamination may impact hand contamination in these settings. Future analyses should focus on determining the strength of the association between fecal contamination of environmental surfaces and hand contamination in different settings.

E. Conclusions

- There were high levels of hand fecal contamination across the different neighborhoods in this study, and it is important to consider hand contamination as a fecal exposure pathway in urban, low-income settings. Old Fadama and Bukom had

higher levels of hand fecal contamination than Alajo and Shiabu. These differences may be attributable to lower socioeconomic status or poorer sanitation and hygiene coverage in Old Fadama and Bukom.

- After normalizing *E. coli* and enterococci concentrations by average hand surface area, children under five had the highest levels of hand contamination per cm^2 of hand surface, while adults had the lowest levels of hand contamination per cm^2 of hand surface. Females had significantly higher levels of enterococci contamination on hands than males, overall and among 5-12 year olds in schools.
- About 25% and 30% of participants had visible dirt on the palms of their hands and on their finger pads, respectively, while 78% of participants had visible dirt under their fingernails. Participants who had visible dirt on the palms of their hands or on their finger pads had significantly higher levels of enterococci hand contamination, compared to those without visible dirt on their hands.
- *E. coli* concentrations in the handrinse samples were consistently lower than enterococci concentrations. There was a moderate, positive correlation between *E. coli* and enterococci concentrations in all handrinse samples. There was a statistically significant linear relationship between *E. coli* and enterococci concentrations in Alajo, Bukom, and Shiabu, in households, nurseries, and public latrines, among males and females, and among adults and children under five. The correlation between *E. coli* and enterococci concentrations was highest for handrinse samples from nurseries and children under five, while there was no correlation between *E. coli* and enterococci concentrations in the handrinse samples from schools and children 5-12 years old. These differences could support previous reports that enterococci has a

longer survival time on hands than *E. coli* and that there is better correlation between these two measures in settings where there is frequent, recent fecal contamination of hands.

- *E. coli* concentrations on swabs were a significant predictor of *E. coli* concentrations in handrinse samples, while soil and stored drinking water *E. coli* concentrations were not significant predictors of handrinse *E. coli* concentrations. Neighborhood and setting were also important predictors of *E. coli* hand contamination because they reflect SES, access to sanitation and hygiene facilities (neighborhood), activities, and age category (setting).

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G. Tables

Table 1. Summary of study neighborhoods[1].

	Alajo	**Bukom**	**Old Fadama**	**Shiabu**
Type of settlement	Formal	Formal	Squatter	Mixed
Location	Inland	Coastal	Inland	Coastal
Flood-prone	Yes	No	Yes	Yes
Near major market	No	Yes	Yes	No

[1]Peprah D, Baker K, Moe C, Robb K, Wellington N, Yakubu H, and Null C. Public toilets and their customers in low-income, urban Accra, Ghana. Environment and urbanization (in press). 2015.

Table 2. *E. coli* concentrations from handrinse samples by demographic and observational characteristics.

	***E. coli* (log_{10} cfu/ pair of hands)**				
	Mean	SD	Min	Max	p-value
Overall (N=276)	2.53	1.23	0.35	5.19	
Sex					0.1961
Male (n=113)	2.65	1.22	0.35	5.00	
Female (n=163)	2.45	1.23	0.35	5.19	
Age Category					**0.0006**
<5 years (n=98)	2.43	1.17	0.35	4.74	
5-12 years (n=61)	2.12	1.14	0.35	4.34	
Adults (n=117)	2.84	1.25	0.35	5.85	
Neighborhood					0.4800
Alajo (n=81)	2.52	1.29	0.35	5.00	
Bukom (n=59)	2.70	1.10	0.35	4.98	
Old Fadama (n=69)	2.58	1.23	0.35	5.00	
Shiabu (n=67)	2.36	1.25	0.35	5.19	
Setting					**0.0015**
Households (n=90)	2.46	1.16	0.35	4.98	
Public Latrines (n=77)	2.95	1.21	0.35	5.19	
Schools (n=56)	2.15	1.16	0.35	4.34	
Nurseries (n=53)	2.46	1.27	0.35	4.74	
Visible dirt on finger pads					0.7440
No (n=203)	2.52	1.26	0.35	5.19	
Yes (n=73)	2.57	1.14	0.35	4.78	
Visible dirt on palms of hands					0.7564
No (n=192)	2.52	1.28	0.35	5.19	
Yes (n=84)	2.57	1.11	0.35	4.98	
Visible dirt under nails					0.8287
No (n=63)	2.50	1.28	0.35	5.00	
Yes (n=213)	2.54	1.21	0.35	5.19	

cfu denotes colony forming units

Table 3. Enterococci concentrations from handrinse samples by demographic and observational characteristics.

	Enterococci (log_{10} cfu/ pair of hands)				
	Mean	SD	Min	Max	p-value
Overall (N=278)	3.08	0.92	0.35	5.85	
Sex					**0.0181**
Male (n=114)	2.92	0.94	0.35	5.00	
Female (n=164)	3.19	0.90	0.35	5.85	
Age Category					**0.0350**
<5 years (n=99)	3.25	0.89	0.70	5.00	
5-12 years (n=63)	2.88	0.80	0.35	4.42	
Adults (n=116)	3.03	0.99	0.35	5.85	
Neighborhood					**<0.0001**
Alajo (n=79)	2.83	0.95	0.35	4.92	
Bukom (n=59)	3.23	1.04	1.35	5.85	
Old Fadama (n=73)	3.50	0.72	1.50	5.00	
Shiabu (n=67)	2.78	0.79	0.35	4.57	
Setting					**0.0154**
Households (n=91)	3.20	0.99	0.35	5.85	
Public Latrines (n=75)	3.04	0.92	0.35	4.99	
Schools (n=59)	2.78	0.78	0.35	4.38	
Nurseries (n=53)	3.27	0.88	0.70	5.00	
Visible dirt on finger pads					**<0.0001**
No (n=199)	2.92	0.93	0.35	5.85	
Yes (n=79)	3.48	0.78	1.35	5.00	
Visible dirt on palms of hands					**0.0002**
No (n=192)	2.94	0.89	0.35	4.99	
Yes (n=86)	3.38	0.94	0.35	5.85	
Visible dirt under nails					0.2821
No (n=62)	2.97	0.76	1.30	4.38	
Yes (n=216)	3.11	0.96	0.35	5.85	

cfu denotes colony forming units

Table 4. Pairwise comparisons of handrinse *E. coli* concentrations[1] for demographic factors[2].

Comparison *E. coli* Concentration	Difference Between Means	95% Confidence Interval		
Age Category				
Adult - <5 years	**0.406**	**0.0197**	**0.7923**	***[3]
Adult - 5-12 years	**0.7119**	**0.2664**	**1.1574**	***
<5 years - 5-12 years	0.3059	-0.1542	0.766	
Setting				
Public latrines – HH[4]	**0.4955**	**0.0147**	**0.9763**	***
Public latrines - nurseries	0.4966	-0.0562	1.0494	
Public latrines - schools	**0.8054**	**0.2615**	**1.3494**	***
HH - nurseries	0.0011	-0.5352	0.5374	
HH - schools	0.31	-0.2172	0.8372	
Nurseries - schools	0.3088	-0.2847	0.9024	

[1] $\log_{10}$ colony forming units/ pair of hands
[2] Variables not included in this analysis had no significant differences in *E. coli* concentrations between categories
[3]*** denotes p-value<0.05
[4]HH denotes household

Table 5. Pairwise comparisons of handrinse enterococci concentrations[1] for demographic factors[2].

Comparison Enterococci Concentration	Difference Between Means	95% Confidence Interval		
Age Category				
<5 years - Adult	0.2211	-0.0739	0.5161	
<5 years - 5-12 years	**0.3703**	**0.0228**	**0.7178**	***[3]
Adult - 5-12 years	0.1492	-0.1883	0.4866	
Neighborhood				
Old Fadama - Bukom	0.2717	-0.1254	0.6688	
Old Fadama - Alajo	**0.6694**	**0.3011**	**1.0376**	***
Old Fadama - Shiabu	**0.7155**	**0.3317**	**1.0992**	***
Bukom - Alajo	**0.3976**	**0.0074**	**0.7879**	***
Bukom - Shiabu	**0.4438**	**0.0388**	**0.8487**	***
Alajo - Shiabu	0.0461	-0.3306	0.4228	
Setting				
Nurseries - HH[4]	0.0768	-0.3298	0.4835	
Nurseries - public latrines	0.2365	-0.1858	0.6588	
Nurseries - schools	**0.4965**	**0.0511**	**0.9419**	***
HH - public latrines	0.1597	-0.2073	0.5267	
HH - schools	**0.4197**	**0.0263**	**0.8131**	***
Public latrines - schools	0.26	-0.1495	0.6695	

[1] $\log_{10}$ colony forming units/ pair of hands

[2] Variables not included in this analysis had no significant differences in enterococci concentrations between categories

[3] *** denotes p-value<0.05

[4] HH denotes household

Table 6. Comparison of *E. coli* concentrations from handrinse samples across neighborhoods by setting.

	***E. coli* (log_{10} cfu/ pair of hands)**				
	Mean	SD	Min	Max	p-value
All settings					0.4800
Alajo (n=81)	2.52	1.29	0.35	5.00	
Bukom (n=59)	2.70	1.10	0.35	4.98	
Old Fadama (n=69)	2.58	1.23	0.35	5.00	
Shiabu (n=67)	2.36	1.25	0.35	5.19	
Households					**0.0381**
Alajo (n=28)	2.21	1.28	0.35	4.27	
Bukom (n=21)	2.99	1.14	1.35	4.98	
Old Fadama (n=23)	2.11	0.88	0.35	4.50	
Shiabu (n=18)	2.67	1.13	1.00	4.78	
Public Latrines					**0.0495**
Alajo (n=20)	3.02	1.32	0.35	5.00	
Bukom (n=19)	2.52	1.14	0.35	4.42	
Old Fadama (n=19)	3.55	1.04	1.70	5.00	
Shiabu (n=19)	2.73	1.17	1.18	5.19	
Schools					0.1178
Alajo (n=15)	2.36	1.20	0.35	4.34	
Bukom (n=8)	2.83	0.82	1.60	3.76	
Old Fadama (n=15)	1.84	1.19	0.35	3.65	
Shiabu (n=18)	1.93	1.17	0.35	3.82	
Nurseries					
Alajo (n=18)	2.57	1.27	0.35	4.23	0.3947
Bukom (n=11)	2.37	1.11	1.35	4.36	
Old Fadama (n=12)	2.85	1.17	1.18	4.74	
Shiabu (n=12)	1.98	1.48	0.35	4.57	

cfu denotes colony forming units

Table 7. Comparison of enterococci concentrations from handrinse samples across neighborhoods by setting.

	Enterococci (log_{10} cfu/ pair of hands)				
	Mean	SD	Min	Max	p-value
All settings					**<0.0001**
Alajo (n=79)	2.83	0.95	0.35	4.92	
Bukom (n=59)	3.23	1.04	1.35	5.85	
Old Fadama (n=73)	3.50	0.72	1.50	5.00	
Shiabu (n=67)	2.78	0.79	0.35	4.57	
Households					0.1073
Alajo (n=28)	2.95	0.94	0.35	4.92	
Bukom (n=21)	3.47	1.32	1.35	5.85	
Old Fadama (n=24)	3.43	0.79	2.02	4.75	
Shiabu (n=18)	2.93	0.76	1.35	3.85	
Public Latrines					**0.0174**
Alajo (n=18)	2.66	0.99	0.35	4.23	
Bukom (n=19)	2.85	0.97	1.35	4.99	
Old Fadama (n=19)	3.56	0.89	1.50	4.98	
Shiabu (n=19)	3.06	0.61	1.96	4.57	
Schools					**0.0003**
Alajo (n=15)	2.60	0.71	1.30	4.38	
Bukom (n=8)	2.92	0.60	2.08	3.67	
Old Fadama (n=18)	3.33	0.38	2.70	3.85	
Shiabu (n=18)	2.31	0.88	0.35	3.54	
Nurseries					**0.0100**
Alajo (n=18)	3.00	1.09	0.70	4.76	
Bukom (n=11)	3.63	0.39	3.04	4.42	
Old Fadama (n=12)	3.79	0.63	2.66	5.00	
Shiabu (n=12)	2.83	0.70	1.74	3.90	

cfu denotes colony forming units

Table 8. Pairwise comparisons of handrinse *E. coli* concentrations[1] across neighborhoods by setting[2].

Comparison *E. coli* Concentration	Difference Between Means	95% Confidence Interval		
Public Latrines				
Old Fadama - Alajo	0.5303	-0.4582	1.5188	
Old Fadama - Shiabu	0.8189	-0.1822	1.82	
Old Fadama - Bukom	**1.0272**	**0.0261**	**2.0283**	***[3]
Alajo - Shiabu	0.2886	-0.6999	1.2771	
Alajo - Bukom	0.4969	-0.4916	1.4854	
Shiabu - Bukom	0.2083	-0.7928	1.2094	

[1] $\log_{10}$colony forming units/ pair of hands
[2] Variables not included in this analysis had no significant differences in *E. coli* concentrations between categories
[3]*** denotes p-value<0.05

Table 9. Pairwise comparisons of handrinse enterococci concentrations[1] across neighborhoods by setting[2].

Comparison Enterococci Concentration	Difference Between Means	95% Confidence Interval		
Schools				
Old Fadama - Bukom	0.4083	-0.3548	1.1713	
Old Fadama - Alajo	**0.7303**	**0.1025**	**1.3581**	***[2]
Old Fadama - Shiabu	**1.0175**	**0.4189**	**1.6161**	***
Bukom - Alajo	0.322	-0.4642	1.1082	
Bukom - Shiabu	0.6092	-0.1539	1.3723	
Alajo - Shiabu	0.2872	-0.3406	0.915	
Nurseries				
Old Fadama - Bukom	0.1604	-0.7325	1.0534	
Old Fadama - Alajo	0.782	-0.0152	1.5793	
Old Fadama - Shiabu	**0.9553**	**0.082**	**1.8286**	***
Bukom - Alajo	0.6216	-0.1971	1.4403	
Bukom - Shiabu	0.7949	-0.098	1.6879	
Alajo - Shiabu	0.1733	-0.6239	0.9705	
Public Latrines				
Old Fadama - Shiabu	0.4999	-0.2487	1.2485	
Old Fadama - Bukom	0.706	-0.0426	1.4546	
Old Fadama - Alajo	**0.8938**	**0.1349**	**1.6528**	***
Shiabu - Bukom	0.2062	-0.5424	0.9548	
Shiabu - Alajo	0.394	-0.3649	1.1529	
Bukom - Alajo	0.1878	-0.5711	0.9467	

[1] $\log_{10}$ colony forming units/ pair of hands
[2] Variables not included in this analysis had no significant differences in enterococci concentrations between categories
[3] *** denotes p-value<0.05

Table 10. Correlation between *E. coli* and enterococci handrinse concentrations[1] by demographic and observational characteristics.

