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Characterizing the microbial load on the hands of children and students: a Systematic Review with Meta-Analysis

By

Joel S. Espinoza
Degree to be awarded: MSPH

Epidemiology

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Bachelor of Science
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2021

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Abstract

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By Joel Espinoza

Hands play a critical role in infectious disease transmission. However, proper hand hygiene behaviors and practices do well to mitigate the spread of pathogens via hands. The presence of microbes on hands can vary by these practices which, in turn, vary across populations based on a combination of environmental and individual-level factors. Furthermore, children and students are uniquely susceptible to hand-mediated disease transmission, yet measures of the microbial load on the hands of children and students remain understudied. This study aims to describe the existing quantifications of microbial loads on the hands of children and students globally, and to assess the relationship between the microbial load on their hands and the setting the samples were collected. Initially, 1370 journal articles were abstracted across three scientific databases which ultimately yielded nineteen studies that met our screening criteria of providing a numeric concentration of the microbial load measured directly from the hands of children and students. A meta-analysis using concentrations from seven of the nineteen articles was conducted to evaluate the association between the microbial load on the hand of a child or student and having been sampled in an outdoor setting compared to an indoor setting. Descriptive analyses of the eighty-nine measurements of the microbial load derived from the nineteen articles included with this review revealed bacteria were the most commonly measured organism comprising 85% of identified measures. These concentrations of the microbial loads, however, appeared to vary greatly across many different microorganisms: bacteria ranged from [0-8.6] log10 CFU per two hands, helminths [0-385] Eggs per two hands, and one protozoa [0-58.3] cysts per 2 hands. Logistic regression analyses found a statistically significant increase ($\beta_1 = 2.27$, 95% CI [0.21, 4.23], $p = 0.0323$) in average log10 CFUs per 2 hands contaminated with generic *E. coli* that were sampled outside compared to those sampled indoors. The results of this study may inform future cross-sectional studies quantifying the microbial load on the hands of children and students, and the meta-analysis indicates that further investigation may yield greater insight into the association of setting with the microbial load.

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Chapter 1. Literature Review

1.1 The Role of Hands in Pathogen Transmission

Hands play a critical role in pathogen transmission, having contributed to deadly epidemics throughout human history [1]. *Yersinia pestis*, more commonly known as the Black Plague, eliminated one third of Europe's population in the 14th century often due to spreading body fluids from the infected via the hands of the uninfected [2]. Typhoid Mary was made infamous for her asymptomatic transmission of *Salmonella typhi* to wealthy families resulting from improper hand hygiene while preparing food at the turn of the 20th century [3]. In as recently as 2014, one of the largest outbreaks of norovirus infected over 700 individuals from a single cruise ship [4]. Pathogenic microorganisms such as these are often spread via hands and cause disease within their host [5]. These microbes can be taxonomically diverse, ranging from bacteria and viruses to helminths and protozoa—though all may be transmitted from the hands of infected and uninfected individuals alike [5].

The importance of hands along multiple transmission routes

Hands serve as an intermediary between a substance or source contaminated with a given pathogen and a portal of entry: often the eyes, nose, mouth, or break in skin [6,7]. The role[6] of hands may include direct contact with an infected individual, ingestion of contaminated food and water, and fomite mediated transmission [7].

Direct contact from the hands of an infected individual and an uninfected individual may lead to the exposed person developing disease. The hands of an uninfected individual may play a role in disease transmission when coming into contact with an infected individual's bodily fluids or pathogens living on the surface of an infected person's skin [8]. The direct transmission of disease

via hands has been well documented, particularly in literature relating to healthcare workers and their patients, often in the form of nosocomial infection [8,9]. One study investigated the presence of gram-negative bacteria among the hands of healthcare personal in the Neonatal Intensive Care Unit (NICU) and confirmed the presence of *E. coli*, *K. pneumoniae*, and *P. aeruginosa*. An extreme example of disease transmission via hands was observed during the Ebola outbreak of 2014 when recommendations were placed by the World Health Organization (WHO) providing emergency guidance for handling human remains in place of culturally and religiously linked burial rites [10]. These practices often involved direct contact and handling of an infected person's corpse [10]. This would result in exposure via post-mortem transmission by which intimate handling and washing of the corpse was associated with an increased risk of Ebola infection, resulting in future infections and death [11].

