On our hands: A systematic review of fecal contamination and enteric pathogen detection on human hands

A thesis submitted by
Molly Cantrell

In partial fulfillment of the requirements for the degree for

Master of Science

in

Civil and Environmental Engineering

Tufts University

February 2021

Advisor/Committee Chair: Amy Pickering

Committee Members: David Gute and Jeffrey Griffiths

Abstract

Despite a significant decrease in diarrheal deaths over the last few decades (an estimated 36% decrease from 1990 to 2016), morbidity continues to be a significant problem worldwide. The transmission of enteric pathogens occurs through contamination of, and subsequent exposure to food, water, flies, surfaces (or fomites), hands, and fields (or soils). The purpose of this thesis is to present evidence on enteric pathogen transmission through hands, a relatively understudied environmental pathway. The objectives are to describe the prevalence and levels of contamination of fecal indicator bacteria (FIB) and enteric pathogens on hands, and to compare contamination levels between five subgroups (country income level, age group, gender, urban/rural, and climate classification). Seventy-eight studies were identified with 48 different types of FIB and enteric pathogens. The most commonly reported pathogens were adenovirus, rotavirus, enterovirus, and norovirus. *E. coli* and fecal coliforms (FC) were the most commonly reported indicators. The average *E. coli* and FC prevalence on hands were 43.8% and 45.6%, and mean contamination levels were 1.59 and 2.22 $\log_{10}$CFU/hand, respectively. We found that low-income countries had significantly greater *E. coli* and FC prevalence than high-income countries. Within low/lower-middle income countries, *E. coli* prevalence and concentration were higher in urban settings as compared to rural areas. Climate classifications could only be compared in upper-middle/high income settings, but may be associated with *E. coli* and FC prevalence. This review highlighted gaps in evidence of hand contamination in rural high-income settings as well as contamination by gender. Additionally, we found that hand rinse samples are likely more sensitive at detecting fecal indicator bacteria than swab and impression samples. This review suggests that hands are frequently contaminated with fecal bacteria, especially in urban low-income settings.

Table of Contents

List of Figures

List of Tables

Introduction

Globally, diarrhea was the eighth leading cause of death among all ages in 2016, with more than 1.6 million estimated deaths (Naghavi et al., 2017). More than a quarter of diarrheal deaths occurred among children under 5 years and the majority of diarrheal deaths (about 90%) occurred in sub-Saharan Africa and south Asia (Troeger et al., 2018). Even though mortality has decreased significantly over the last three decades, from an estimated 2.5 million deaths in 1990 to 1.6 million is 2016 (a 36% decrease), diarrheal disease morbidity is still high (Kosek et al., 2003; Naghavi et al., 2017). Globally there are an estimated 1.7 billion cases of diarrheal disease in children under five every year (Walker et al., 2013). This shows that "the primary drivers of change in diarrhea mortality have been ones that preferentially reduce the risk of dying from the disease rather than those that reduce the risk of infection" (Troeger et al., 2018). To reduce risk of infection, it is important to understand how pathogens are transmitted.

A recent meta-analysis of almost 90,000 individual participant data points from 20 studies suggested that fecal indicator bacteria (FIB) concentrations in households were associated with child diarrhea and stunting (Goddard et al., 2020). This finding highlights the importance of understanding the various transmission pathways in order to implement effective interventions. Enteric pathogen transmission occurs through contamination of, and subsequent exposure to, environmental reservoirs. The environmental reservoirs demonstrated to be of concern include food, water, flies, surfaces (or fomites), hands, and fields (or soils). These exposure pathways are visualized in the fecal-diagram, or F-diagram (Figure 1). Enteric pathogens may also be spread as aerosols, though there is limited data on infectivity of airborne exposures (Barker & Jones, 2005; Bing-Yuan et al., 2018).

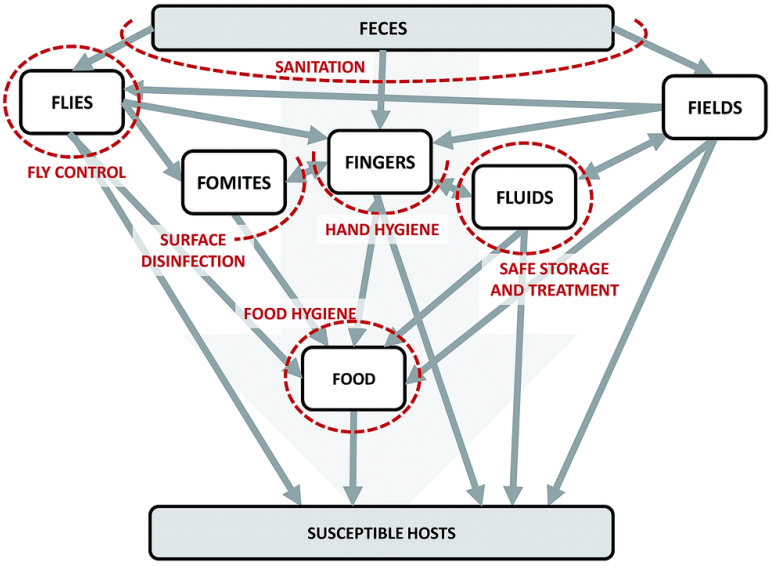

Figure 1: F-Diagram (Julian, 2016)

As shown in Figure 1 and as described in the literature, these transmission pathways do not act independently and are affected by social and etiological factors and the relative importance of each transmission pathway is likely contextual and site-specific (Eisenberg et al., 2012; Wagner et al., 1958).

Despite awareness of the importance of multiple potential reservoirs, previous research predominately focused on transmission of enteric pathogens through drinking water and food. Hands have been a relatively understudied pathway. A few studies have suggested the critical importance of hands in direct and indirect exposure to diarrheal diseases (M. C. M. Mattioli et al., 2015; Pickering et al., 2018). Direct risks refer to hand-to-mouth contacts and indirect risks refer to contaminated hands touching drinking water, food, and fomites (Julian, 2016). A recent study of households in rural Bangladesh measured fecal contamination in hands, soil, water, flies, and food, and found that, hands were the most strongly associated with increased risk of subsequent diarrheal illness among children (Pickering et al., 2018). Additionally, based on hand rinse and stored water FIB concentrations along with child specific exposure data from USEPA

2011 Exposure Factors Handbook, a quantitative fecal exposure assessment model created for a study in Tanzania showed that children ingest a significantly greater amount of feces each day from hand-to-mouth contacts than from drinking water, 0.93 and 0.098 mg, respectively (M. C. M. Mattioli et al., 2015). Another study in Tanzania found that hands were important in the transmission of viral pathogens (M. C. Mattioli et al., 2013). These studies' findings motivated this thesis, which aimed to compile evidence on enteric pathogen transmission through hands. The research objectives were:

1) To describe the prevalence and level of contamination with fecal indicator bacteria and enteric pathogens on hands globally.
2) To compare hand contamination levels between 1) low- and high-income countries, 2) children and adults, 3) urban and rural areas, 4) males and females, and 5) climate classifications
3) To determine the association between level of hand contamination and sampling method
4) To generate a comprehensive list of all enteric pathogens that have been detected on human hands

Methods

Search strategy and selection criteria

We conducted a systematic review of fecal contamination and enteric pathogen detection on hands from studies identified in an electronic search of PubMed, Embase, and Web of Science databases following the Preferred Reporting Items for Systematic review and Meta-Analyses for Protocols (PRISMA-P) guidelines (*PRISMA-P-Checklist.Pdf*, n.d.). The search was conducted twice: in March 2018 and then again in June 2020. Co-reviewers Lou Curchod and

Rahel Schneidegger conducted the review in 2018 and Molly Cantrell conducted the review in 2020. The following search strings were used for both sets of queries: (((fecal OR pathogenic OR enteric) AND bacteria) OR e. coli OR enterococci OR helminth OR protozoa OR virus OR phage) AND hand AND contamination.