	Pearson Correlation Coefficient	p-value
Overall (n=271)	0.33	**<0.0001**
Sex		
Male (n=110)	0.38	**<0.0001**
Female (n=161)	0.34	**<0.0001**
Age Category		
<5 years (n=97)	0.57	**<0.0001**
5-12 years (n=60)	0.11	0.3974
Adults (n=114)	0.27	**0.0042**
Neighborhood		
Alajo (n=79)	0.25	**0.0239**
Bukom (n=57)	0.47	**0.0003**
Old Fadama (n=68)	0.19	0.122
Shiabu (n=67)	0.48	**<0.0001**
Setting		
Households (n=90)	0.33	**0.0013**
Public Latrines (n=74)	0.25	**0.0331**
Schools (n=55)	0.08	0.5443
Nurseries (n=52)	0.69	**<0.0001**
Visible dirt on finger pads		
No (n=198)	0.35	**<0.0001**
Yes (n=73)	0.34	**0.0038**
Visible dirt on palms of hands		
No (n=189)	0.37	**<0.0001**
Yes (n=82)	0.28	**0.0122**
Visible dirt under nails		
No (n=62)	0.33	**0.0081**
Yes (n=209)	0.34	**<0.0001**

[1]$\log_{10}$ colony forming units/ pair of hands

Table 11. Simple and multivariable linear regression for modeling *E. coli*[1] hand contamination from environmental contamination[2] and demographic characteristics.

Simple Linear Regression	R^2	p-value
Environmental Sample Type		
Stored Drinking Water (n=121)	0.0042	0.4803
Swabs (n=204)	0.2625	**<.0001**
Soil (n=150)	0.0001	0.929
Demographic Characteristics		
Neighborhood (n=261)	0.0028	0.3916
Setting (n=262)	0.0003	0.7703
Sex (n=262)	0.0060	0.2110
Age Category (n=262)	0.0113	0.0861

Multivariable Regression (n=59)	Parameter Estimate	Partial t-test p-value
Stored Drinking Water	0.1286	0.3902
Swabs	0.6473	**<0.0001**
Soil	-0.0704	0.5263
Neighborhood	-0.1754	0.2962
Setting	-0.0358	0.2186
Sex	-0.1998	0.5171
Age Category	0.1966	0.3807

Summary of Full and Reduced Models	F-statistic	p-value
Full Model (7 variables) (n=59)	3.33	**0.0054**
Swabs, Neighborhood, Setting (n=203)[3]	**27.21**	**<0.0001**
Swabs, Setting (n=204)[3]	37.54	**<0.0001**
Swabs[4] (n=204)	71.91	**<0.0001**

[1]$\log_{10}$ colony forming units (cfu) / pair of hands

[2]Soil: $\log_{10}$ cfu/ gram; Stored Drinking Water: $\log_{10}$ cfu/ 100mL; Swabs: $\log_{10}$ cfu/ swab

[3]model selected by All Possible/ Best Subsets method

[4]model selected by Backwards Elimination, Forward Selection, and Stepwise Selection methods

H. Figures

Figure 1. Distribution of $\log_{10}$ *E. coli* concentrations in all handrinse samples.

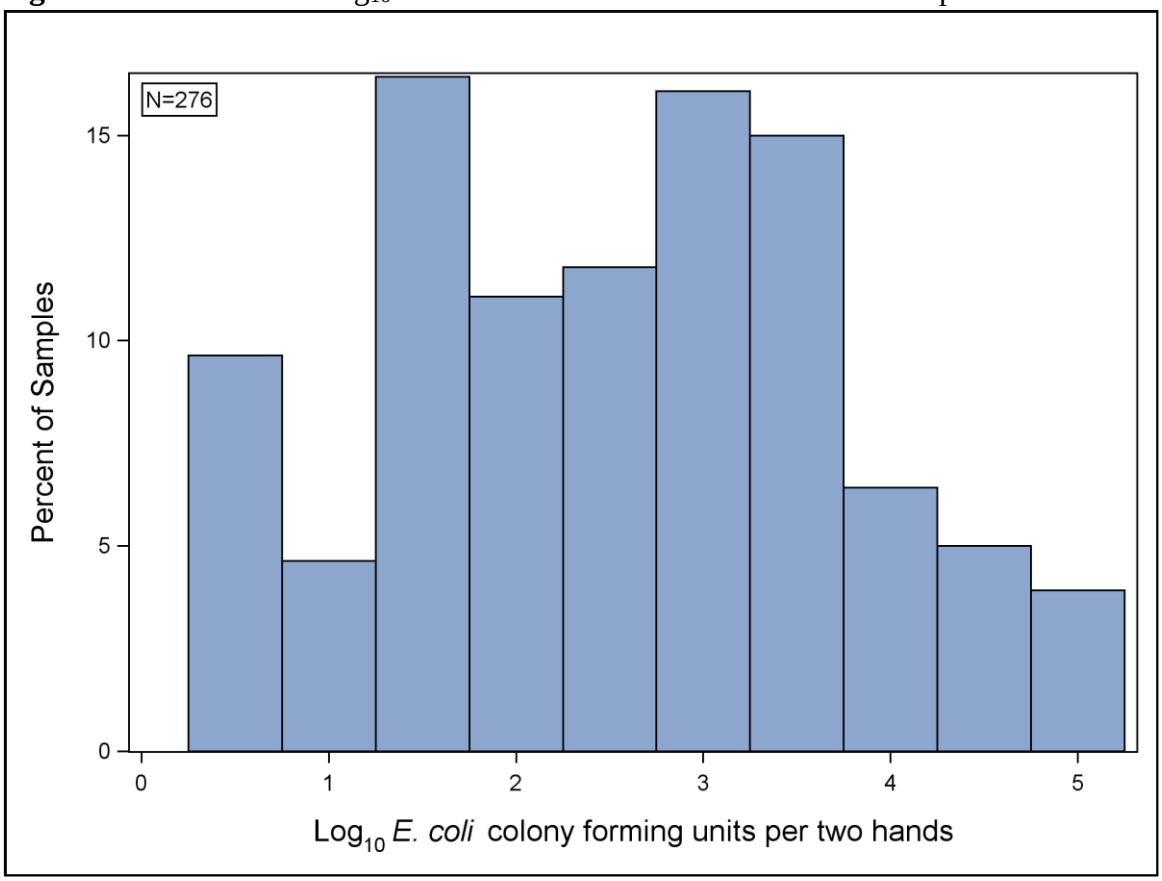


Figure 2. Distribution of $\log_{10}$ enterococci concentrations in all handrinse samples.

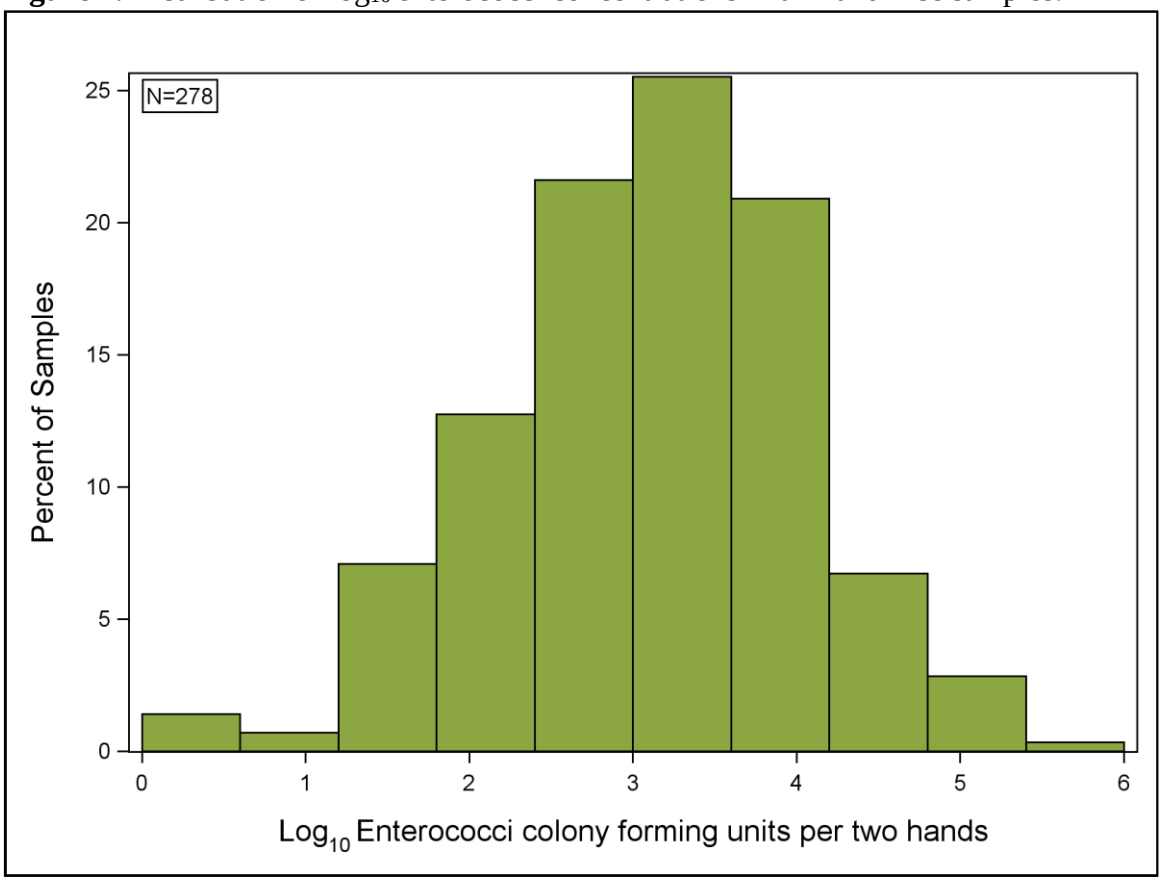


Figure 3. Distribution of $\log_{10}$ *E. coli* concentrations in handrinse samples by participant sex.

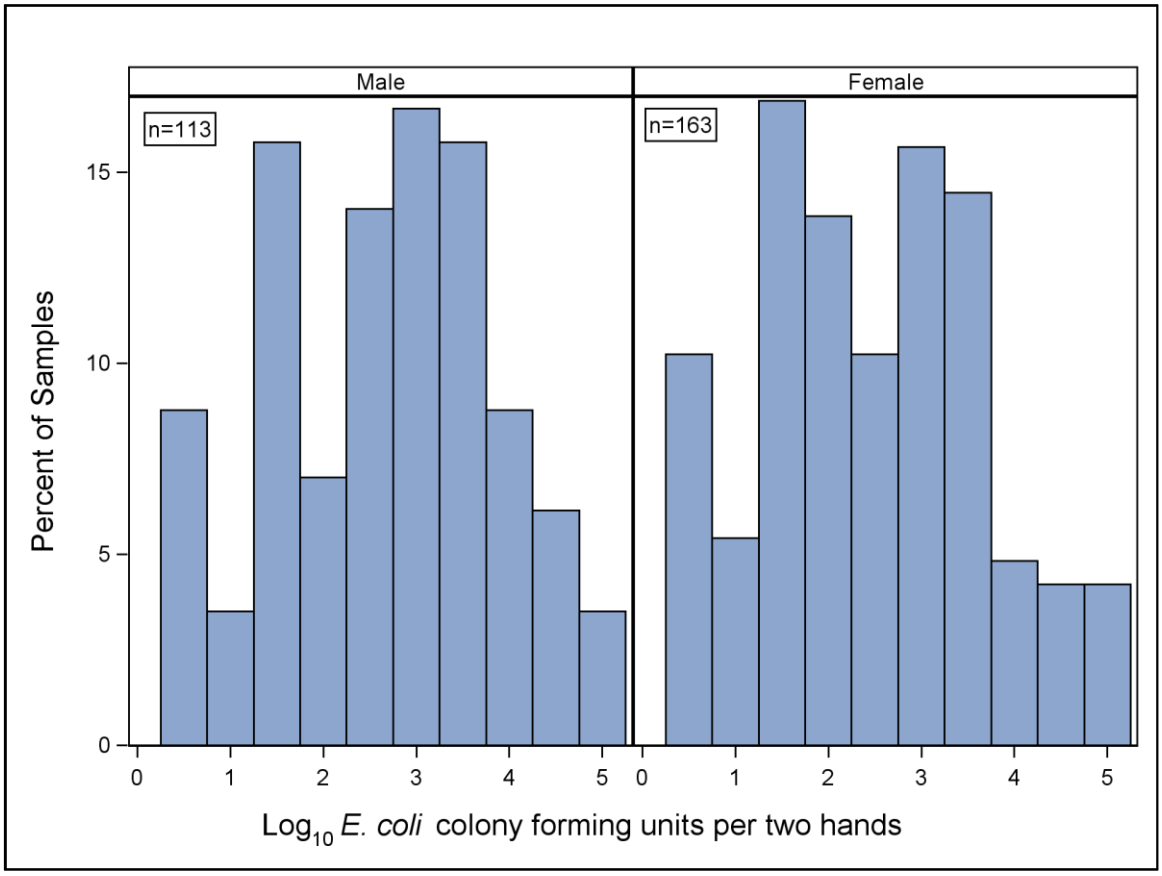


Figure 4. Distribution of $\log_{10}$ enterococci concentrations in handrinse samples by participant sex.