Hands also play a significant role in the spread of pathogens transmitted via the fecal-oral route. Improper handling of food with hands contaminated with fecal bacteria and enteric pathogens presents an opportunity for transmission along the fecal-oral route to occur [6,7]. Microscopic fecal matter may remain on the hands or under the fingernails of improperly washed hands. If pathogenic microorganisms are present, such as when shed through an infected individual's stool, they may be spread to a susceptible individual via the fecal-oral route and potentially cause disease [7]. Indeed, several outbreaks have been linked to improper food handling and inadequate hand hygiene specifically, including hepatitis A virus (HAV) and norovirus [12,13]. New developments have also revealed that pathogens primarily thought to spread via the inhalation of respiratory droplets, such as SARS-CoV-2 or COVID-19, can be shed through human stool and ultimately transferred to the hands [14]. Work investigating wastewater surveillance and stool sampling among individuals with

active COVID-19 continues to expand the knowledge and understanding of the role of hands in the transmission of SARS-CoV-2 through the fecal-oral route [14,15].

Fomite transmission occurs when hands come into contact with an inanimate surface that is contaminated by pathogens, often from a previously infected individual [7]. For example, a hospital patient with MRSA may transfer pathogens onto their clothes or their bed, or someone infected with HAV with improper hand hygiene may leave traces of the virus on a doorknob or table [7,9]. These surfaces can then serve as sources of exposure if touched by an uninfected person [7]. Some pathogens have the capacity to survive on surfaces outside of a human host for extended periods of time, increasing the likelihood of coming into contact with hands of a susceptible individual [7]. This ability depends upon a variety of intrinsic and environmental factors such as temperature, and exposure to UV light [7]. Persistence in the environment ultimately allows pathogenic microorganisms to be picked up by hands of unsusceptible individuals and enter then body through a portal of entry. One behavioral observational study conducted by the University of New South Wales found that on average, some individuals touched their face with their hands up to 23 times/hour [16]. When taken into the context of disease transmission via contaminated hands, each contact with the face holds the potential of the introduction of a pathogen to a portal of entry such as the eyes or mouth [7,16]. Although much is known about hand-related transmission routes, questions remain in quantifying the degree of hand contamination necessary to transmit a pathogen [8]. There also remains a gap in understanding how the quantity of pathogens on hands varies on an individual basis [8].

1.2 Hand Hygiene and the prevention of infection

Overview of hand hygiene practices

Hand hygiene practices are largely accepted as an effective means of infectious disease prevention [17]. Multiple interventions exist to reduce the likelihood of pathogen transmission via hands. The two most common hand hygiene practices globally include washing hands with soap and water and using alcohol-based hand sanitizers [17]. Both handwashing with soap and the use of hand sanitizers have proven efficacious in eliminating pathogens from hands and are most-often required among food service and healthcare workers [8,17]. Discrepancies do exist, however, as to which hand hygiene method may be more effective [17]. While some evidence has demonstrated a preference for traditional handwashing with soap and water, these assertions are often applied in the context of food handling [17]. In 2002, the Healthcare Infection Control Practices Advisory Committee (HICPAC) revised guidelines establishing the use of alcohol-based hand sanitizers as an effective hand hygiene standard of care for healthcare personnel [17,18]. Additionally, handwashing may only be effective depending on the exact method used [17]. The efficacy of handwashing may vary by the amount of soap used, the duration of time spent washing, the amount of friction applied on and between hands, and temperature of water used [8,16]. A study by Larson et al. found that even an increase from 1 mL of soap to 3mL used while washing one's hands could yield in several reductions in the log colony-forming units (CFUs) remaining on hands [19]. This finding reinforces the idea that improper handwashing techniques may prove ineffective in reducing the number of pathogens found on hands [8,19].

Barriers to achieving adequate hand hygiene

Hand hygiene is not uniformly perceived nor practiced throughout populations across the globe and is dependent on several structural and cultural factors. Attitudes surrounding hand hygiene practices differs across people of varying ages, genders, socio-economic status, ability, occupation,

and country-income level [20-23]. Structural factors, such as access to water, soaps, and sanitizers contribute to realized hand hygiene behaviors [20-23]. For example, one qualitative study interviewed individuals of varying ability in Malawi regarding barriers to accessing water, sanitation, and hygiene [20]. Investigators found interactions between societal expectations of women to fetch water, menstrual health, and ability to travel long distances as having the greatest impact on inaccessibility to WASH practices [20].