Two independent reviewers did the initial title and abstract screening. A third reviewer resolved discrepancies. We included peer-reviewed published studies of all study designs that measured fecal indicators or enteric pathogens on human hands. Studies were excluded if they measured microorganisms that were not enteric pathogens or fecal indicators; artificially contaminated hands with bacteria, such as through randomized controlled trials; did not present primary data (i.e. reviews that included secondary data from previously conducted studies); were conducted in food handling, farm, clinical, or laboratory settings; dealt with food and animal contamination. However, studies were included if a subset of the data set met the inclusion and exclusion criteria. In that case, just the subset of relevant results from the study was included and other results were excluded. For example, control groups in case/control studies or people whose hands were sampled when they arriving at a hospital were included.

After reviewing titles and abstracts, full texts were reviewed against the same inclusion and exclusion criteria. Figure 2 is a flow diagram presenting the number of studies in each stage of the selection process. In the initial search, 243 duplicate studies were excluded. In the title and abstract review, 1574 studies were excluded for various reasons including they reported only water or food samples, did not report fecal indicator bacteria or enteric pathogens, and were in the wrong study setting. In the full text review, 110 studies were excluded mainly due to duplicate primary data and exclusion criteria such as wrong study setting (ex: food handling and clinics).

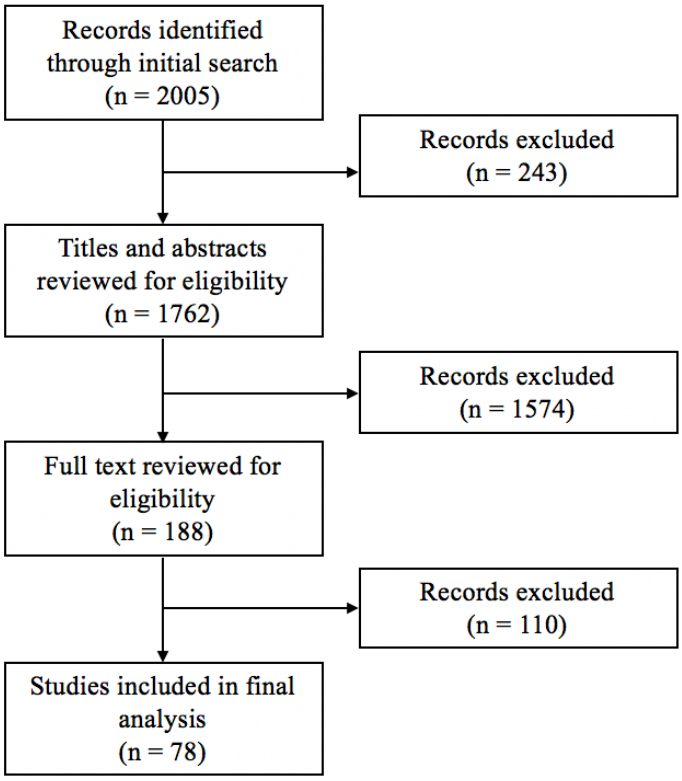

Figure 2: Study Selection Steps

Data management

Databases were searched directly and records were downloaded into Endnote. Endnote software was used to remove duplicates, and then to create a master file for importing into Covidence (www.covidence.com), a commercial software providing a collaborative environment for screening and managing records.

A standardized form was used to extract relevant results, demographics, and other study information such as year of publication, microbiological indicator or pathogen, number of samples collected, and sampling method. Two reviewers, Tim Julian and Rahel Schneidegger, extracted data from 3 studies to pilot the standardized prior to use. After the initial data extraction, reviewers checked data with 10% record duplication to ensure consistency and the few errors found were corrected.

Data analysis

There were two different types of contamination measurements reported: prevalence, the percent of hand samples positive for a fecal indicator, and the mean concentration of an organism in the samples. The concentrations were not always reported in the same units. Therefore, all of the concentration values were synthesized to $\log_{10}$CFU/hand. For this analysis, quantification of bacteria in units of most probable number (MPN) was treated as equivalent to quantification using units of colony forming unit (CFU). As a consequence of the method used to derive MPN estimates, MPN is generally characterized by higher intra-sample variability and "somewhat higher" estimates than CFU (Gronewold & Wolpert, 2008). However, the analytical difference between methods is generally thought to be much lower than the intrinsic variability of the assay (Buckalew et al., 2006; Gronewold & Wolpert, 2008; Noble et al., 2003). Additionally, to standardize results for studies reporting concentration per unit area instead of "per hand", an adult hand was assumed to be 160 cm^2 (*Exposure Factors Handbook - Chapter 7: Dermal Exposure Factors*, 2011). Notably, no studies on child hand contamination reported results per unit area other than "per hand", so a child hand estimate was not needed. Subgroup analyses included age (adults/children), urban/rural, gender (male/female), country income level, and climate classification. The age of children was study dependent, but overall, age ranges spanned from birth to 15 years. Urban and rural classifications were a result of the investigators of each study making a designation. If no designation was made in the study, then we assigned based on population density. Country income levels were based on World Bank country income classifications for the year the study was conducted (*World Bank Country and Lending Groups – World Bank Data Help Desk*, n.d.). These classifications are low-income, lower-middle income, upper-middle income, and high-income economies, which are defined by gross national income

per capita. The climate classifications were based on Köppen-Geiger Climate Classifications where zone A is tropical or equatorial, zone B is arid or dry, zone C is warm/mild temperate, zone D is continental, and zone E is polar (Society, 2019). Based on the Köppen-Geiger climate classifications and the World Bank country income levels, we assigned climate classifications and temporally correct country income levels based on the year the study was conducted.

Results

We identified 78 studies in total using the above search criteria. The majority of the studies were published between 2010 and 2020 (Figure 3). Most studies (71%) had at least 100 samples and only 9 studies had fewer than 30 samples. Forty-six of the studies (49%) used the hand rinse method to collect samples. Nineteen (24%) used the swab method and twelve used impressions. One study (1%) did not report the sampling method used.

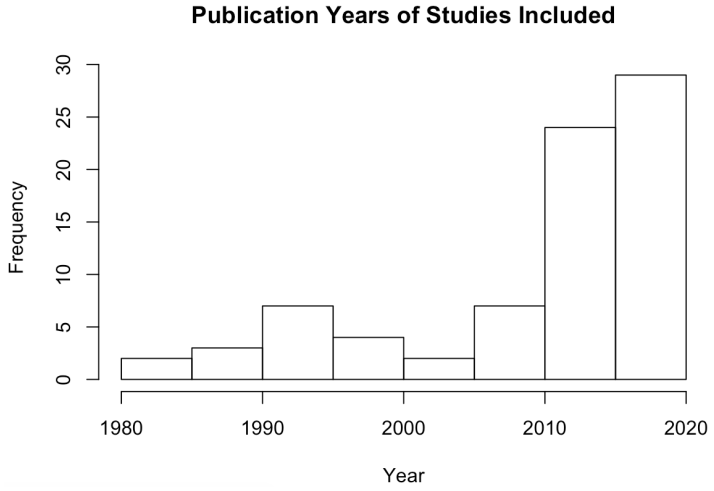

Figure 3: Publication years of studies included in the review

FIB and enteric pathogens

Forty-eight different fecal indicator bacteria and enteric pathogens were found on hands. The most common indicators were *E. coli* and fecal coliforms (FC). Fifty-one studies (65% of

the studies) reported *E. coli* and 23 studies (29%) reported FC. Enterococci was also a common indicator with 9 studies (12%). All indicators are given in Table 1.