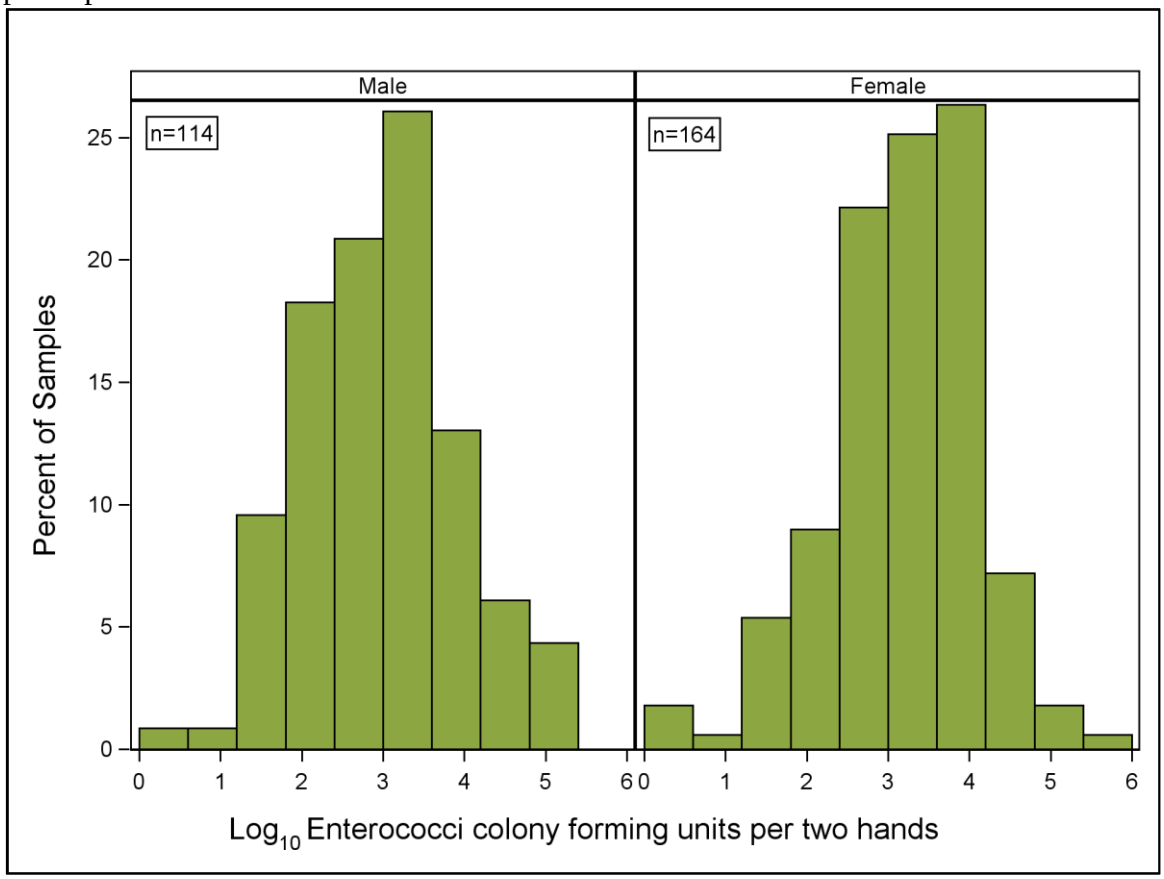


Figure 5. Distribution of $\log_{10}$ *E. coli* concentrations in handrinse samples by age category.

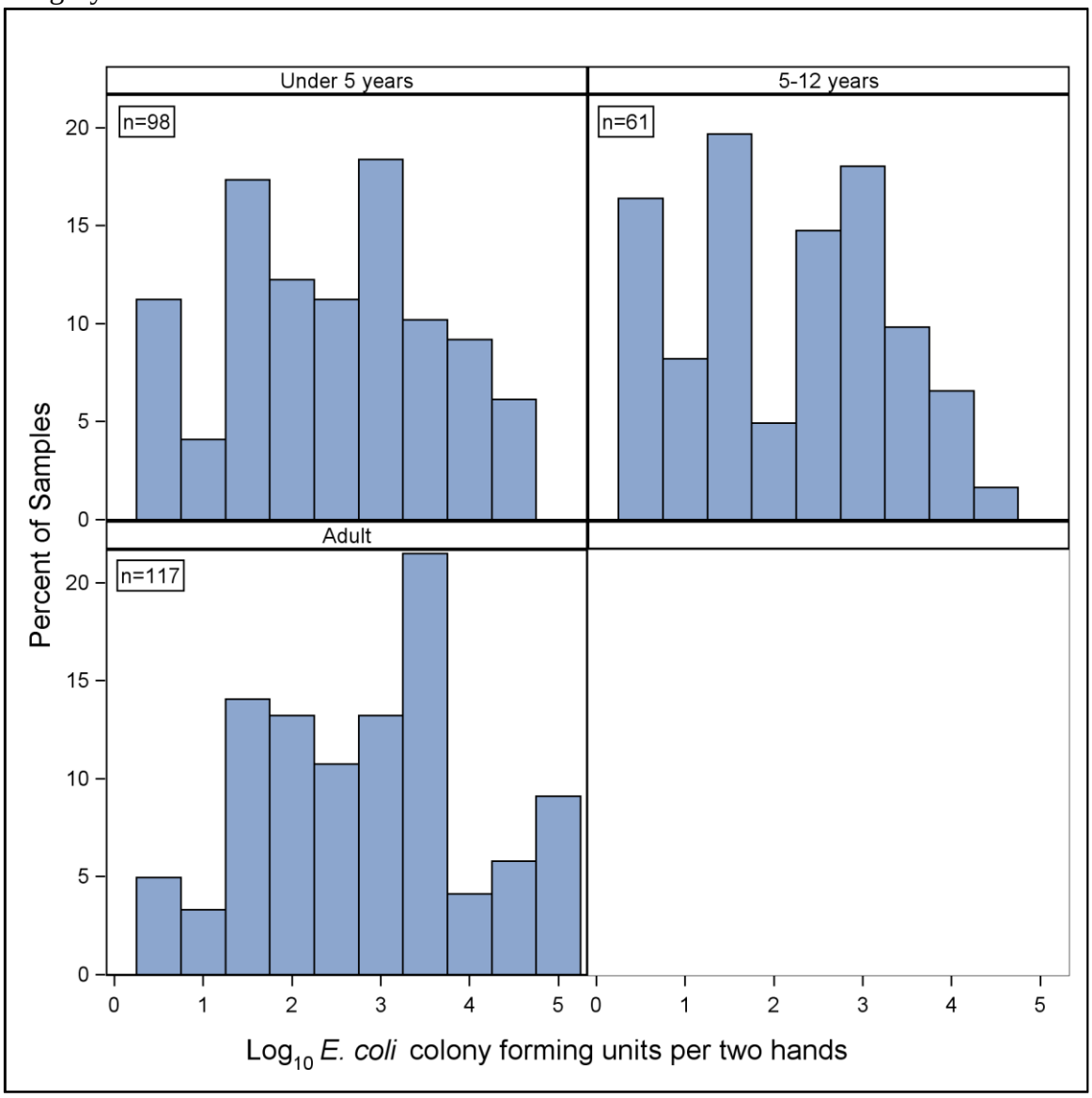


Figure 6. Distribution of log_{10} enterococci concentrations in handrinse samples by age category.

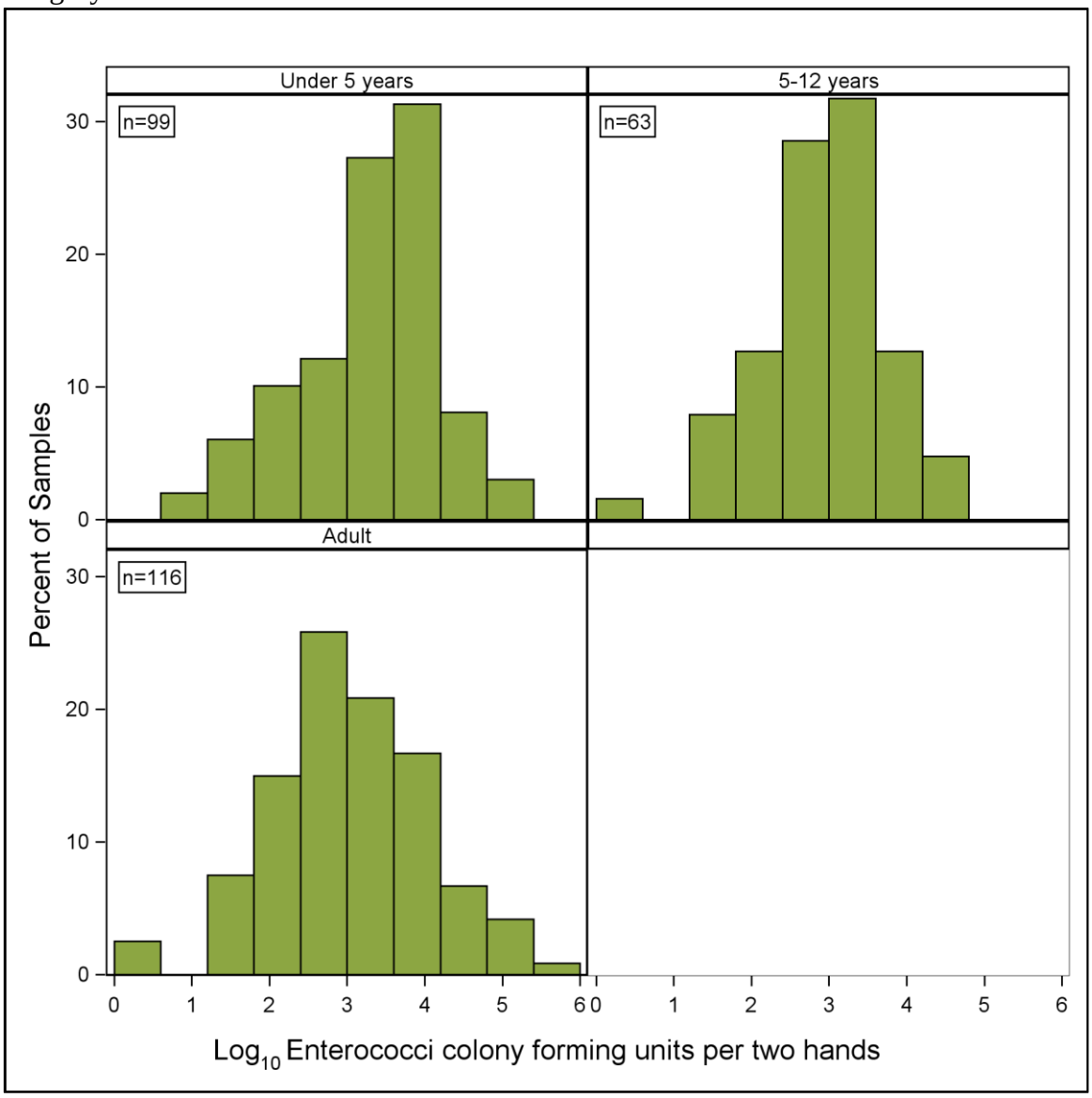


Figure 7. Distribution of $\log_{10}$ *E. coli* concentrations in handrinse samples by neighborhood.

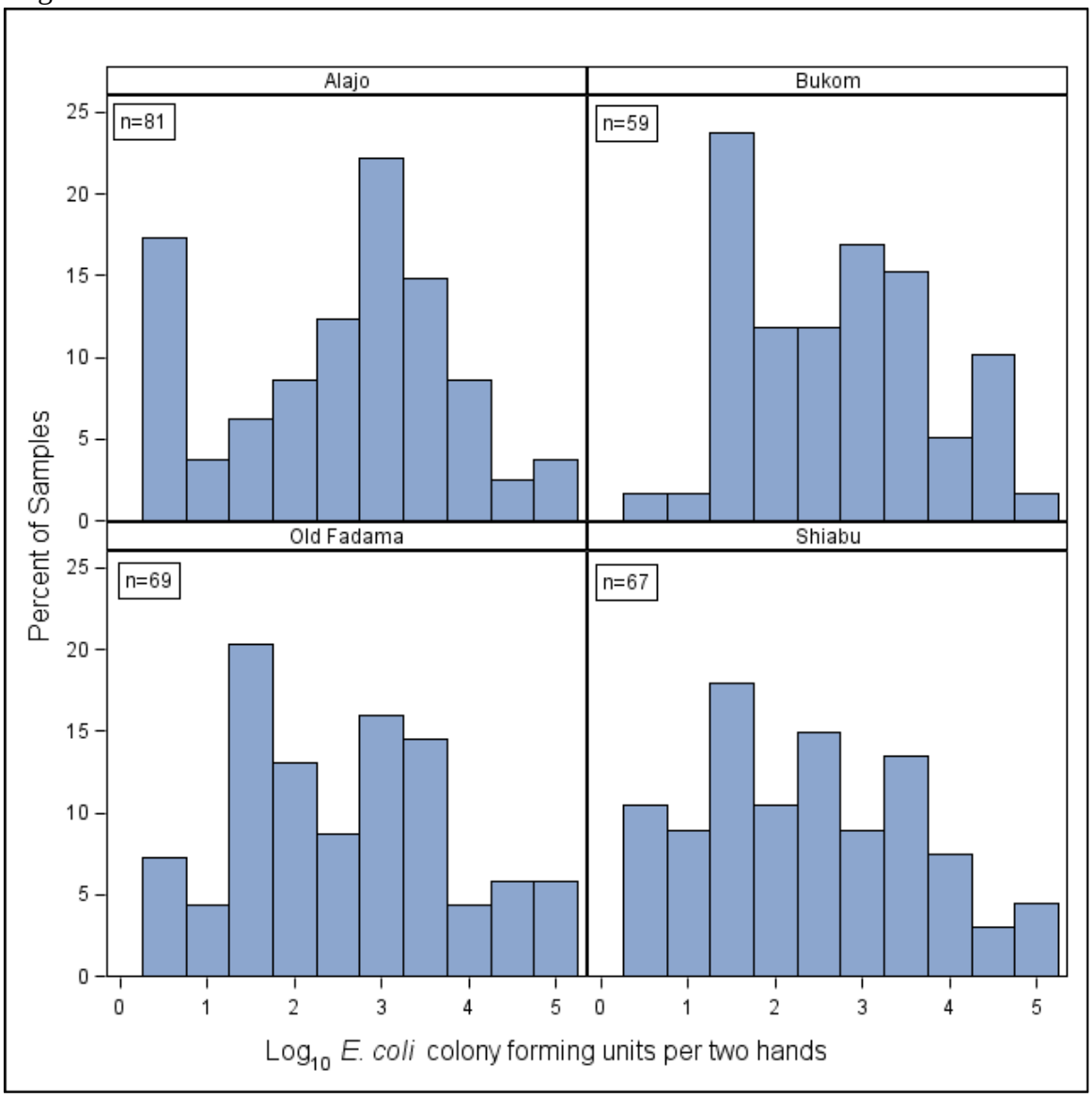


Figure 8. Distribution of log_{10} enterococci concentrations in handrinse samples by neighborhood.