Social and cultural perceptions and attitudes surrounding hygiene also contribute to the realized hand hygiene behaviors [20-23]. Interestingly, two independent investigations into the attitudes towards handwashing among healthcare professionals in Iran and Türkiye both cited lack of time, lack of institutional incentive, and availability of handwashing stations and supplies as barriers despite the majority demonstrating an understanding of the role of hand hygiene in infection prevention [21,22]. Behaviors and perceptions varied still in a study of sixth-eighth grade children in Bogota, Colombia which found lack of adequate access to soap and perceived control over one's personal hygiene impacted their realized hand hygiene behavior [23]. Granted the multitude of factors that contribute to demonstrated hand hygiene, the combination of physical and social components of one's environment and their individual characteristics undeniably shape the circumstances by which one may be exposed to and infected with pathogen organisms [7].

1.3 Child and student susceptibility to hand-mediated diseases

Children and adolescents are at an increased risk of being exposed to and infected with diseases spread via hands, especially in low and middle income countries [24,25]. In 2013 alone, there were about 900,000 cases of Hand Foot and Mouth Disease (HFMD), a disease primarily impacting

young children in daycare facilities and primary schools [26]. A variety of factors ranging from diet to environment have been found to contribute not only to exposure to pathogenic organisms but also the experienced severity of a given illness among young children [24-26]. The combination of these factors play a critical role in overall child development such as through the development of the immune system through early exposure and resistance to infections [24,25]. One study in 2021 identified that among children, their unique contact patterns place them at a greater risk of being exposed to pathogens, mostly through prolonged and intense contacts with other children such as in schools [27].

Setting contributes to child exposure to pathogens

Schools and daycare facilities present a unique set of challenges in preventing disease transmission among children and students. In the United States, students spend over six hours a day on average (about 1200 hours per year) in the company of other students—time in which these children may be exposed to another infected student [29]. This frequent, repetitive, and prolonged exposure to potentially contagious classmates leaves schoolchildren increasingly vulnerable to a variety of the transmission dynamics described above [30]. Students' likelihood of exposure is then increased by the amount of time spent in contact with other children in and outside of the classroom and by contact with fomites (such as doorknobs, desks, and school supplies) [28,30]. Even when compared with other settings such as offices and nursing homes, prevention measures such as regular sanitation of open surfaces were not found to be as effective among children in schools and daycare centers [31]. Enteric pathogens and hand foot and mouth disease, for example, have all been found to be easily transmissible in these particular environments via fomite transmission and via the fecal-oral route [27,28,30]. HFMD—so named for its common transmission route and for the

expression of its symptoms—is highly contagious [26]. In 2018, multiple related outbreaks of HFMD even occurred among young adult students enrolled across multiple universities in the United States [32]. These outbreaks were found to have transmitted both through direct contact with infected individuals and through hand-mediated transmission via common surfaces in shared living spaces. Infectious disease outbreaks including HFMD, mumps, and norovirus have been found to occur throughout college campuses with multiple studies citing their unique social networks, hand-fomite transmission within shared living spaces, and frequency of person-to-person contacts as factors contributing to the risk exposure to infectious diseases [32-34].

Assessing exposure to pathogens spread among children via hands

Understanding the quantified measurement of pathogens to which hands are exposed to plays an essential role in understanding child and student susceptibility to infectious disease. When considering the hand-mediated transmission dynamics among this population, one must be able to ascertain whether exposure to infectious pathogens crosses the threshold of an individual's infectious dose [35]. Methods to quantify the microbial load vary and may depend upon the microorganism one wishes to quantify. Bacteria are most often enumerated via culture to produce "colony forming units" or CFUs while for some larger parasites, the number of eggs or cysts may simply be counted using light microscopy. Oftentimes, infectious viral load (VL)—with the limitation that this measure only extends to viruses—is quantified from a nasopharyngeal swab or via stool sample in the instance of viral diarrheas, but is not often tested nor reported on the hands of child and adult subjects alike [35]. Many studies also use proxies to estimate the pathogenic load on hands, often in the form of RNA load rather than viral particles in instances of viruses due to the difficulty of quantifying focus forming units [35,36]. Previous literature has also been found to

collapse quantified measures of the microbial load into categories such as low vs high without reporting the individual numeric quantity of pathogens found on hands [35].

Therefore, it is critical to gain an understanding of the measured quantification and characterization of the microbial and potentially pathogenic load on the hands of children, adolescents, and any young adult that engages or will engage in person-to-person interactions in a shared space such as a daycare facility, school, or university.

1.4 Need Statement

It is necessary to summarize the quantifiable microbial load and to identify factors associated with pathogen transmission via hands among children and students across the world.