Table 1: All indicators reported in the studies included

Type of indicator	
Bacteria (commonly used fecal indicator bacteria underlined)	Aerobic plate count, Any multidrug-resistant organism (MDRO+), *Bacillus* spp, *Bacteroidales* Cow, *Bacteroidales* General, *Bacteroidales* Human, *Camplyobacter jejuni, Clostridium perfringens,* Coagulase negative *Staphylococcus spp., Commensal flora, CrAssphage, E. coli,* enteroaggregative *E. coli* (EAEC), *Enterococci, Enterococcus faecalis, Enterococcus* spp., enterohemorragic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E coli* (ETEC), shiga-toxin producing *E. coli* (STEC), *Enterobacter* spp., *Enterobacteriaceae,* fecal bacteria, fecal coliform, *Fecal Streptococci, Klebsiella* spp., Pathogenic *E. coli, Proteus* spp., *Pseudomonas* spp., Resistant gram-negative *bacilli* (R-GNB), *Salmonella* spp., *Serratia* spp., *Shigella* spp., *Shigella* spp. / EIEC, *Staphylococcus aureus, Streptococcus Faecalis, Streptococcus* spp., total coliform, *Vancomycin-resistant enterococci (VRE+), Vibrio cholerae*
Viruses	Adenovirus, Enterovirus, Hepatitis A virus, Norovirus, Norovirus GII, Rotavirus
Protozoa	*Giardia Lamblia*

Prevalence and concentration levels

Sixty-eight studies (87%) reported prevalence, the percent of hand samples positive for a fecal indicator. Forty-one (53%) studies reported indicator concentration levels. Of the 51 studies with *E. coli* as an indicator, 44 studies reported *E. coli* prevalence data and 32 reported *E. coli* concentration data. The average *E. coli* prevalence was 43.8% and the average *E. coli* concentration was 1.59 $\log_{10}$CFU/hand. Of the 23 studies with FC as an indicator, 19 reported FC prevalence data and 11 reported FC concentrations. The average FC prevalence was 45.6% and the average FC concentration was 2.22 $\log_{10}$CFU/hand.

Demographics

The global regions most represented in this review are South-East Asia and Africa, with 24 and 23 studies (31% and 29% of all studies included) respectively. Seventeen studies (22%)

were conducted in North America. The countries with the highest number of studies were the United States (14 studies, 18%), India (11 studies, 14%) and Bangladesh (10 studies, 14%).

Thirty-four of the studies (44%) reported contamination values for adults only, 22 studies (28%) reported for children only, 17 studies (22%) reported both adults and children individually, and 5 studies (6%) only reported adults and children together. Only one study did not report an urban/rural classification. Forty-seven studies (60%) reported urban data only, 23 studies (29%) reported rural data, one study (1%) report both urban and rural data individually, and 6 studies (8%) reported a combination of rural, urban, and/or peri-urban data. Three studies (4%) reported contamination values for males and females and 4 additional studies (5%) reported the general comparison between genders (i.e. which gender group, if either, had higher hand contamination levels) without including quantitative data.

Subgroup contamination comparisons for E. coli

The subgroups examined within the data were country income levels, children vs. adults, rural vs. urban, climate, and male vs. female. Statistical significance was defined as alpha equals 0.05. Within the analysis of the *E. coli* data, t-tests showed significant differences in *E. coli* prevalence levels in low/lower-middle income countries compared to high/upper-middle income countries. Low/lower-middle income countries had significantly higher *E. coli* prevalence than high/middle-upper income countries (48.2% mean prevalence in low income versus 23.2% in high income, $p = 0.02$), as shown in Figure 4. There was not a significant difference in *E. coli* prevalence between adults and children even when subgrouping by income level or specifically comparing adults with children under five-years-old ($p = 0.95$ for all adults and children, $p = 0.24$ for low income, $p = 0.49$ for high income, $p = 0.3$ for adults versus children under 5).

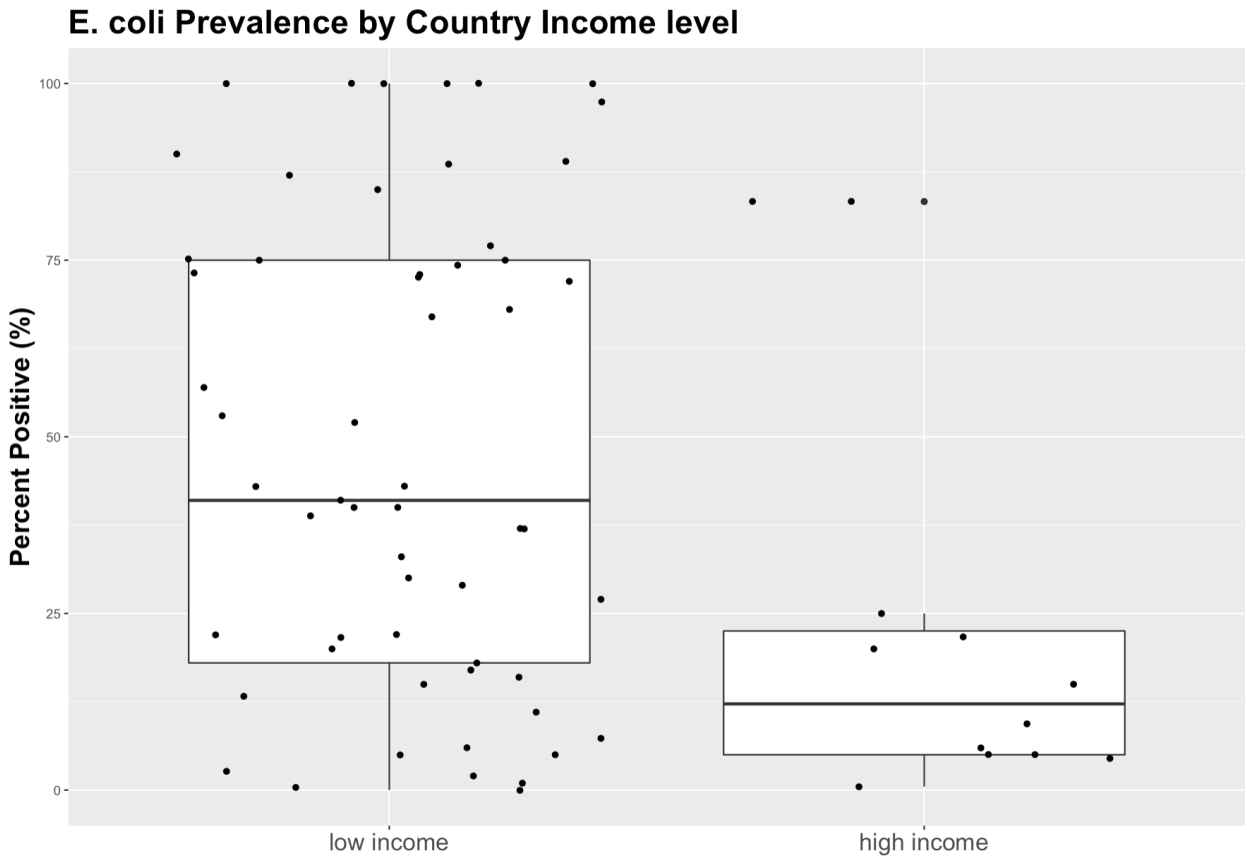

Figure 4: Subgroup with statistical significance: *E. coli* prevalence by country income level (n = 69 observations, from 44 studies)

Initially, there was not a significant difference in *E. coli* prevalence between rural and urban areas. All upper-middle/high country income studies that reported *E. coli* prevalence were conducted in urban areas. However, studies in low/lower-middle income countries were conducted in both rural and urban areas. Within this subgroup of low/lower-middle income countries, urban areas had higher *E. coli* prevalence values (64.5% mean prevalence in urban versus 33.5% in rural, $p = 5.0 \times 10^{-4}$), as shown Figure 5A.