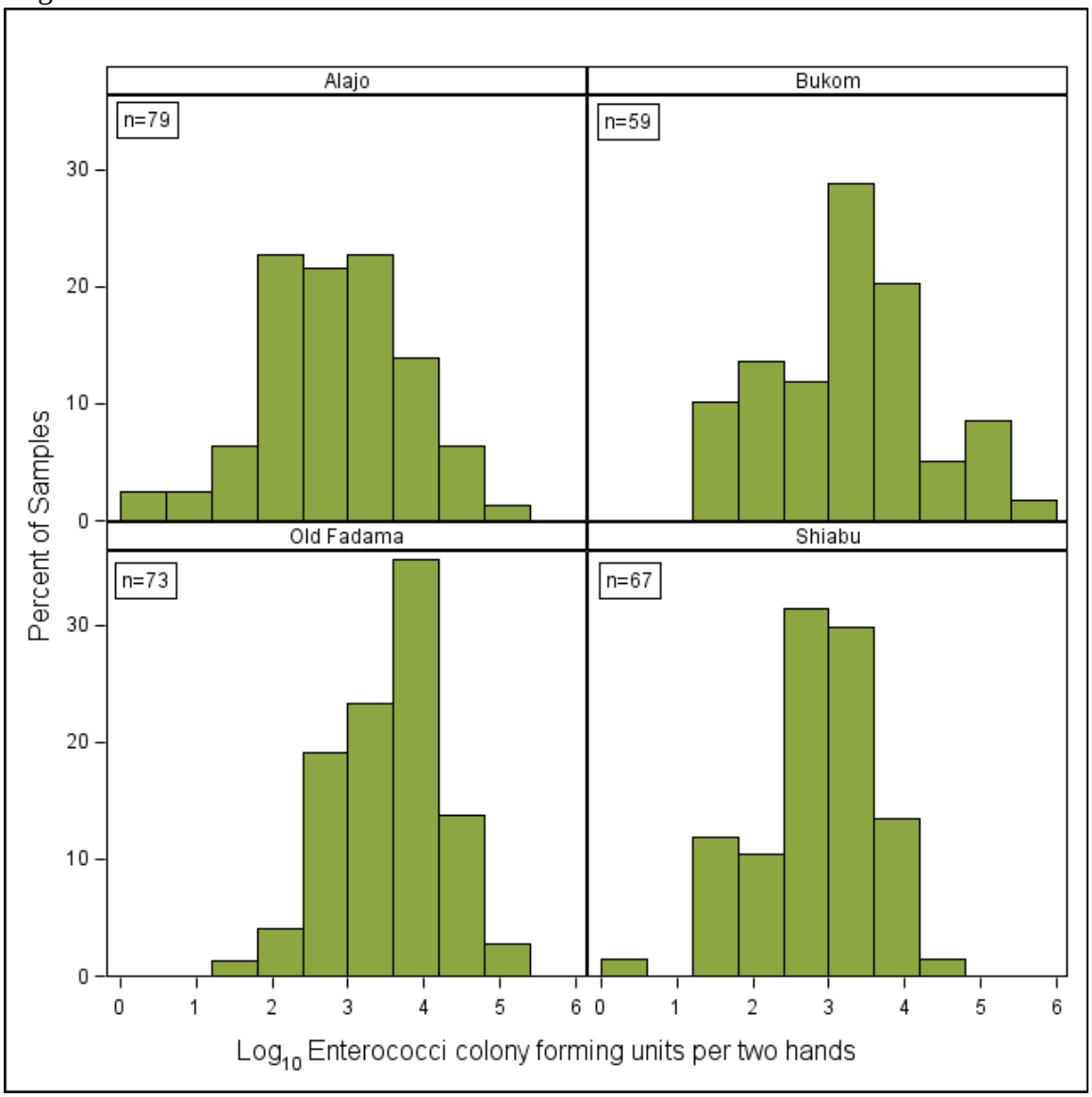


Figure 9. Distribution of $\log_{10}$ *E. coli* concentrations in handrinse samples by setting.

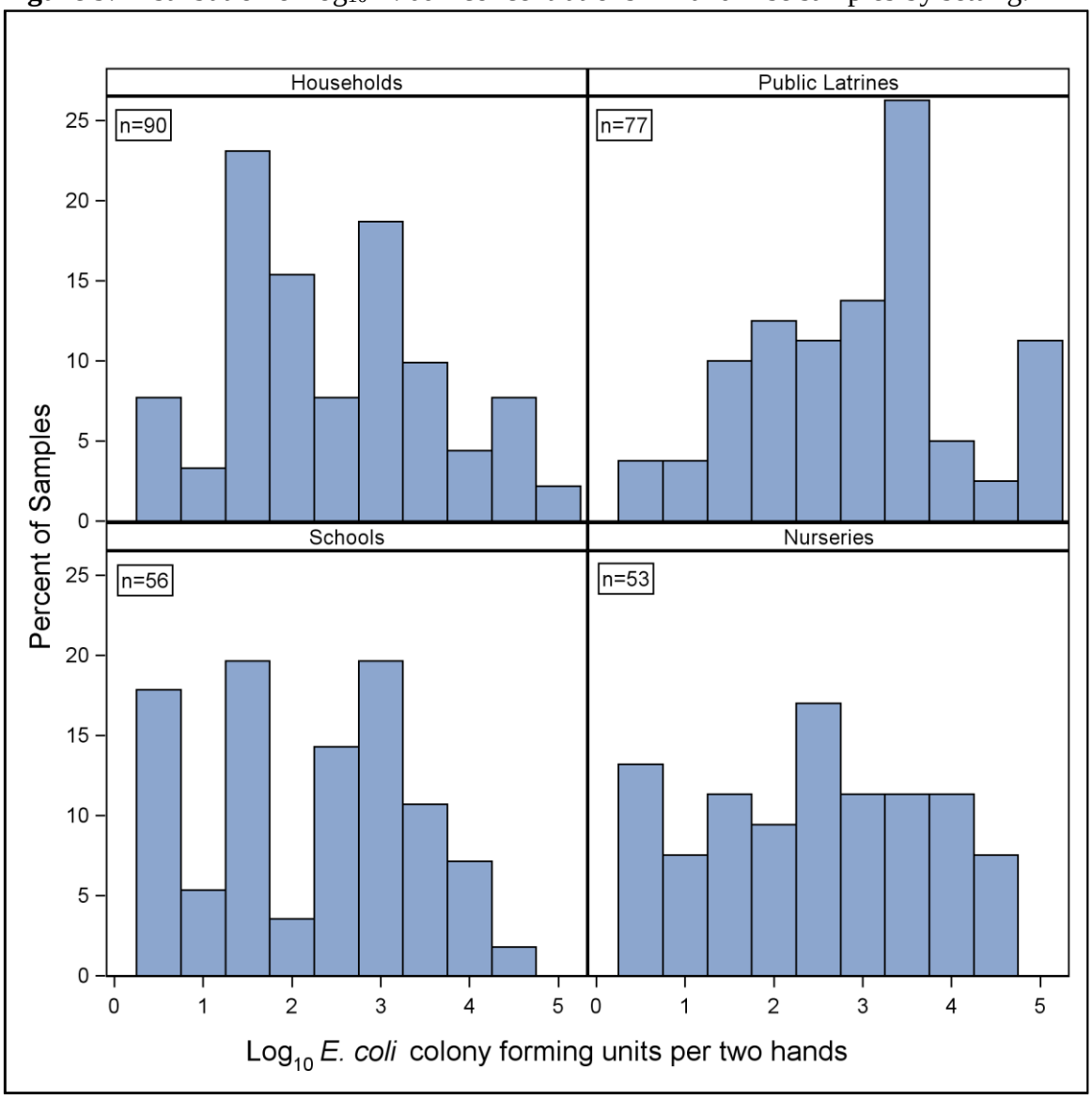


Figure 10. Distribution of log_{10} enterococci concentrations in handrinse samples by setting.

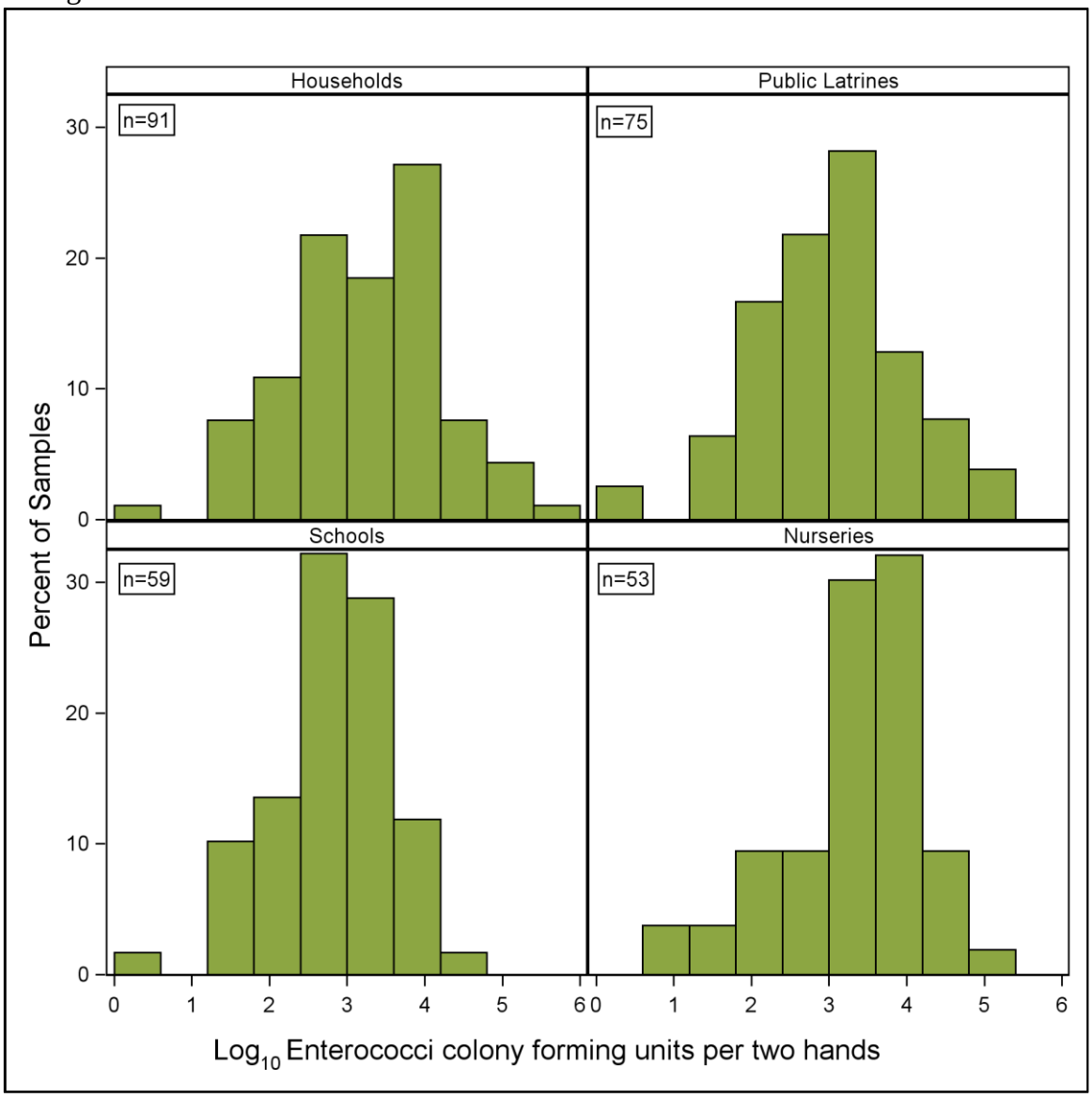


Figure 11. Distribution of handrinse samples by participant sex and setting (N=283).

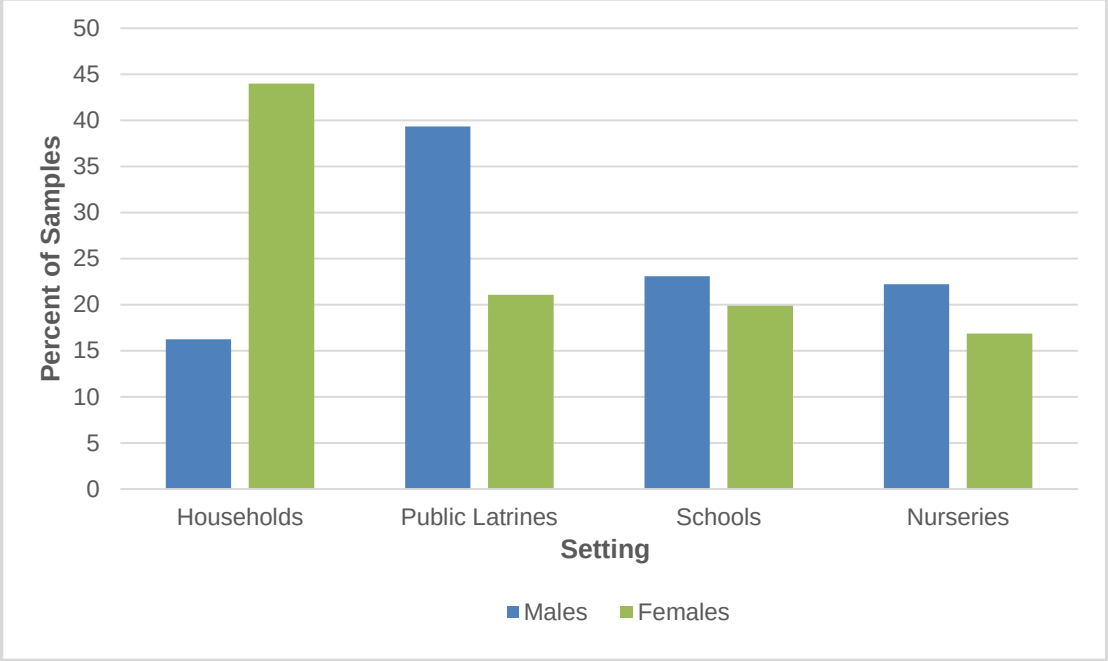


Figure 12. Distribution of handrinse samples by age category and setting (N=283).