1.5 Goal Assessment and Significance

The goal of this project is to describe the existing quantifications of microbial loads on the hands of children and students globally, and to assess the relationship between the microbial load on their hands and the setting the samples were collected.

The results of this study may highlight the existing knowledge gaps in the classes of microorganisms which have been historically quantified on hands, for example, if bacteria have been sampled and reported more frequently than viruses or helminths. This thesis can identify inconsistencies, if any, in the measurements collected from hands that have been used to report the same and different pathogenic and/or indicator organisms. This understanding may inform the design of future interventions through the identification of best practices for sampling, quantifying,

and summarization of the microbial load on the hands of children and students. The results of this systematic review may also go on to inform dose-response analyses such as studying potential relationships between the microbial load on hands and disease incidence or conversely, absence of disease given the presence of pathogens on hands.

Chapter 2. Methods

2.1 Identification

Defining our population of interest

We were initially interested in children and students within daycare facilities, schools, and colleges/universities in the United States and Mexico. Our exclusion and inclusion criteria, as provided below, were expanded to provide a global perspective and account for a nuanced approach to conduct our meta-analysis on factors pertaining to setting.

Search strategy

In order to identify relevant documents, three databases were supplied the same search string: PubMed, Web of Science, and Embase.

Search of PubMed, Web of Science, and Embase databases

The searches used across these three databases we conducted using a unique search string tailored to the research objective of this thesis.

> String for searching: (((pathogen* OR microb* OR viral OR virus) AND (child OR student)) AND (hand OR hands)) AND (contamination)

No other search strings were used or modified for this search.

2.2 Screening

The screening of identified documents across all three databases was performed in two steps. In the initial screening, due to the varying methods of displaying data in different sections of publications that might not be captured in an initial reading of the title or abstract, the methods and

results sections were also reviewed. The first screening was conducted using the following inclusions and exclusion criteria:

Inclusion criteria for the first screening

(i) published studies of all study designs, as well as observational studies nested within intervention studies if data was abstracted at baseline or

(a) if measurements were provided for a control group (in the absence of an intervention)

(ii) microorganisms measured in papers meeting our criteria included pathogenic and non-pathogenic bacteria, viruses, and parasites (protozoa and helminths)

(iii) to meet the definition of a child, studies must have included participants under the age of 18, including infants and children under 5 years old.

(iv) The definition of a student required school enrollment by as indicated by:

(a) measurement being performed during a class break for which participants were an attendee of that school or

(b) being formally enrolled within an academic program and

(c) being aged 5 years old to beyond 18 years of age to include ages that approximate to the undergraduate level in the United States in their equivalent academic level of study

(v) the microbial load of at least one microorganism was measured on the hands children and students on a quantitative and continuous scale

Exclusion criteria for the first screening

(i) microbial load was quantified by presence or prevalence measures as a percent or proportion of measured samples

(ii) measurements were from an intervention study in which investigators measured microbial load among different intervention groups after the initiation of an intervention

(iii) measurements came from young parents of infants rather than infants and children themselves

(iv) the hands of clinicians and health professionals were used to measure microbial exposure of children

(v) a substitute for a hand (fake skin/cloth) was used as a stand-in for a hand

(vi) studies were not excluded on the basis of country, country income level, nor setting type.

(vii) duplicate journal articles across databases were excluded

After the first round of screening, full texts were reviewed against the same inclusion and exclusion criteria. Additionally, the following exclusion criteria are the specific justifications for excluding some of the remaining literature in the second screening.

Exclusion criteria for the first screening

(i) investigators artificially contaminated the hands which were measured

(ii) a mathematical model was created to approximate the measured microbial load on the hand of a child

(iii) measurements of the microbial load from adults and adolescents that would otherwise meet our inclusion criteria could be disaggregated

Notably, one article excluded from this review required participants to wear gloves from which measurements were obtained, due to ethical considerations. While our study did not allow for the use of proxies of hands, the unique circumstances surrounding the nature of specimen collection are relevant to the research objective of this thesis but could not be included within the metanalysis. The table of reported measurements from this study can be found in appendix A.

2.3 Database extraction and formatting

Publication related variables

Data from each publication was collected on the year of publication, approximate year of sample collection (if across multiple years, the initial year of collection was used), country and subregion in which the samples were collected, sample methods including hand rinsing method and plating/replication techniques, and behavioral health data if available (often when accompanied by a behavioral health survey). This table may be found in Appendix A.