Additionally, there was a statistically significant difference in *E. coli* prevalence between climate classification subgroups (for A to C comparison, $p = 0.04$). Again, since income level is significant, we conducted further analysis while controlling for country income level. Both low/lower-middle income countries and high/upper-middle income countries were in tropical

areas (classification A), dry areas (classification B), and temperate areas (classification C). In the low/low-middle income group, there were 23 studies in tropical areas, 3 studies in dry areas, and 12 studies in temperate areas. There was not a significant difference in *E. coli* prevalence levels between climate classifications in low/lower-middle income countries. In the high/upper-middle income group, there were 2 studies in tropical areas, 2 studies in dry areas, and 4 studies in temperate areas. There was a moderately significant difference between dry (B) and temperate (C) areas (62.8% mean prevalence in B versus 12.0% in C, p=0.039), which is shown in Figure 5B.

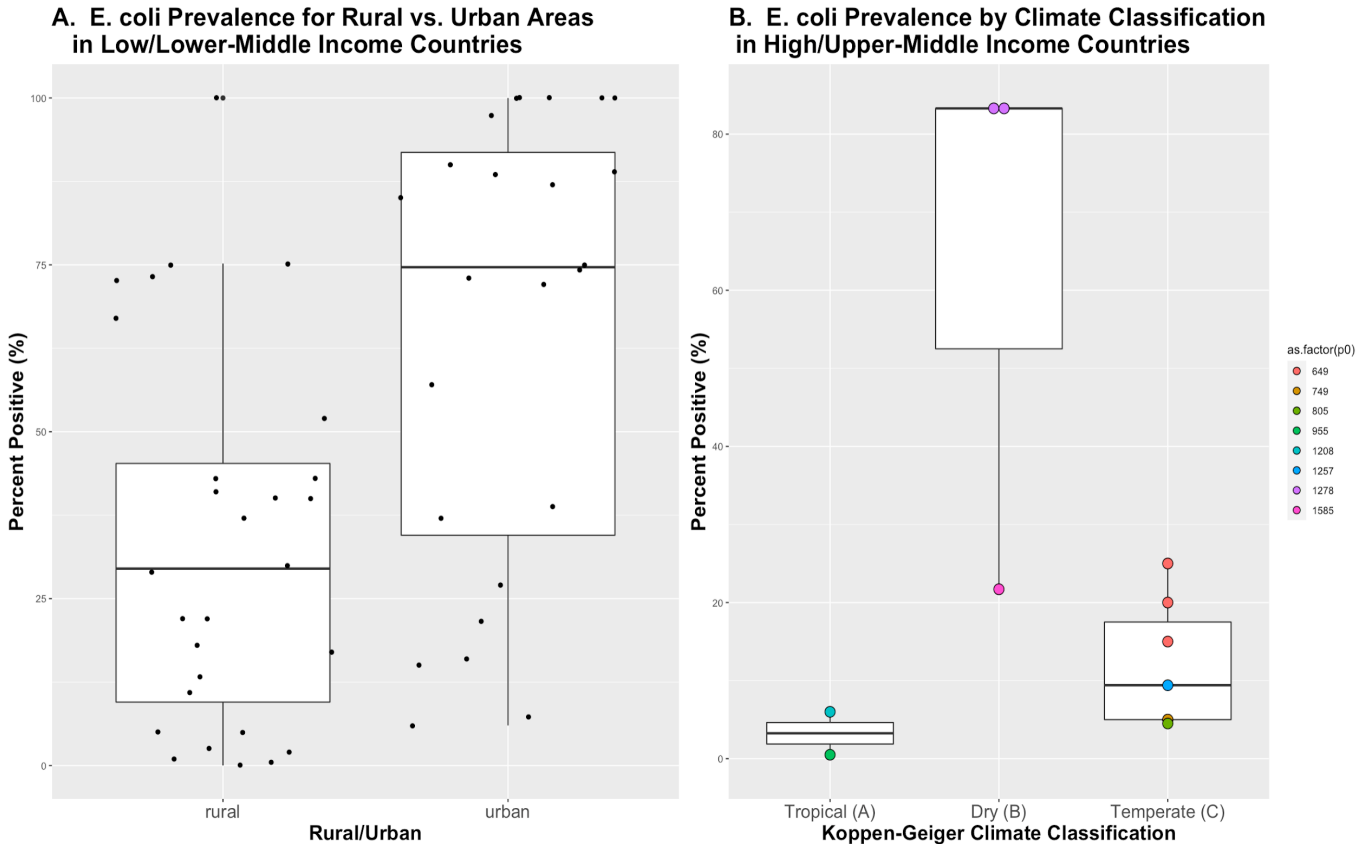

Figure 5: Subgroups with statistically significant differences in *E. coli* prevalence. A) *E. coli* prevalence for rural vs. urban areas within low/lower-middle income countries (n = 56 observations, from 36 studies); B) *E. coli* prevalence by climate classification within high/upper-middle income countries, colored by study number (n = 12 observations, from 8 studies)

For *E. coli* concentration levels, the only subgroup that showed significant differences was rural/urban. Urban areas had significantly higher *E. coli* concentrations levels than rural areas (2.26 $\log_{10}$CFU/hand mean concentration in urban versus 0.75 $\log_{10}$CFU/hand in rural, $p=5.5x10^{-6}$), as shown in Figure 6.

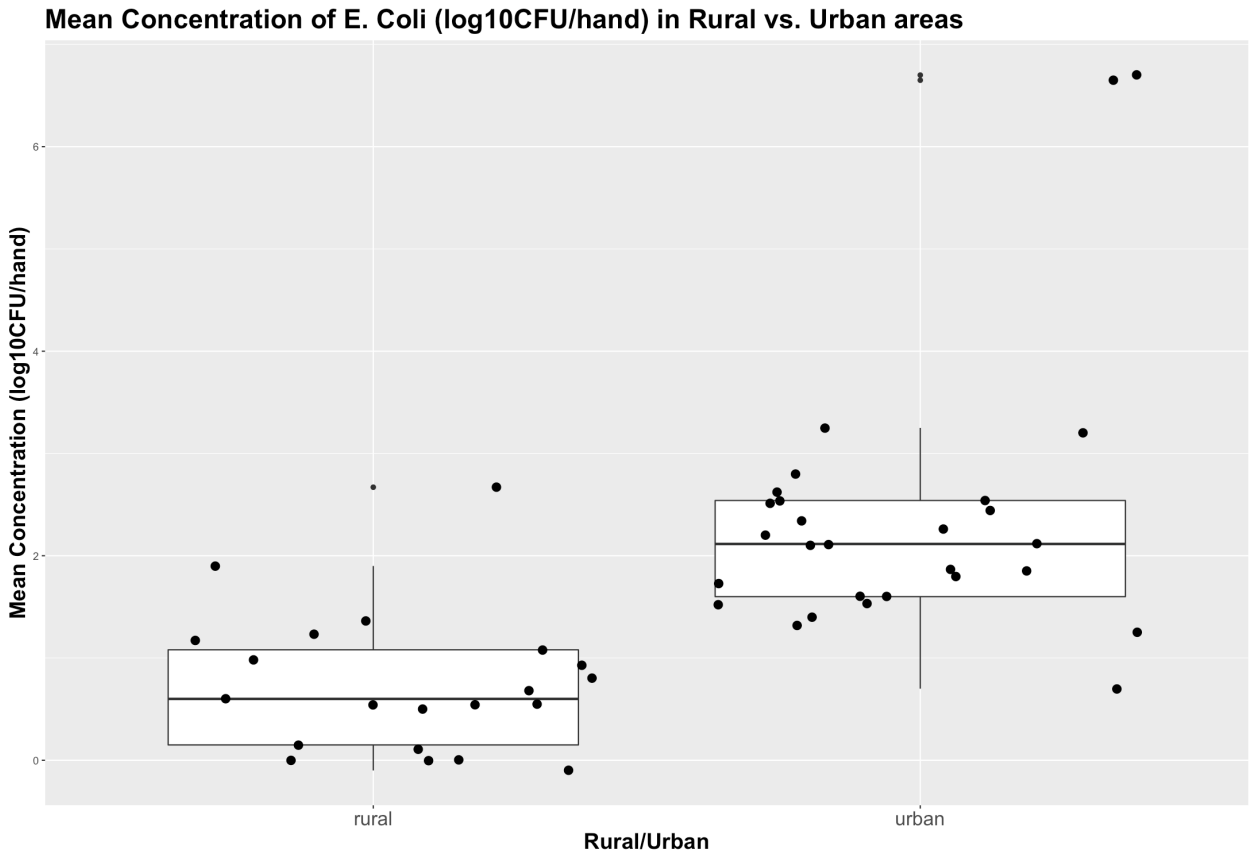

Figure 6: Mean concentration of *E. coli* in Rural vs. Urban areas (log10CFU per hand) (n = 51 observations, from 32 studies)

We approached the gender subgroup analysis differently because, as mentioned, there were few studies that reported gender. Three studies reported prevalence values for males and females and 4 additional studies reported the general comparison between genders without qualitative data. Six of these studies looked at school children. Within these 6 studies, 4 found that male students exhibited higher levels of FIB, one found that females had higher levels of FIB, and one found no statistical difference between boys and girls. The 7th study was of the

hands of adult commuter in the UK, which showed that overall prevalence was similar between men and women.