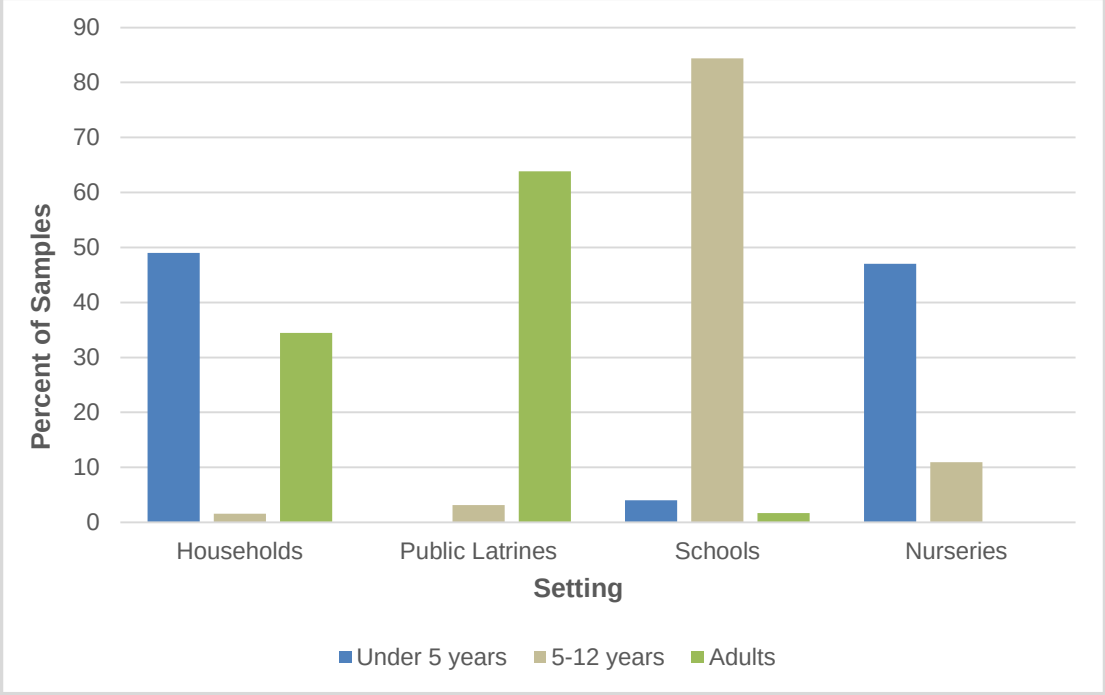


Figure 13. Correlation between $\log_{10}$ *E. coli* concentrations and $\log_{10}$ enterococci concentrations in handrinse samples.

Figure 14. Correlation between $\log_{10}$ *E. coli* concentrations and $\log_{10}$ enterococci concentrations in handrinse samples, by participant sex.

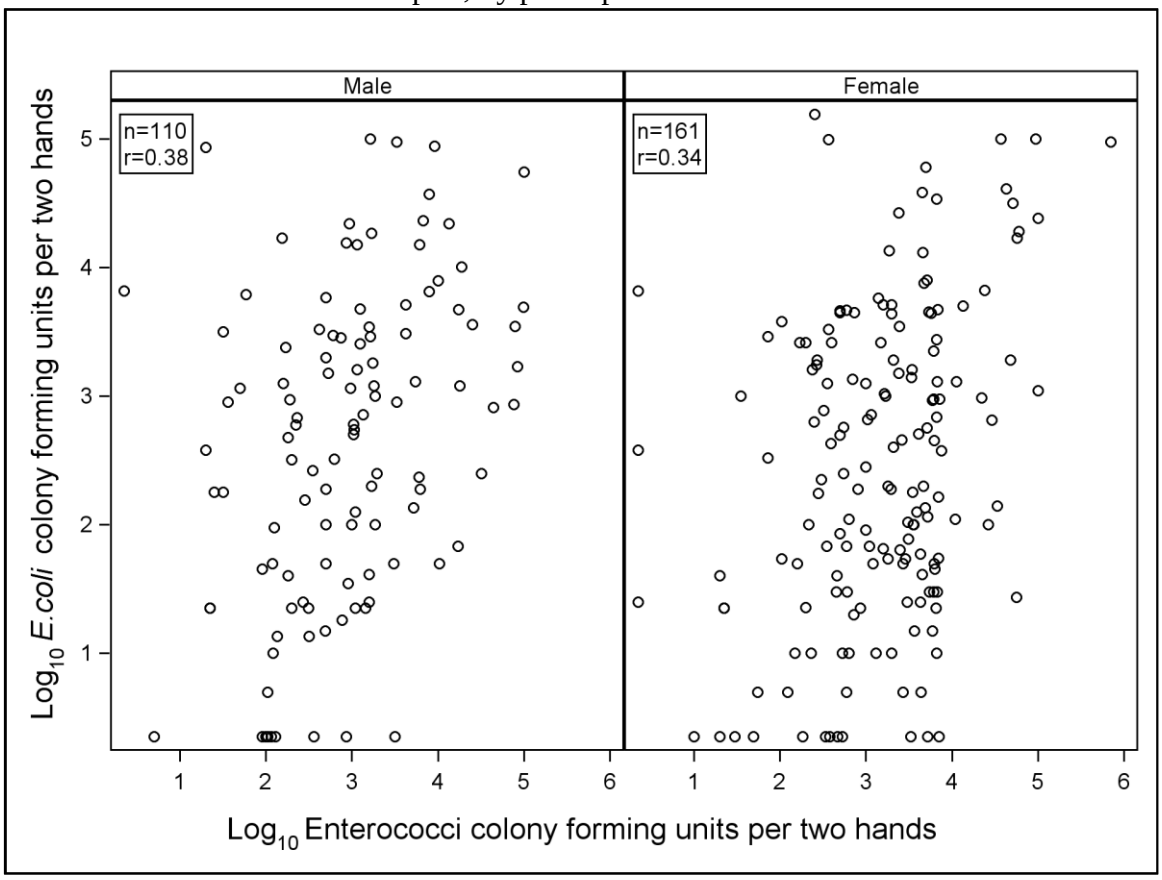


Figure 15. Correlation between $\log_{10}$ *E. coli* concentrations and $\log_{10}$ enterococci concentrations in handrinse samples, by age category

.

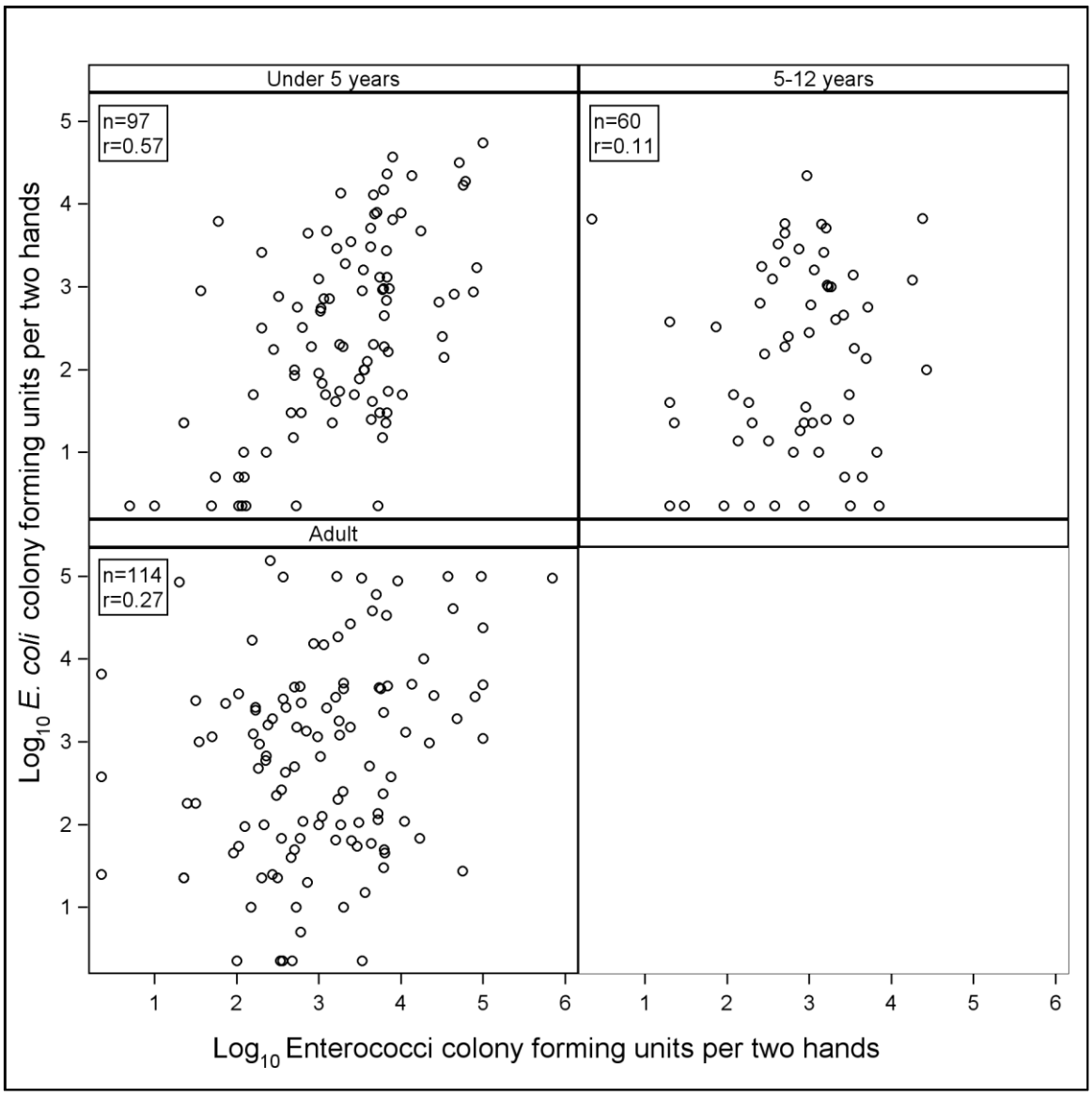


Figure 16. Correlation between $\log_{10}$ *E. coli* concentrations and $\log_{10}$ enterococci concentrations in handrinse samples, by neighborhood.

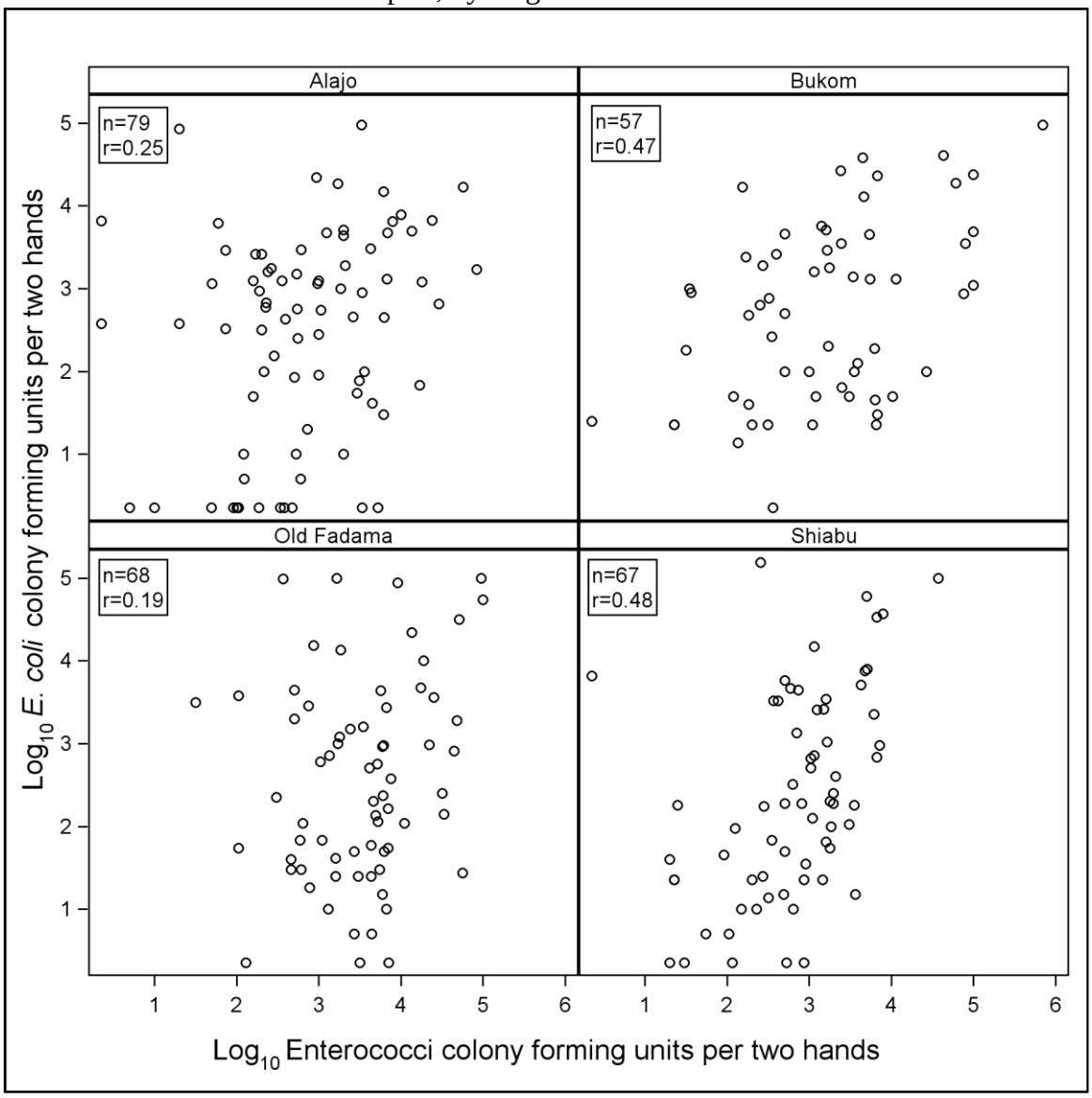


Figure 17. Correlation between $\log_{10}$ *E. coli* concentrations and $\log_{10}$ enterococci concentrations in handrinse samples, by setting.