Demographic Variables

The following details the aggregation and categorization of the available data used for the meta-analysis of this thesis:

(i) Low, low-middle, upper-middle, and high income respectively [37]

(ii) Rurality and outside setting were coded as dichotomous variables from where 0 indicated rural or inside and 1 indicated urban or outside respectively

(a) One paper, for which six observations were gathered, was collapsed from a suburban setting to being categorized as an urban setting [38].

(iii) Setting was classified as a three-category nominal variable:

(a) school was defined as having occurred inside a classroom or on school grounds during scheduled breaks or under teacher supervision, or in a childcare/day care center

(b) household was loosely defined as having occurred within a household, compound, home, or within an area near one's home (such as parks and sidewalks)

(c) clinical settings included medical and dental care centers from which samples were collected from students or child patients.

(iv) for specifying outdoors, all household, school, and clinical settings were initially set inside by default. An observation was categorized as "outside" if at least one of the following two conditions were met:

(a) hand water rinse samples were stated to be collected outside (in the instance of parks and sidewalks) or

(b) subjects had markedly returned from "outdoor activities" moments prior to sample collection.

(v) age of subjects was collapsed into four groups consistent with the United States education system: children under 5 years of age (pre-K), children aged 6-12 (primary school), adolescents aged 13-17, and young adults aged 18 and up consistent with undergraduate age.

(vi) gender was not collected and, in some instances, collapsed to prioritize the consideration of age for our analysis.

A quantitative summary of the demographic variables can be found within the results section of this thesis in table 2.

Specimen-specific variables

Organisms were classified by specimen name, specimen type (bacteria or parasite), and pathogenicity as a dichotomous variable where one indicated being pathogenic. All of the reported sample concentrations were extrapolated to a denominator of per two hands: the final measurements included in the database are included in the summary of the specimen specific tables found within the results section of this thesis in table 2.

Microbial load measurement formatting

Microbial loads were converted to one of the following measures for consistency: log10 CFU/2 hands, log10 MPN/2 hands, Cysts/2 hands, Eggs/2 hands, and Eggs/gram. For studies that did not report the measured CFUs or MPNs on a log scale or reported the denominator by only one hand, calculations were performed in excel to adjust these values to uniformly interpret these measurements. This included converting measurements from a log scale to a numeric value, multiplying the measurements by two, and reconverting to a log scale if the values were initially recorded on a log scale per one hand. If the values were not reported on a log scale, then they were simply converted to a log if the values had a denominator of two hands. If the values were only recorded per one hand and not on a log scale, then the values were first multiplied by two then converted to a log measurement.

2.4 Data analysis

I conducted an analysis in SAS 9.4 (SAS Institute Inc., Cary, NC, USA) using data extracted from the selected literature which can be found within table 3 of the results section of this thesis. Analyses were restricted to specimen type and each respective unit of measurement. Twenty observations reporting the same organism and unit of measurement were used to conduct a meta-analysis performing a linear regression on factors identified through existing literature and factors of interest relating to setting that may affect the outcome of interest: measured microbial load. A t-value of $t = 2.101$ was used opposed to the typical $z = 1.96$ when calculating the 95% confidence interval to account for our small sample size within our regression model.

Chapter 3. Results

A total of 1370 articles were initially identified, from which 19 studies were ultimately selected for review after the iterative screening process (Fig. 1, Table 1). Studies from a total of 14 countries across four continents conducted from 1993-2020 were included within this systematic review. From these studies, 7147 children and students informed the sample measurements providing a total of 89 groups of children and students with reported microbial loads. The total populations sampled included children, infants, schoolchildren, hospitalized children, and university dental students. Measurements of bacteria, helminths, and protozoa were obtained using culture-based assays, polymerase chain reaction (PCR), and light microscopy (Table 1). Article name, author, sampling mechanism, and enumeration methods are provided in Table A.1 in Appendix A.

A breakdown of the characteristics of the populations included (Table 2), concentrations of measured samples (Table 3), and analysis of the association between setting and the microbial load (Table 4) are provided.