Contamination comparisons for fecal coliforms

Within the analysis of the FC data, t-tests showed that low/lower-middle income countries had significantly higher FC prevalence levels than high/upper-middle income countries (64.8% mean in low-income versus 35.0% in high-income $p = 0.0074$), as shown in Figure 7A. Additionally, there were statistically significant differences within the climate classification subgroup. Low/lower-middle income studies were in tropical areas (classification A, 7 studies) and dry areas (classification B, 2 studies), while high/upper-middle income studies were in dry areas (classification B, 3 studies), temperate areas (classification C, 7 studies), and continental areas (classification D, 2 studies). In the low/low-middle income group, there was not a significant difference in FC prevalence levels ($p = 0.08$). In the high/upper-middle income group, there were significant differences between temperate and dry areas (19.2% mean prevalence in temperate versus 53.5% in dry, $p = 0.04$), as well as temperate and continental areas (38% mean prevalence in continental, $p = 0.03$), shown in Figure 7B.

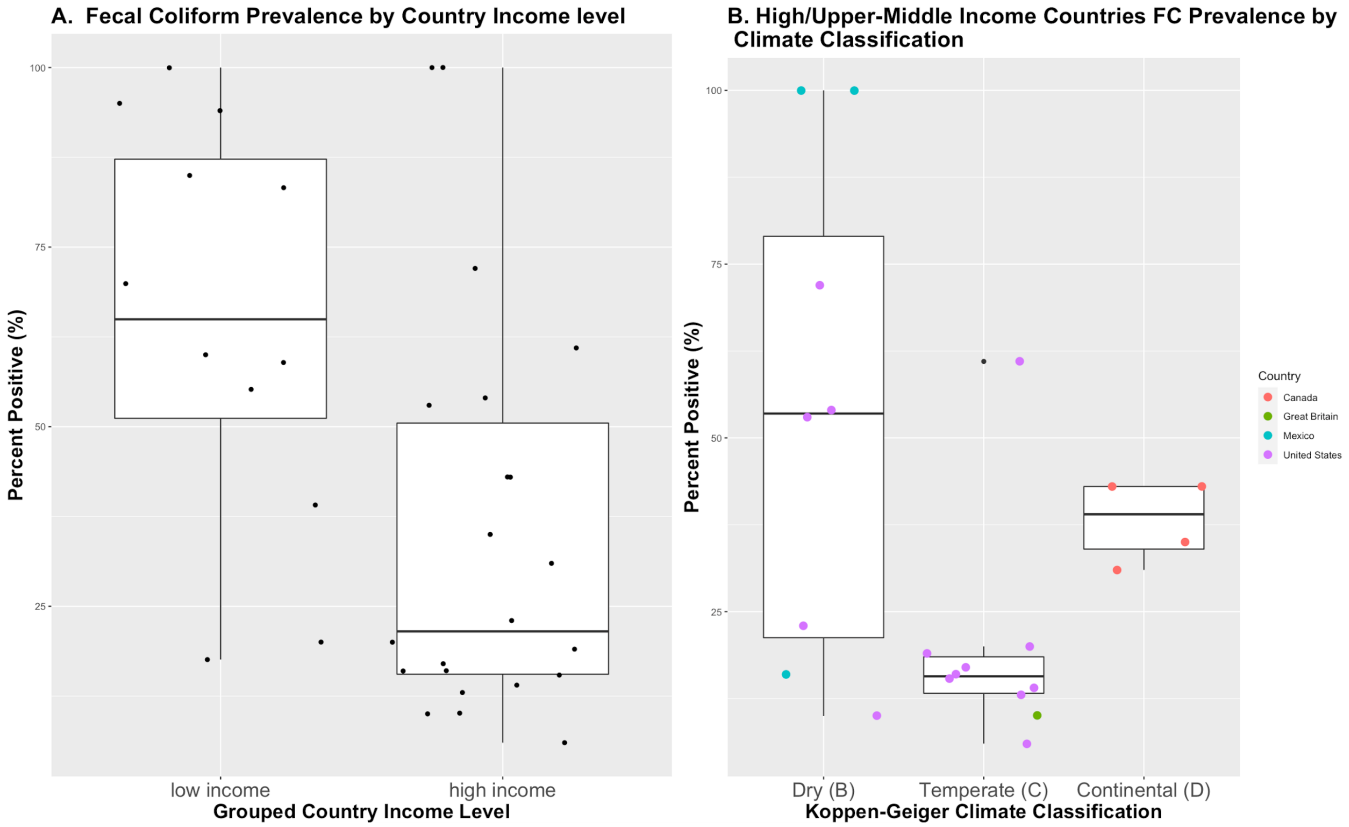

Figure 7: Subgroups with statistically significant differences in fecal coliform prevalence. A) FC prevalence by country income level (n = 34 observations, from 19 studies); B) FC prevalence by climate classification within high/upper-middle income countries (n = 22 observations, from 11 studies)

Similar to the *E. coli* prevalence analysis, FC prevalence did not show statistical significance between rural and urban areas when analyzing the raw data. Since income levels were a significant factor for FC prevalence as well, we conducted rural/urban analysis while controlling for income. This showed that all of the samples from rural areas were from low/lower-middle income countries and almost all of the sample from urban areas were from upper-middle/high income countries. Therefore, no analysis could be conducted in regards to urban/rural FC prevalence comparisons since this subgroup aligned with the income level subgroup. There were not significant differences in FC prevalence in between adults and children, even when specifically comparing adults with children under five-years-old.

Additionally, there were no statistically significant differences between FC contaminations levels in any of the subgroups.

Detection methods

In the 78 studies, 46 studies (59%) used hand rinse samples, 19 studies (24%) used swab samples, and 12 studies (15%) used impressions. Only one study did not report the detection method used for sampling hand contamination. Rinse samples had significantly higher prevalence than swab samples for *E. coli* prevalence and significantly higher prevalence than impression samples for FC prevalence ($p = 0.001$ and 2.3×10^{-5}, respectively). Therefore, the hand rinse method may be more sensitive of a sampling method than swabs and impressions.

We planned to examine comparable studies containing hand contamination values for school children in low and high-income countries. However, there were an insufficient number of studies for such an analysis. There were seven studies that reported *E. coli* prevalence on school children's hands in low/lower-middle income countries, but only one study in an upper-middle income setting. No studies reported fecal coliform prevalence for school children and the other indicators used also produced limited data.

Discussion

We reviewed studies that measured FIB and enteric pathogens on hands to describe the prevalence and level of contamination in many different global locations (Odonkor & Ampofo, 2013; US EPA, 2015). Prevalence of *E. coli* and fecal coliforms were fairly high among the study populations, 43.8% and 45.6% respectively. Previous studies have shown that hands, "play a pivotal role in fecal microbe transfer, linking environmental sources to oral ingestion" (Wang et al., 2017) and have associated *E. coli* concentration on hands with increased diarrhea

incidence among children (Pickering et al., 2018). Given the high observed prevalence rates, and the likely contributions of hand contamination to diarrheal disease, hands may play an important role in transmission of fecal bacteria and enteric pathogens.