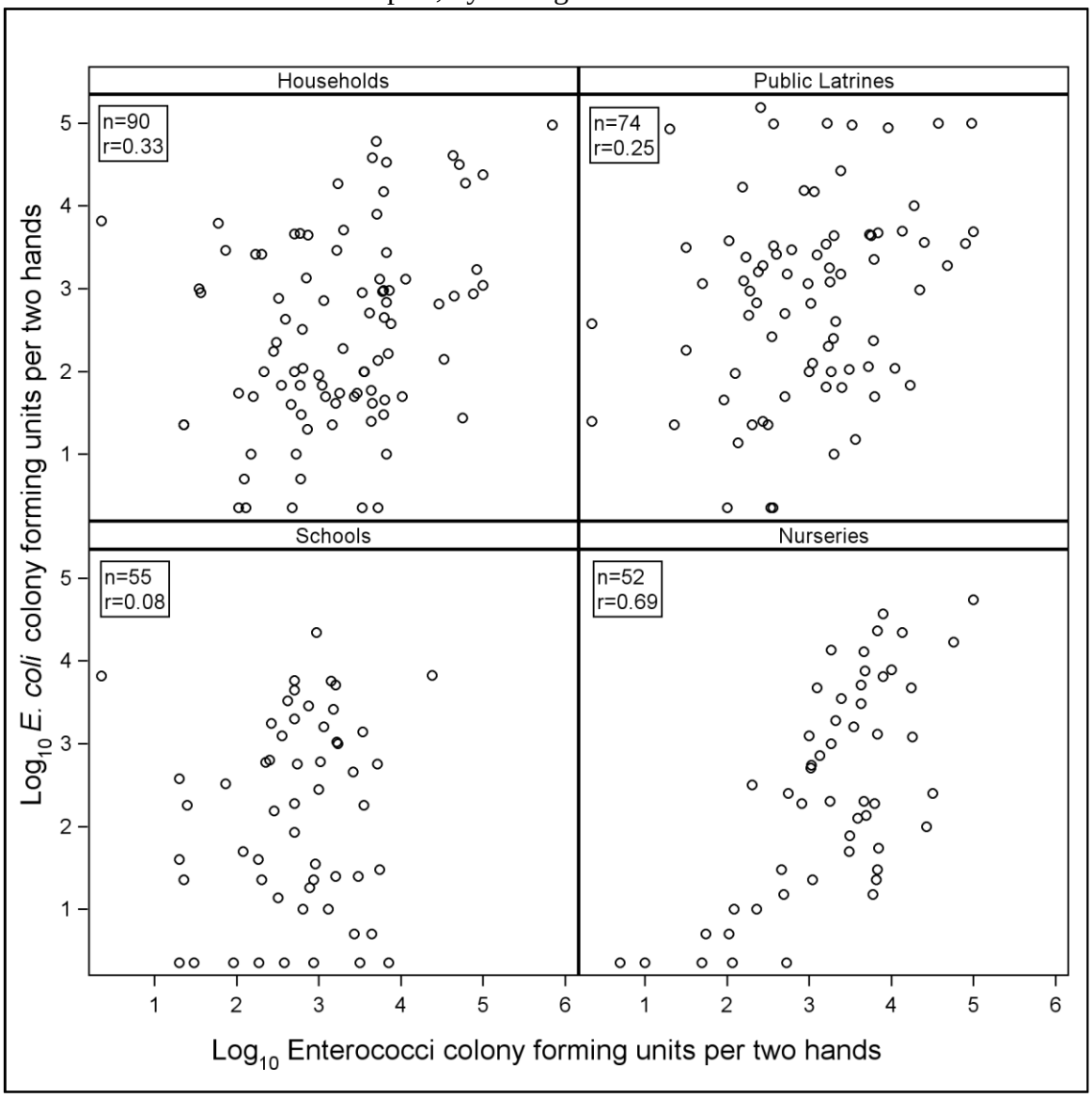
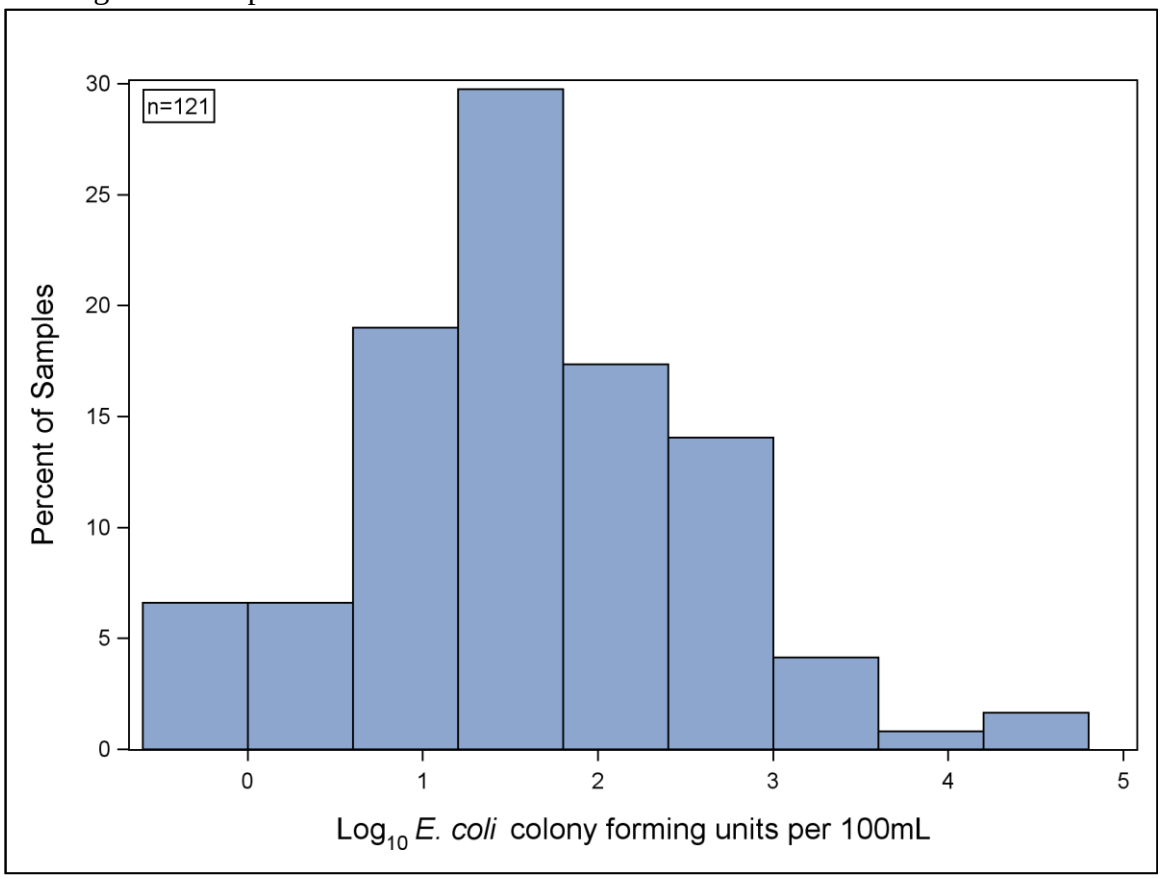
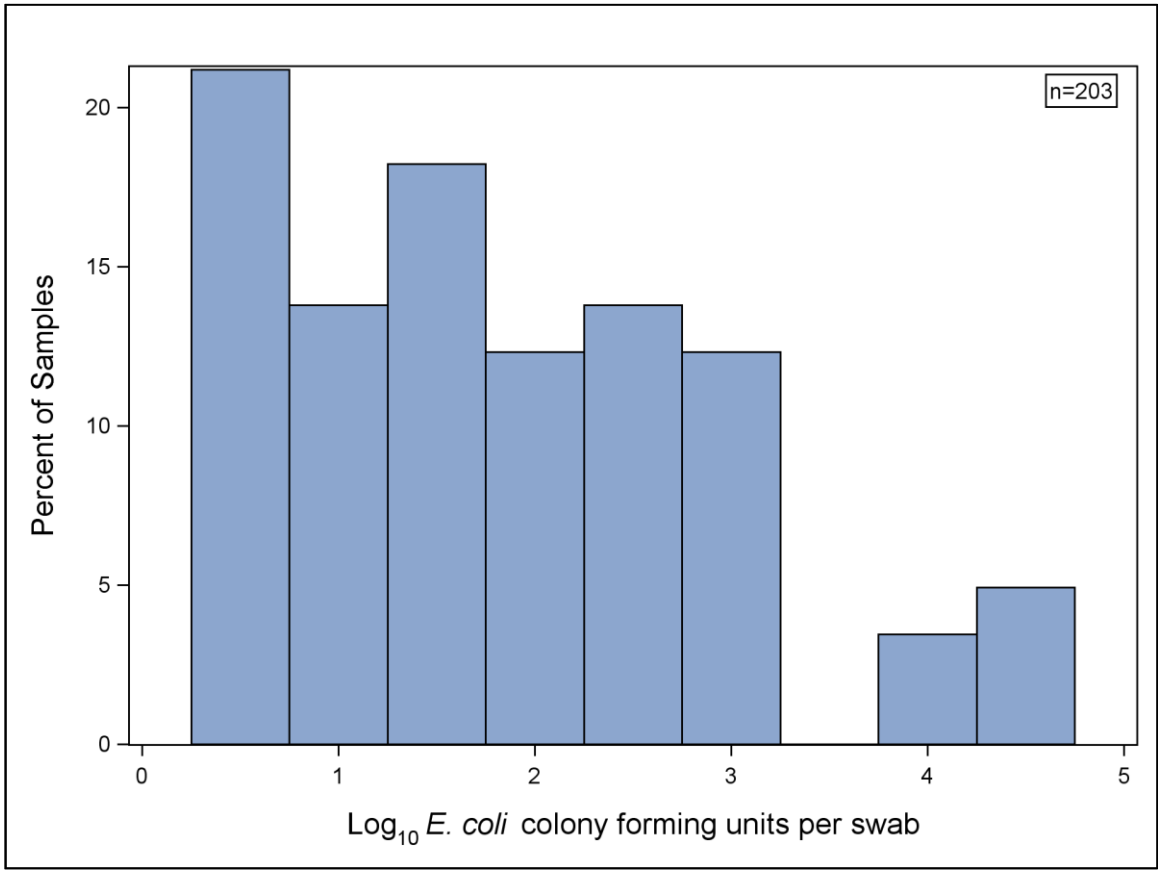


Figure 18. Distribution of $\log_{10}$ *E. coli* in stored drinking water for subset of stored drinking water samples[1].



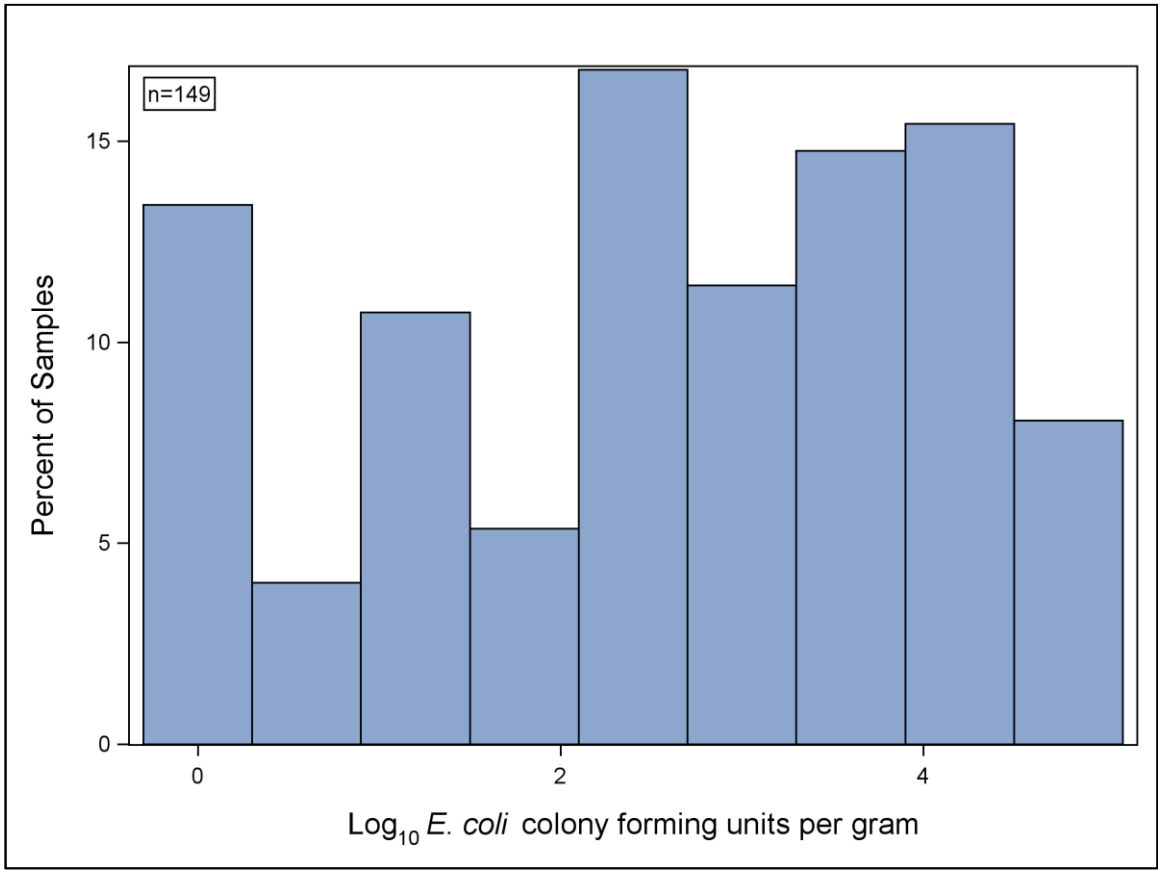
[1]subset of stored drinking water samples from specific locations where handrinse samples were taken

Figure 19. Distribution of $\log_{10}$ *E. coli* from swabs for subset of swab samples[1].