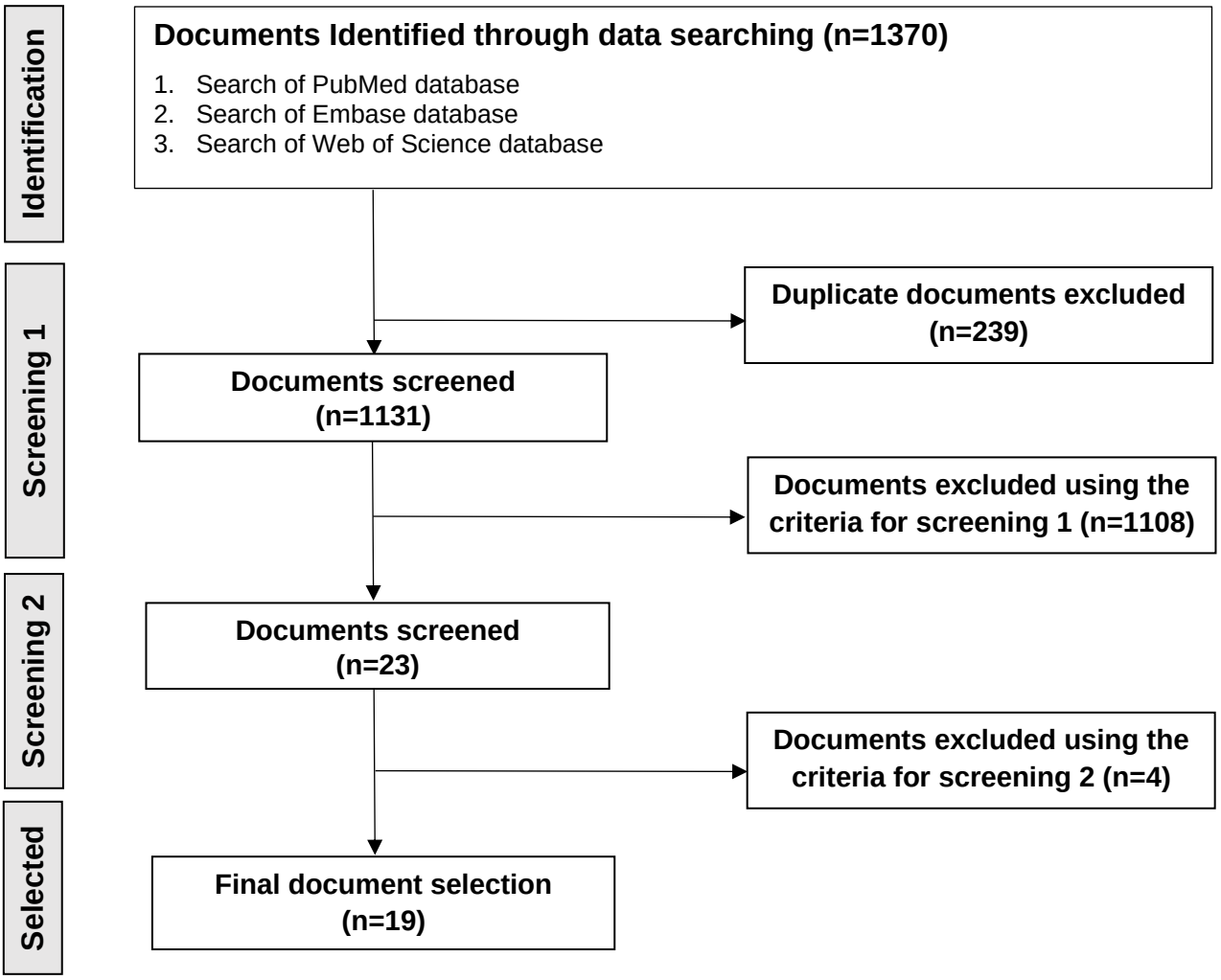

Figure 1. Flow diagram depicting the searching and screening of journal articles

Table 1. Selected article characteristics

Characteristic	Description
Articles Included	19
Countries where studies took place	Bangladesh, Democratic Republic of the Congo, Greece, India, Japan, Kenya, Mauritius, Mexico, Nepal, Saudi Arabia, South Africa, Tanzania, Vietnam, United States
Date range of studies	1993-2020
Total children sampled	7147
Groups of children analyzed	89
Populations studied	Children, infants, schoolchildren, hospitalized children, university dental students
Microbes enumerated	Bacteria, helminths, protozoa
Measurement techniques	Culture-based assays, PCR, light microscopy