A previous systematic analysis found that primary intervention strategies to reduce diarrhea incidence are necessary to continue to improve the global burden of diarrheal disease (Troeger et al., 2018). Our finding that hands may play an important role in transmission of fecal bacteria and enteric pathogens highlights the significant need of primary interventions, such as handwashing, that focus on decreasing hand contamination.

It is important to note that there has not been research to determine the optimal fecal indicators for measuring hand contamination. As a result, most studies rely on indicators, such as fecal coliforms and *E. coli*, which are commonly used in water sampling to indicate whether other potentially harmful bacteria may be present in drinking water (US EPA, 2015). There are two factors that have led to *E. coli* being the preferred detection method for fecal contamination in drinking water and other matrices: "first, the finding that some fecal coliforms were not fecal in origin, and second, the development of improved testing methods for *E. coli*" (Odonkor & Ampofo, 2013).

Country income significantly influences hand contamination

As we expected, low income countries have significantly higher prevalence of *E. coli* and fecal coliforms than high income countries. One factor that may be contributing to the observed higher prevalence levels in low income countries is lack of access to water, sanitation, and hygiene (WASH), including hand washing facilities with soap and water. Access to handwashing facilities is strongly related to sociodemographic index (SDI; a composite measure including income per capita, education, and fertility) (Brauer et al., 2020). A surprising finding in this

subgroup was that high-income countries also had relatively high prevalence levels with 23% mean prevalence for *E. coli* and 35% mean prevalence for fecal coliforms.

It is important to recognize the inherent limitations of using the World Bank Country Income classifications, which are based solely on gross national income per capita. Critics of this classifications system have said "it produces results that do not reflect real-world situations," since it does not account for issues such as inequality, human development, and government capacity (*Do World Bank Country Classifications Hurt the Poor?*, n.d.). The cut-offs between classifications also mean that there could be a $1 GNI difference between countries in one category and another. As a result, we focused our analysis on two groupings: low/lower-middle and upper-middle/high income. Additionally, future research could also incorporate more specific income levels within countries, such as community income levels.

Urban areas have higher levels of hand contamination

Comparison of hand contamination between urban and rural settings could only be conducted for low/lower-middle income countries because there were no studies in rural areas in upper-middle/high income countries. In low/lower-middle income countries, there were 19 studies in rural settings and 22 studies in urban settings that reported *E. coli* contamination. *E. coli* prevalence was higher in urban areas than in rural areas. Various factors could have caused urban or rural areas to have higher contamination. Educational disparities are common between urban and rural areas, with rural areas underperforming their urban counterparts (Zhang, 2006). Studies have shown that children whose parents have a higher education level, will have lower prevalence of fecal contamination on their hands (Kyriacou et al., 2009). Additionally, more people in urban areas have piped water systems, which could decrease hand contamination (UN-Water, n.d.). Studies have also shown that animal ownership, including livestock and domestic

animals, contributes to fecal contamination (Ercumen et al., 2017). Animal ownership is common in low/lower-middle income countries, but the impact of animal ownership in urban compared to rural areas on fecal contamination is not yet fully understood (Penakalapati et al., 2017). Moreover, population density may be correlated with higher rates of fecal contamination as shown in a drinking water study in Egypt (Fakhr et al., 2016). However, a study in Guatemala found that population density was not a key determinant in risk of enteric infection, which is likely associated with environmental contamination (Jarquin et al., 2016).

Additionally, country-level or city-level data can mask further disparities within urban areas, where the urban poor may fare similarly to rural communities (*Urban and Rural Disparities Remain Despite Progress in Closing Health and Development Gaps – Population Reference Bureau*, n.d.). For instance, Kibera, a slum within Nairobi, Kenya is an area included in this dataset. Other studies included likely represented a range of socioeconomic status in their study populations as well. Therefore, this is another area where it would be beneficial to factor community income levels into future research.

Only one of the studies reported prevalence for both urban and rural areas. This study, which was conducted in India and did not test for significance, found higher levels of *E. coli* prevalence on adult hands in urban areas, but higher levels FC prevalence on adult hands in rural areas. Further research is needed to understand hand contamination variations between urban and rural settings.

Climate may influence hand contamination

There were no significant differences based on climate classifications in low/lower-middle income countries, but there were in high/upper-middle income countries. Both *E. coli* and FC prevalence were significantly lower in temperate areas (classification C) than in dry areas

(classification B) for high/upper-middle income countries. Additionally, FC prevalence was also significantly lower in temperate areas than in continental areas (classification D). There has not yet been research published on the impact temperature and humidity have on fecal indicator bacteria on hands. However, our results showed that temperature may influence contamination levels. For *E. coli* prevalence, both studies in dry areas were also categorized as warm climates (BSh sub classifications) where the average annual temperature is greater than or equal to 18°C. While, the studies in temperate areas had varying levels of precipitation with the warmest month of the year averaging greater than 22°C (these studies were Cfb and Csc sub classifications). Therefore, consistently warm, dry climate may provide a better environment for *E. coli* on hands than a milder climate. This trend was also seen in the FC prevalence analysis. There were warm and dry sub classifications within the dry area studies, but warm, dry studies increased the prevalence in this group significantly compared to temperate areas. It is important to note that these climate classifications did not consider seasonal variation for time of sampling. In temperate areas, for examples, temperature and precipitation vary significantly throughout the year. Therefore, seasonality, including temperature and precipitation of the location at the time of sampling, should be considered in further research.

Adults and children have similar levels of hand contamination

Surprisingly, there was no clear trend between children and adult prevalence or concentrations, even when comparing children under 5 to adults. A potential explanation is that hand contamination reflects environmental contamination more than hygiene behavior. Moreover, similar levels of contamination between adults and children could be a result of how rapidly hands are contaminated after washing and how difficult they are to keep clean, which

have been found in previous studies of mothers in Bangladesh and Tanzania (Pickering et al., 2011; Ram et al., 2011).

Within this review, 17 studies (22%) reported both adult and child hand contamination. These 17 studies, similar to our overall review, did not show a clear trend of either adults or children having higher hand contamination levels. The indicators used in these studies were *E. coli* prevalence and concentration, FC prevalence and concentration, and total coliform. Looking at all of these indicators, 5 studies reported children with higher levels and 12 studies reported adults with higher levels.

One interesting finding is that all 6 of the studies that reported *E. coli* prevalence showed adults with higher prevalence than children. However, of the 8 studies that reported FC prevalence, 4 showed children with higher levels, 3 showed adults with higher levels, and 1 changed depending on which child age group was being compared to adults (infants had the highest levels, followed by adults, then toddlers, then 4-year-olds). These results support the overall finding that there is no clear difference in hand contamination levels between adults and children, but also suggest that child hand contamination by age may be an interesting focus for future research. Moreover, the prevalence difference between groups was often fairly small. Eleven of the 14 studies that reported prevalence of *E. coli* or FC had a prevalence difference of 15% or less between adults and children. There were two studies that reported concentration levels, one for *E. coli* and one for FC, and both reported adults with higher concentration levels.

It is important to note that all 17 of these studies reported contamination for mothers, caregivers, day care center workers, or teachers (4 studies, 6 studies, 6 studies, and 1 study, respectively) as well as the children that they cared for or taught. Since the adults were caring for the children in most of these scenarios, it was likely that adults were frequently in contact with

the same objects as the children. This supports the explanation that hand contamination reflects environmental contamination.