[1]subset of swab samples from specific locations where handrinse samples were taken

Figure 20. Distribution of $\log_{10}$ *E. coli* in soil for subset of soil samples[1].



[1]subset of soil samples from specific locations where handrinse samples were taken

III. LESSONS LEARNED AND FUTURE ANALYSES

A. Lessons Learned

- Although handrinse samples were usually collected near the end of a structured observation period, this study was limited by lack of information on the activity participants were engaged in just prior to handrinse sample collection. Since hand fecal contamination can strongly associated with the activity done prior to testing, I recommend that future handrinse sample collection forms include a description of what the participants were doing just prior to sample collection. The collection of observational data on daily activities and surfaces/ objects touched would be helpful for addressing this limitation, but could be costly and time-intensive to obtain. In lieu of structured observations, the simple recording of the activity done just prior to handrinse sample collection could be useful for assessing predictors of hand contamination.
- Looking back, it would have been helpful to include structured observations data in this thesis to better understand the differences in daily activities and contacts with environmental media (surfaces, floors, objects) among people of different ages. As daily activities and contacts likely vary widely by age category, accounting for differential behavior in the predictive models would have been valuable.
- This thesis followed the assumption that transfer of fecal contamination from environmental media to hands was additive. However, the collection of handrinse samples was not conducted in a way that made it possible to examine this assumption. This study could have attempted to examine this assumption by collecting handrinse samples from participants before and after touching different surfaces or objects in

their environments. This may have made it possible to measure the transfer of microbes onto/ off of hands after contact with the environment. A better understanding of the transfer of microbes from the environment to hands could inform more accurate and detailed predictive models for hand contamination in our study settings.

- If the analyses for this specific study were planned prior to data collection, I would create specific definitions for categories of swab samples to be collected. This would attempt to place each surface or object to be measured into a meaningful category based on the type and size of the area swabbed and the ease with which the sample was collected. An alternative would be to swab a smaller variety of objects and collect more swab samples from these target objects. Looking back, if I had known that *E. coli* concentrations in swab samples would be predictive of handrinse *E. coli* concentrations, and realized how difficult it would be to separate the swab samples into meaningful categories for inclusion in predictive modeling, I would have sought guidance sooner. I feel that this piece of the analysis had the potential for further assessment, but could not be developed given the time constraints and level of data collected.
- It would have been interesting to link hand contamination levels with frequency and occurrence of handwashing. For this thesis, I assumed that handwashing was low for all participants, based on previous structured observations data. However, as hand contamination levels varied across different study settings and sub-groups, I would have liked to consider the influence of handwashing on fecal contamination of hands. Additionally, handwashing has been an important focus in hygiene promotion

activities in low-income settings and its inclusion as a focus in this study could have provided additional relevance to public health practice.

- During this process, I learned how difficult it is to accurately measure fecal contamination on hands and in the environment. There is no perfect method for measuring real-world hand contamination levels. Further, fecal contamination of hands represents just one of the multiple fecal exposure pathways in these settings. Through this thesis, I gained confidence in my ability to draw conclusions from microbiological data, but I also learned that there will always be unknown or unmeasurable variables impacting fecal-oral transmission of microbes in low-income urban settings.

B. Future Analyses

- This study only considered the effects of demographic variables and *E. coli* concentrations of environmental samples (surface swabs, soil, and drinking water) on handrinse contamination. The larger SaniPath study collected data on a number of different structural characteristics, such as presence of latrines and handwashing stations in the homes, which were not included in this analysis. Future analyses could consider linking this data to the environmental samples to model the impact of both structural characteristics and environmental fecal contamination on hand contamination.
- This study assessed the relationship between *E. coli* and enterococci concentrations in handrinse samples, but did not actually study the rate at which *E. coli* and enterococci concentrations in the handrinse samples decline. Future studies could calculate the rates at which *E. coli* and enterococci die off to provide further evidence for the

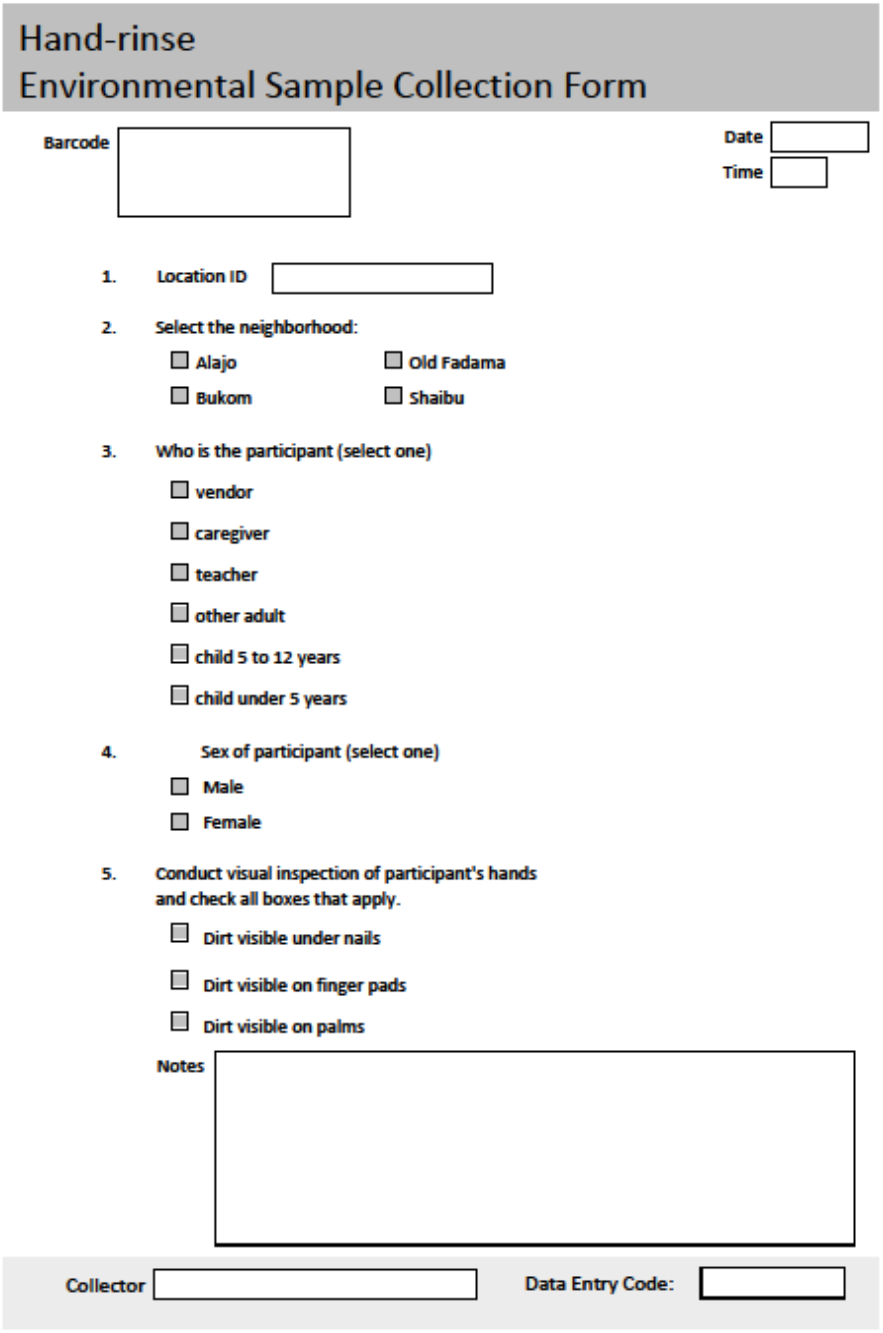
hypothesis that faster die off of *E. coli* results in a weaker correlation between *E. coli* and enterococci concentrations in handrinse samples from settings where exposures to fecal contamination occur less often.

- This study found that *E. coli* contamination on swabs were a significant predictor of handrinse *E. coli* contamination. However, this study was limited in its ability to break down the wide variety of swab samples into meaningful categories for predictive modeling. This was due, in part, to the large variety of objects that were sampled and the different sizes of areas swabbed. Future studies interested in linking environmental contamination and hand contamination should consider collecting a smaller range of swab samples in larger numbers, with distinct categories for analysis. This may allow for a more accurate and illuminating categorization of contamination among the swab samples. Additionally, this study was creative when designing specific protocols for swabbing objects that were not flat. Future studies should further this work by focusing on the creation of standardized methods to accurately measure environmental contamination of irregular objects.
- While not included in this thesis, an analysis of structured observations of child behaviors in homes and nurseries is currently underway. The findings from this analysis will provide insight into daily activities of children, including surfaces touched most often and the frequency with which children put objects and hands into their mouths. Once complete, these findings could be paired with the results from this thesis to better explain how fecal contamination spreads from the environment onto children's hands. This data could also be useful for modeling the amount of fecal contamination ingested from hands and objects.

- The models for this thesis have assumed that the amount of fecal contamination on hands is additive after each contact with a contaminated surface or other environmental media. Future analyses could consider potential alternatives, such as contact with objects and surfaces actually detaches fecal contamination from hands or that some saturation point is reached, whereby additional contacts do not alter the amount of fecal contamination on hands.

IV. APPENDICES

A. Handrinse Sample Collection Form



Hand-rinse
Environmental Sample Collection Form

Barcode

Date

Time

1. Location ID

2. Select the neighborhood:
   - ☐ Alajo
   - ☐ Bukom
   - ☐ Old Fadama
   - ☐ Shaibu

3. Who is the participant (select one)
   - ☐ vendor
   - ☐ caregiver
   - ☐ teacher
   - ☐ other adult
   - ☐ child 5 to 12 years
   - ☐ child under 5 years

4. Sex of participant (select one)
   - ☐ Male
   - ☐ Female

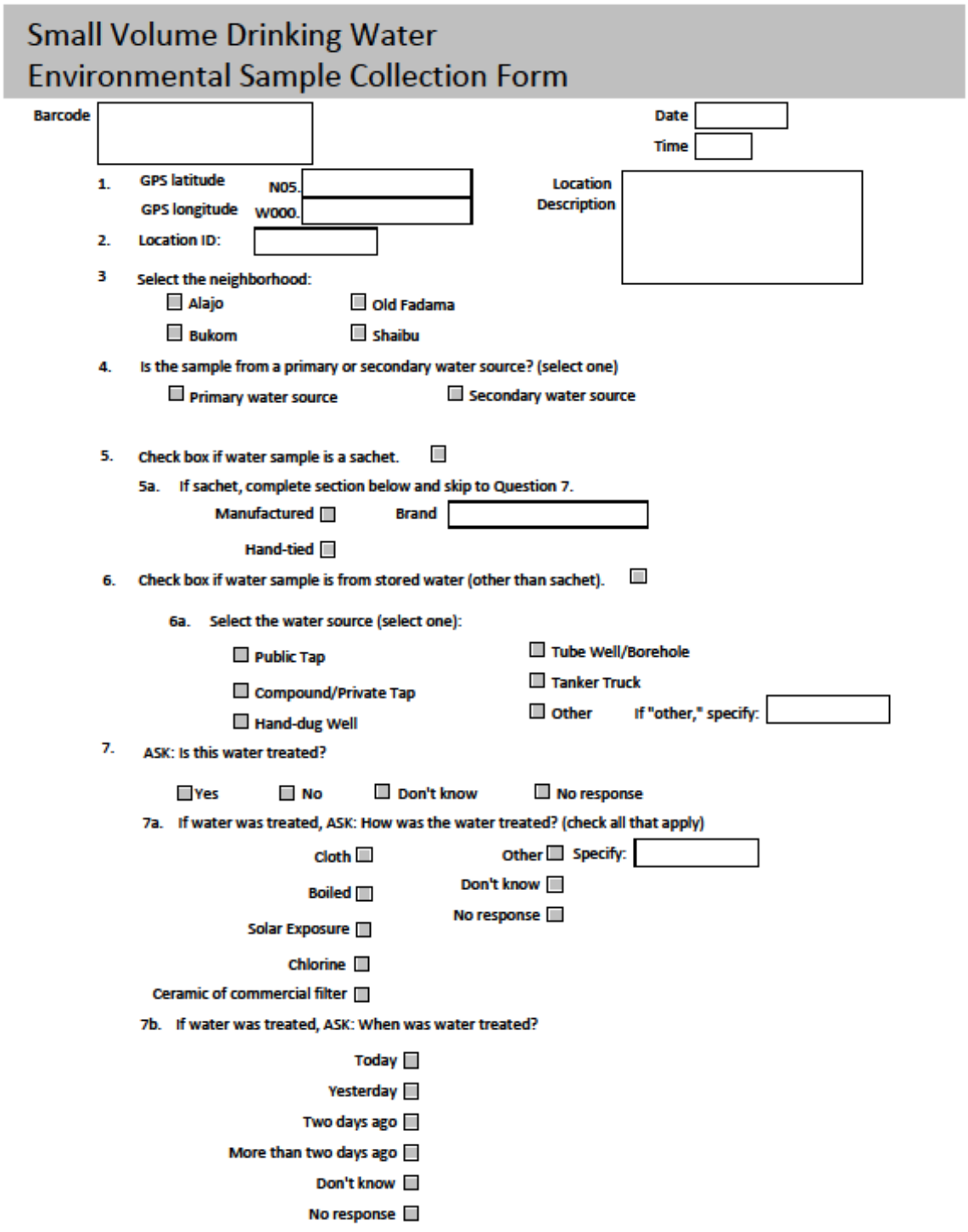
5. Conduct visual inspection of participant's hands and check all boxes that apply.
   - ☐ Dirt visible under nails
   - ☐ Dirt visible on finger pads
   - ☐ Dirt visible on palms

Notes

Collector

Data Entry Code:

B. Stored Drinking Water Sample Collection Form



Small Volume Drinking Water
Environmental Sample Collection Form

Barcode

Date

Time

1. GPS latitude N05.
   GPS longitude W000.

Location Description

2. Location ID:

3 Select the neighborhood:

- ☐ Alajo
- ☐ Old Fadama
- ☐ Bukom
- ☐ Shaibu

4. Is the sample from a primary or secondary water source? (select one)

- ☐ Primary water source
- ☐ Secondary water source

5. Check box if water sample is a sachet. ☐

5a. If sachet, complete section below and skip to Question 7.