3.1 Characteristics of samples abstracted from selected articles

To understand the context in which measurements of the microbial loads on hands were quantified, Table 2 provides a demographic overview of the 89 groups of children and students that were sampled globally. Among these groups of children, no measurements of the viral load on hands were obtained while a majority of the sample measurements were derived from bacteria followed by parasitic microorganisms. The characteristics pertaining to each sample: country income level, rurality, the setting in which the sample was taken, physical sampling location, age categories of the sample populations, units of measurement, and pathogenicity, were near evenly distributed across organisms. A majority of the measurements were obtained in upper-middle income and lower-middle income countries which each combined contributed almost two thirds of the observations in Table 2. Just over one third of the 89 sample populations were sampled in high-income countries while only two observations from the same study were reported in a low-income country. Notably, all the observations obtained from high income countries measured bacteria and not any other kind of microorganism (Table 2). About one fifth of observations were sampled in rural areas compared to urban areas across both bacteria and parasites. Roughly 30% of all

concentrations were obtained in an outside setting compared to an inside setting. Almost all of the samples within our analysis were collected in either a household or school setting compared to a clinical setting. Only two articles, contributing four of the 89 measurements included in this thesis, collected data in clinical settings, both of which measured bacteria [39,40]. Approximately 90% of samples across bacteria and parasites were obtained from children less than or equal to the age of 12 (Table 2). The reported measurements of bacteria were measured in units of CFUs and MPNs (colony-forming units and most probable number). Of the 76 bacterial measurements, about one-fifth 15 (21%) were pathogenic while 58 observations (79%) were indicator organisms (Table 2). Ultimately, the availability of measurements of the microbial load varied by country income-level, location of the sampled subjects, and age. The proportion of identified measurements across bacteria and parasites did not vary by rurality nor outdoor sample setting within this dataset.

Table 2. Population sample characteristics

Variable	Total (N=89)	Bacteria (N=76)	Parasite (N=13)
Geography			
Income Level			
Low	2 (2%)	2 (2%)	0 (0%)
Lower-Middle	29 (33%)	21 (28%)	8 (61%)
Upper-Middle	27 (30%)	22 (29%)	5 (39%)
High	31 (35%)	31 (41%)	0 (0%)
Rurality			
Rural	19 (21%)	16 (21%)	3 (23%)
Urban	70 (79%)	60 (79%)	10 (77%)
Sample Setting			
Inside	61 (69%)	52 (68%)	9 (69%)
Outside	28 (31%)	24 (32%)	4 (31%)
Location			
Clinical	4 (5%)	4 (5%)	0 (0%)
Household	42 (47%)	38 (50%)	4 (31%)
School	43 (48%)	34 (45%)	9 (69%)
Individuals			
Age Category			
≤5 years	22 (25%)	22 (29%)	0 (0%)
6-12 years	58 (65%)	47 (62%)	11 (85%)
13-17 years	3 (3%)	1 (1%)	2 (15%)
≥18 years	6 (7%)	6 (8%)	0 (0%)
Microbes			
Unit of Measurement			
Cysts/2 hands	4 (5%)	0 (0%)	4 (31%)
Eggs/2 hands	7 (8%)	0 (0%)	7 (54%)
Eggs/gram	2 (2%)	0 (0%)	2 (15%)
log10 CFU/2 hands	57 (64%)	57 (75%)	0 (0%)
log10 MPN/2 hands	17 (19%)	17 (22%)	0 (0%)
**	2 (2%)	2 (3%)	0 (0%)
Pathogenicity* **			
Pathogenic	28 (33%)	15 (21%)	13 (100%)
Non-Pathogenic	58 (67%)	58 (79%)	0 (0%)
**	3	3	0

* Columns are N (column percentages)
** Characteristic was not applicable to the sample (e.g., the organisms in a sample were not stated)
*** A complete list of microbes and their pathogenicity can be found in Appendix B

3.2 Characterization of sampled microbes

To describe the existing quantifications of microbial loads on the hands, a breakdown of the specific organisms that were quantified, their concentrations, and units of measurement were analyzed in Table 3. The concentrations listed in table 3 were all converted to the same unit of measurement, when applicable, on the log10 scale per two hands as described in section 2.3 of this thesis. Three concentrations measured in log 10 CFUs were reported that did not specify which bacteria were quantified all of which came from the same study [40]. This prevented conclusions to be drawn regarding pathogenicity from these measures of the microbial load. The documented concentrations of bacteria cultured from the hands of children and students varied across many species from 0 to a maximum of 8.6 log10 CFU per two hands. Additionally, the concentrations of worms ranged from 0 to 385 Eggs per two hands while the concentration of cysts per 2 hands ranged from 0 to 58.3. Among this list of microbial concentrations, CFUs were used to quantify bacteria a majority of the time, followed by MPNs which were used in about one fourth of the bacterial samples listed. Twenty observations across studies recorded *E. coli* measured in CFUs (n=14) and MPNs (n=6), which are indicated by the red box in Table 3. Twenty observations were also found measuring the microbial load of faecal streptococcus, these were all found in the same study and study population. Among the 13 measures of parasites, four observations from the same study quantified *Giardia lambia* while the remaining nine measures captured varying species of helminths. These organisms were quantified in units of Eggs/hand, or Eggs/gram. Of the measured quantifications of the microbial load, bacteria had the greatest range of individual organisms measured, followed by helminths, then cysts while no viral concentrations were identified in this review.