Hand contamination is rarely reported by gender

Any analysis on hand contamination between gender groups was limited due to the small number of studies that reported on gender. However, 4 out of the 6 school studies showing boys with higher levels of hand contamination than girls suggests that this could be a trend. This could suggest that girls have better hand hygiene behaviors than boys or it could be a result of boys playing in sports and/or in soil more often than girls. There was not much literature on children hand contamination or hygiene behavior, but there have been a few studies comparing hand hygiene behaviors between men and women. A 2003 study on a university campus found that females washed their hands more often than males, 61% to 37% (Johnson et al., 2003). A study in US airports found the same result, 83% of females versus 74% of males washed their hands (*Another US Airport Travel Hazard - Dirty Hands*, n.d.). This could suggest that hygiene habits in between genders is similar in children and adults. However, much more research is necessary to have a better understanding of potential differences. For example, hand contamination studies with results reported by gender group could be conducted of girls and boys in households and schools and of women and men at home and in their places of workplace.

Rinse may be the most sensitive hand sampling method to FIB

As presented earlier, the review showed that hand rinse samples may be more sensitive to fecal indicator bacteria than impression or swab samples. Studies on other types of transmission pathways, including food items such as broiler carcasses at slaughter, have shown that rinse methods have had higher recovery of *E. coli* than swab methods (Nagel Gravning et al., 2021). Other methods to estimate dermal exposure of hands, including interception methods, such as

cotton gloves, and fluorescent tracer techniques, would not have been appropriate for this review because they require artificial contamination (Ng et al., 2013).

Limitations

This study had a few limitations. First, we had to convert MPN to CFU for the concentration synthesis. As mentioned earlier, MPN can have 'somewhat higher' estimates than CFU, so this equivalence assumption could have increased CFU concentrations slightly. Since all 7 studies that reported concentration in units of MPN were in low/lower-middle income countries, treating MPN as equivalent to CFU may have slightly increased mean concentrations in this group. Another limitation is the quality of the underlying data set, which has limited breadth due to biases in the type of studies conducted. For example, there were few studies on male adults and not many studies on children aggregated by gender. There was also only one study in rural high-income countries, which meant the urban/rural subgroup could only be analyzed in low-income settings. However, limitations in this study were minimal because we followed PRISMA-P guidelines, which facilitated the preparation and reporting for this systemic review and allowed for greater transparency in the research process.

Conclusion

Hands are frequently contaminated with fecal bacteria, and contamination levels are influenced by country-level income, urban/rural, and potentially climate. This review showed that hands are an especially critical pathway in low/lower-middle income countries and particularly in urban settings. Further research should be conducted to understand potential relationships between climate and hand contamination; and, seasonality, including temperature and precipitation at the time of sampling, needs to be considered. Additionally, a review similar

to this one, but of respiratory pathogens on hands, such as rhinovirus and SARS-CoV-2, would be another valuable addition to the research record.

In terms of policy implications, this review highlights the importance of studying hand contamination and conducting hand hygiene interventions in countries of all income levels, since prevalence levels in upper-middle/high income countries were around 20-35%. Hand hygiene interventions typically focus on caregivers in low-income settings, yet our results suggest that interventions aiming to reduce enteric pathogen transmission should also focus on child hand contamination.

References

Another US airport travel hazard—Dirty hands. (n.d.). EurekAlert! Retrieved December 5, 2020, from http://www.eurekalert.org/pub_releases/2003-09/asfm-aua091103.php

Barker, J., & Jones, M. V. (2005). The potential spread of infection caused by aerosol contamination of surfaces after flushing a domestic toilet. *Journal of Applied Microbiology*, *99*(2), 339–347. https://doi.org/10.1111/j.1365-2672.2005.02610.x

Bing-Yuan, Zhang, Y.-H., Leung, N. H. L., Cowling, B. J., & Yang, Z.-F. (2018). Role of viral bioaerosols in nosocomial infections and measures for prevention and control. *Journal of Aerosol Science*, *117*, 200–211. https://doi.org/10.1016/j.jaerosci.2017.11.011

Brauer, M., Zhao, J. T., Bennitt, F. B., & Stanaway, J. D. (2020). Global Access to Handwashing: Implications for COVID-19 Control in Low-Income Countries. *Environmental Health Perspectives*, *128*(5). https://doi.org/10.1289/EHP7200

Buckalew, D. W., Hartman, L. J., Grimsley, G. A., Martin, A. E., & Register, K. M. (2006). A long-term study comparing membrane filtration with Colilert® defined substrates in detecting fecal coliforms and Escherichia coli in natural waters. *Journal of Environmental Management*, *80*(3), 191–197. Scopus. https://doi.org/10.1016/j.jenvman.2005.08.024

Do World Bank Country Classifications Hurt the Poor? (n.d.). Retrieved January 1, 2021, from https://www.globalpolicy.org/social-and-economic-policy/the-three-sisters-and-other-institutions/the-world-bank/51412-do-world-bank-country-classifications-hurt-the-poor.html

Eisenberg, J. N. S., Trostle, J., Sorensen, R. J. D., & Shields, K. F. (2012). Toward a Systems Approach to Enteric Pathogen Transmission: From Individual Independence to Community Interdependence. *Annual Review of Public Health*, *33*(1), 239–257. https://doi.org/10.1146/annurev-publhealth-031811-124530

Ercumen, A., Pickering, A. J., Kwong, L. H., Arnold, B. F., Parvez, S. M., Alam, M., Sen, D., Islam, S., Kullmann, C., Chase, C., Ahmed, R., Unicomb, L., Luby, S. P., & Colford, J. M. (2017). Animal Feces Contribute to Domestic Fecal Contamination: Evidence from E. coli Measured in Water, Hands, Food, Flies, and Soil in Bangladesh. *Environmental Science & Technology*, *51*(15), 8725–8734. https://doi.org/10.1021/acs.est.7b01710

Exposure Factors Handbook—Chapter 7: Dermal Exposure Factors. (2011). 77.

Fakhr, A. E., Gohar, M. K., & Atta, A. H. (2016). Impact of Some Ecological Factors on Fecal Contamination of Drinking Water by Diarrheagenic Antibiotic-Resistant Escherichia coli in Zagazig City, Egypt. *International Journal of Microbiology*, *2016*. https://doi.org/10.1155/2016/6240703

Goddard, F. G. B., Pickering, A. J., Ercumen, A., Brown, J., Chang, H. H., & Clasen, T. (2020). Faecal contamination of the environment and child health: A systematic review and individual participant data meta-analysis. *The Lancet Planetary Health*, *4*(9), e405–e415. https://doi.org/10.1016/S2542-5196(20)30195-9

Gronewold, A. D., & Wolpert, R. L. (2008). Modeling the relationship between most probable number (MPN) and colony-forming unit (CFU) estimates of fecal coliform concentration. *Water Research*, *42*(13), 3327–3334. https://doi.org/10.1016/j.watres.2008.04.011

Jarquin, C., Arnold, B. F., Muñoz, F., Lopez, B., Cuéllar, V. M., Thornton, A., Patel, J., Reyes, L., Roy, S. L., Bryan, J. P., McCracken, J. P., & Colford, J. M. (2016). Population Density, Poor Sanitation, and Enteric Infections in Nueva Santa Rosa, Guatemala. *The American Journal of Tropical Medicine and Hygiene*, *94*(4), 912–919. https://doi.org/10.4269/ajtmh.15-0555

Johnson, H. D., Sholcosky, D., Gabello, K., Ragni, R., & Ogonosky, N. (2003). Sex Differences in Public Restroom Handwashing Behavior Associated with Visual Behavior Prompts. *Perceptual and Motor Skills*, *97*(3), 805–810. https://doi.org/10.2466/pms.2003.97.3.805

Julian, T. R. (2016). Environmental transmission of diarrheal pathogens in low and middle income countries. *Environmental Science: Processes & Impacts*, *18*(8), 944–955. https://doi.org/10.1039/C6EM00222F

Kosek, M., Bern, C., & Guerrant, R. L. (2003). The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. *Bulletin of the World Health Organization*, *81*(3), 197–204.