Manufactured ☐ Brand

Hand-tied ☐

6. Check box if water sample is from stored water (other than sachet). ☐

6a. Select the water source (select one):

- ☐ Public Tap
- ☐ Compound/Private Tap
- ☐ Hand-dug Well
- ☐ Tube Well/Borehole
- ☐ Tanker Truck
- ☐ Other If "other," specify:

7. ASK: Is this water treated?

- ☐ Yes
- ☐ No
- ☐ Don't know
- ☐ No response

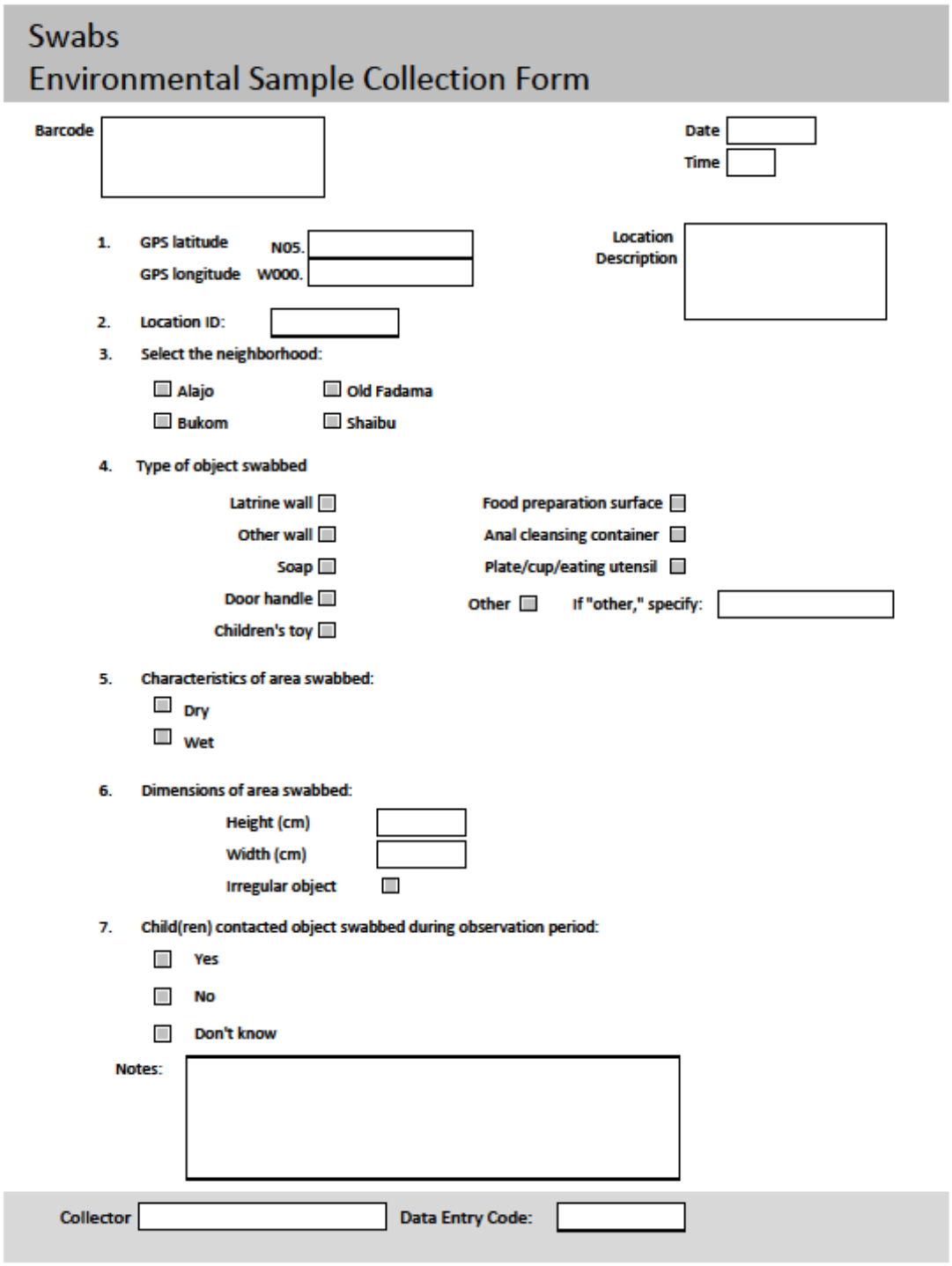
7a. If water was treated, ASK: How was the water treated? (check all that apply)

- Cloth ☐
- Boiled ☐
- Solar Exposure ☐
- Chlorine ☐
- Ceramic of commercial filter ☐
- Other ☐ Specify:
- Don't know ☐
- No response ☐

7b. If water was treated, ASK: When was water treated?

- Today ☐
- Yesterday ☐
- Two days ago ☐
- More than two days ago ☐
- Don't know ☐
- No response ☐

C. Swabs Sample Collection Form



Swabs
Environmental Sample Collection Form

Barcode

Date
Time

1. GPS latitude N05.
   GPS longitude W000.

Location Description

2. Location ID:

3. Select the neighborhood:
   - ☐ Alajo
   - ☐ Bukom
   - ☐ Old Fadama
   - ☐ Shaibu

4. Type of object swabbed
   - ☐ Latrine wall
   - ☐ Other wall
   - ☐ Soap
   - ☐ Door handle
   - ☐ Children's toy
   - ☐ Food preparation surface
   - ☐ Anal cleansing container
   - ☐ Plate/cup/eating utensil
   - ☐ Other — If "other," specify:

5. Characteristics of area swabbed:
   - ☐ Dry
   - ☐ Wet

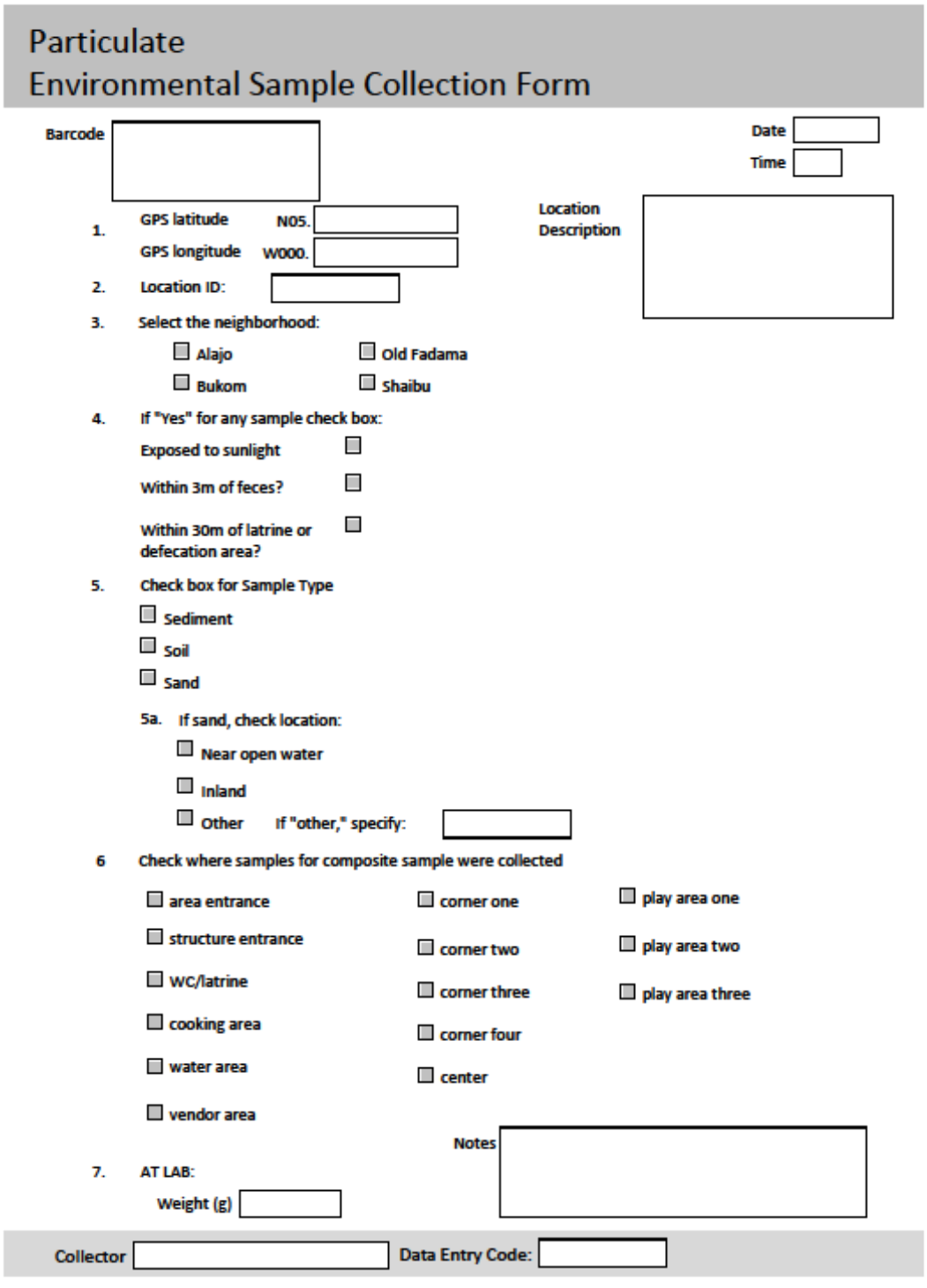
6. Dimensions of area swabbed:
   - Height (cm)
   - Width (cm)
   - ☐ Irregular object

7. Child(ren) contacted object swabbed during observation period:
   - ☐ Yes
   - ☐ No
   - ☐ Don't know

Notes:

Collector Data Entry Code:

D. Particulate Sample Collection Form



Particulate
Environmental Sample Collection Form

Barcode

Date

Time

1. GPS latitude N05.
   GPS longitude W000.

Location Description

2. Location ID:

3. Select the neighborhood:
   - ☐ Alajo
   - ☐ Old Fadama
   - ☐ Bukom
   - ☐ Shaibu

4. If "Yes" for any sample check box:
   - Exposed to sunlight ☐
   - Within 3m of feces? ☐
   - Within 30m of latrine or defecation area? ☐

5. Check box for Sample Type
   - ☐ Sediment
   - ☐ Soil
   - ☐ Sand

5a. If sand, check location:
   - ☐ Near open water
   - ☐ Inland
   - ☐ Other If "other," specify:

6 Check where samples for composite sample were collected
   - ☐ area entrance
   - ☐ structure entrance
   - ☐ WC/latrine
   - ☐ cooking area
   - ☐ water area
   - ☐ vendor area
   - ☐ corner one
   - ☐ corner two
   - ☐ corner three
   - ☐ corner four
   - ☐ center
   - ☐ play area one
   - ☐ play area two
   - ☐ play area three

Notes

7. AT LAB:
   Weight (g)

Collector Data Entry Code:

E. Recommended Values for Surface Area of Body Parts

Table 7-2. Recommended Values for Surface Area of Body Parts

Age Group	Head	Trunk[a]	Arms[b]	Hands	Legs[c]	Feet	Source
	Mean Percent of Total Surface Area						
Male and Female Children Combined							
Birth to <1	18.2	35.7	13.7	5.3	20.6	6.5	U.S. EPA (1985)
1 to <3 months[d]	18.2	35.7	13.7	5.3	20.6	6.5	
3 to <6 months[d]	18.2	35.7	13.7	5.3	20.6	6.5	
6 to <12 months[d]	18.2	35.7	13.7	5.3	20.6	6.5	
1 to <2 years[d]	16.5	35.5	13.0	5.7	23.1	6.3	
2 to <3 years[e]	8.4	41.0	14.4	4.7	25.3	6.3	Boniol et al. (2008) (average of data for males and females)
3 to <6 years[f]	8.0	41.2	14.0	4.9	25.7	6.4	
6 to <11 years[g]	6.1	39.6	14.0	4.7	28.8	6.8	
11 to <16 years[h]	4.6	39.6	14.3	4.5	30.4	6.6	
16 to <21 years[i]	4.1	41.2	14.6	4.5	29.5	6.1	
Adult Male							U.S. EPA Analysis of NHANES 2005–2006 data and U.S. EPA (1985)
21+ years	6.6	40.1	15.2	5.2	33.1	6.7	
Adult Female							
21+ years	6.2	35.4	12.8	4.8	32.3	6.6	
	Mean Surface Area by Body Part[j] m^2						
Male and Female Children Combined							
Birth to <1 month[d]	0.053	0.104	0.040	0.015	0.060	0.019	U.S. EPA Analysis of NHANES 1999–2006 data and U.S. EPA (1985)
1 to <3 months[d]	0.060	0.118	0.045	0.017	0.068	0.021	
3 to <6 months[d]	0.069	0.136	0.052	0.020	0.078	0.025	
6 to <12 months[d]	0.082	0.161	0.062	0.024	0.093	0.029	
1 to <2 years[d]	0.087	0.188	0.069	0.030	0.122	0.033	
2 to <3 years[e]	0.051	0.250	0.088	0.028	0.154	0.038	U.S. EPA Analysis of NHANES 1999–2006 data and Boniol et al. (2008)
3 to <6 years[f]	0.061	0.313	0.106	0.037	0.195	0.049	
6 to <11 years[g]	0.066	0.428	0.151	0.051	0.311	0.073	
11 to <16 years[h]	0.073	0.630	0.227	0.072	0.483	0.105	
16 to <21 years[i]	0.075	0.759	0.269	0.083	0.543	0.112	
Adult Male							U.S. EPA Analysis of NHANES 2005–2006 data and U.S. EPA (1985)
21+ years	0.136	0.827	0.314	0.107	0.682	0.137	
Adult Female							
21+ years	0.114	0.654	0.237	0.089	0.598	0.122	