Kyriacou, A., Drakopoulou, S., Georgaki, I., Fountoulakis, M., Mitsou, E., Lasaridi, K. E., Manios, Y., & Manios, T. (2009). Screening for faecal contamination in primary schools in Crete, Greece. *Child: Care, Health and Development*, *35*(2), 159–163. https://doi.org/10.1111/j.1365-2214.2008.00911.x

Mattioli, M. C. M., Davis, J., & Boehm, A. B. (2015). Hand-to-Mouth Contacts Result in Greater Ingestion of Feces than Dietary Water Consumption in Tanzania: A Quantitative Fecal

Exposure Assessment Model. *Environmental Science & Technology*, *49*(3), 1912–1920. https://doi.org/10.1021/es505555f

Mattioli, M. C., Pickering, A. J., Gilsdorf, R. J., Davis, J., & Boehm, A. B. (2013). Hands and water as vectors of diarrheal pathogens in Bagamoyo, Tanzania. *Environmental Science & Technology*, *47*(1), 355–363. https://doi.org/10.1021/es303878d

Nagel Gravning, G. E., Røtterud, O.-J., Bjørkøy, S., Forseth, M., Skjerve, E., Llarena, A.-K., Lian, A., Johannessen, G. S., & Hauge, S. J. (2021). Comparison of four sampling methods for microbiological quantification on broiler carcasses. *Food Control*, *121*, 107589. https://doi.org/10.1016/j.foodcont.2020.107589

Naghavi, M., Abajobir, A. A., Abbafati, C., Abbas, K. M., Abd-Allah, F., Abera, S. F., Aboyans, V., Adetokunboh, O., Afshin, A., Agrawal, A., Ahmadi, A., Ahmed, M. B., Aichour, A. N., Aichour, M. T. E., Aichour, I., Aiyar, S., Alahdab, F., Al-Aly, Z., Alam, K., … Murray, C. J. L. (2017). Global, regional, and national age-sex specific mortality for 264 causes of death, 1980–2016: A systematic analysis for the Global Burden of Disease Study 2016. *The Lancet*, *390*(10100), 1151–1210. https://doi.org/10.1016/S0140-6736(17)32152-9

Ng, M. G., Poot, S. de, Schmid, K., Cowie, H., Semple, S., & Tongeren, M. van. (2013). A preliminary comparison of three dermal exposure sampling methods: Rinses, wipes and cotton gloves. *Environmental Science: Processes & Impacts*, *16*(1), 141–147. https://doi.org/10.1039/C3EM00511A

Noble, R. T., Weisberg, S. B., Leecaster, M. K., McGee, C. D., Ritter, K., Walker, K. O., & Vainik, P. M. (2003). Comparison of beach bacterial water quality indicator measurement methods. *Environmental Monitoring and Assessment*, *81*(1–3), 301–312. Scopus. https://doi.org/10.1023/A:1021397529041

Odonkor, S. T., & Ampofo, J. K. (2013). Escherichia coli as an indicator of bacteriological quality of water: An overview. *Microbiology Research*, *4*(1), e2–e2. https://doi.org/10.4081/mr.2013.e2

Penakalapati, G., Swarthout, J., Delahoy, M. J., McAliley, L., Wodnik, B., Levy, K., & Freeman, M. C. (2017). Exposure to Animal Feces and Human Health: A Systematic Review and Proposed Research Priorities. *Environmental Science & Technology*, *51*(20), 11537–11552. https://doi.org/10.1021/acs.est.7b02811

Pickering, A. J., Ercumen, A., Arnold, B. F., Kwong, L. H., Parvez, S. M., Alam, M., Sen, D., Islam, S., Kullmann, C., Chase, C., Ahmed, R., Unicomb, L., Colford, J. M., & Luby, S. P. (2018). Fecal Indicator Bacteria along Multiple Environmental Transmission Pathways (Water, Hands, Food, Soil, Flies) and Subsequent Child Diarrhea in Rural Bangladesh. *Environmental Science & Technology*, *52*(14), 7928–7936. https://doi.org/10.1021/acs.est.8b00928

Pickering, A. J., Julian, T. R., Mamuya, S., Boehm, A. B., & Davis, J. (2011). Bacterial hand contamination among Tanzanian mothers varies temporally and following household activities. *Tropical Medicine & International Health: TM & IH*, *16*(2), 233–239. https://doi.org/10.1111/j.1365-3156.2010.02677.x

PRISMA-P-checklist.pdf. (n.d.). Retrieved December 30, 2020, from http://www.prisma-statement.org/documents/PRISMA-P-checklist.pdf

Ram, P., Jahid, I., Halder, A., Nygren, B., Islam, S., Granger, S., Molyneaux, J., & Luby, S. (2011). Variability in Hand Contamination Based on Serial Measurements: Implications for Assessment of Hand-Cleansing Behavior and Disease Risk. *The American Journal of Tropical Medicine and Hygiene*, *84*, 510–516. https://doi.org/10.4269/ajtmh.2011.10-0299

Society, N. G. (2019, October 24). *Köppen Climate Classification System*. National Geographic Society. http://www.nationalgeographic.org/encyclopedia/koppen-climate-classification-system/

Troeger, C., Blacker, B. F., Khalil, I. A., Rao, P. C., Cao, S., Zimsen, S. R., Albertson, S. B., Stanaway, J. D., Deshpande, A., Abebe, Z., Alvis-Guzman, N., Amare, A. T., Asgedom, S. W., Anteneh, Z. A., Antonio, C. A. T., Aremu, O., Asfaw, E. T., Atey, T. M., Atique, S., … Reiner, R. C. (2018). Estimates of the global, regional, and national morbidity, mortality, and aetiologies of diarrhoea in 195 countries: A systematic analysis for the Global Burden of Disease Study 2016. *The Lancet Infectious Diseases*, *18*(11), 1211–1228. https://doi.org/10.1016/S1473-3099(18)30362-1

UN-Water. (n.d.). Urbanization. *UN-Water*. Retrieved November 13, 2020, from https://www.unwater.org/water-facts/urbanization/

Urban and Rural Disparities Remain Despite Progress in Closing Health and Development Gaps – Population Reference Bureau. (n.d.). Retrieved November 11, 2020, from https://www.prb.org/urban-rural-datasheet/

US EPA, O. (2015, November 30). *National Primary Drinking Water Regulations* [Overviews and Factsheets]. US EPA. https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations

Wagner, E. G., Lanoix, J. N., & Organization, W. H. (1958). *Excreta disposal for rural areas and small communities*. World Health Organization. https://apps.who.int/iris/handle/10665/41687

Walker, C. L. F., Rudan, I., Liu, L., Nair, H., Theodoratou, E., Bhutta, Z. A., O'Brien, K. L., Campbell, H., & Black, R. E. (2013). Global burden of childhood pneumonia and diarrhoea. *The Lancet*, *381*(9875), 1405–1416. https://doi.org/10.1016/S0140-6736(13)60222-6

Wang, Y., Moe, C. L., Null, C., Raj, S. J., Baker, K. K., Robb, K. A., Yakubu, H., Ampofo, J. A., Wellington, N., Freeman, M. C., Armah, G., Reese, H. E., Peprah, D., & Teunis, P. F. M. (2017). Multipathway Quantitative Assessment of Exposure to Fecal Contamination for Young Children in Low-Income Urban Environments in Accra, Ghana: The SaniPath Analytical Approach. *The American Journal of Tropical Medicine and Hygiene*, *97*(4), 1009–1019. https://doi.org/10.4269/ajtmh.16-0408

World Bank Country and Lending Groups – World Bank Data Help Desk. (n.d.). Retrieved October 30, 2020, from https://datahelpdesk.worldbank.org/knowledgebase/articles/906519

Zhang, Y. (2006). Urban-Rural Literacy Gaps in Sub-Saharan Africa: The Roles of Socioeconomic Status and School Quality. *Comparative Education Review*, *50*(4), 581–602. https://doi.org/10.1086/507056