Table 3. List of organisms and their concentrations

Organism (n = 89)	Concentration	
*Ancylostoma duodenale, Enterobius Vermicularis, Toxocara canis***	0.8	Eggs/2 hands
Aerobic Mesophilic (2)	[7.2-7.4]*	log10 CFU/2 hands
Ascardia galli	1	Eggs/2 hands
Ascaris lumbricoides	305	Eggs/gram
Ascaris lumbricoides, Enterobius vermicularis	0	Eggs/2 hands
Ascaris lumbricoides, Enterobius vermicularis, Trichuris trichiura, Taenia sp.	1.9	Eggs/2 hands
Bacteroidales (Genbac3)	6.0	log10 MPN/2 hands
Bacteroidetes (BacR)	4.5	log10 MPN/2 hands
Escherichia coli (6)	1.0	log10 CFU/2 hands
Escherichia coli (14)	[0.8-2.8]*	log10 MPN/2 hands
Enterococci (ENT)	2.0	log10 MPN/2 hands
Enterococcus spp	1.3	log10 CFU/2 hands
Enterobius vermicularis	1.3	Eggs/2 hands
Enterobius vermicularis, Trichuris trichiura	3.3	Eggs/2 hands
faecal streptococci (20)	[0.3-0.9]*	log10 CFU/2 hands
Fecal coliform (8)	[0.0-8.6]*	log10 CFU/2 hands
Giardia lambia (4)	[0.0-58.3]*	Cysts/2 hands
Klebsiella pneumoniae (4)	[1.7-4.7]*	log10 CFU/2 hands
***	[1.8-1.9]*	log10 CFU/2 hands
Staphylococcus aureus (6)	[2.5-6.7]*	log10 CFU/2 hands
Staphylococcus (2)	1.5	log10 CFU/2 hands
Staphylococcus, Micrococcus, Klebsiella pneumonia (3)	[1.9-2.1]*	log10 CFU/2 hands
Streptococcus pyogenes	[0.0-5.2]*	log10 CFU/2 hands
Taenia sp.	1	Eggs/2 hands
Trichuris trichiura	34	Eggs/gram

* (n-n) Indicates a range of the minimum and maximum summary measurements across studies with the same organism

** (n) Reflects pooled result of multiple organisms and not the individual measures for each

*** The exact cultured organism was not discernable in this study

3.3 Meta-analysis

In the previous table, we identified a range of pathogens and a wide distribution of microbial loads. To determine if there was any association between microbial load and setting, we conducted an exploratory analysis on a subset of the sample measurements from Table 3. Non-pathogenic *E. coli* microbial load and outside setting were selected for the regression analysis in Figure 2. We chose *E. coli* because it was the only organism for which there were more than 10 measurements across multiple sample populations to conduct a linear regression incorporating data from 7 of the 19 unique studies [41-47]. Twenty observations measuring the microbial load of faecal streptococcus were also found. However, these were all found in the same study and the study population preventing an analysis from being conducted to compare concentrations of the microbial load across any of the characteristics listed in Table 2 including setting. The binary variable for outside setting served as our independent exposure variable. The linear regression resulted in a statistically significant association between sample setting and increased microbial load [$\beta_1 = 2.27$, 95% CI (0.21, 4.23), $p = 0.0323$]. Our analysis found that microbial load of non-pathogenic *E. coli* on the hands of children and students that were sampled outside or upon the completion of outdoor activities is expected to be 2.27 log10 CFUs greater than the quantity measured on the hands of children and students that were sampled indoors (Table 4). This thesis was also interested in testing other relationships with viral load including school age category, rurality, and structural location in which sampling took place, but none demonstrated a statistically significant association (data not shown). The statistically significant association between sampling the hands of children and students outdoors and increased microbial load on hands supports that greater associations may also exist between setting and the microbial load if a more diverse population and robust dataset.

Figure 2. Mean E. coli concentration was different for samples measured outside compared to inside

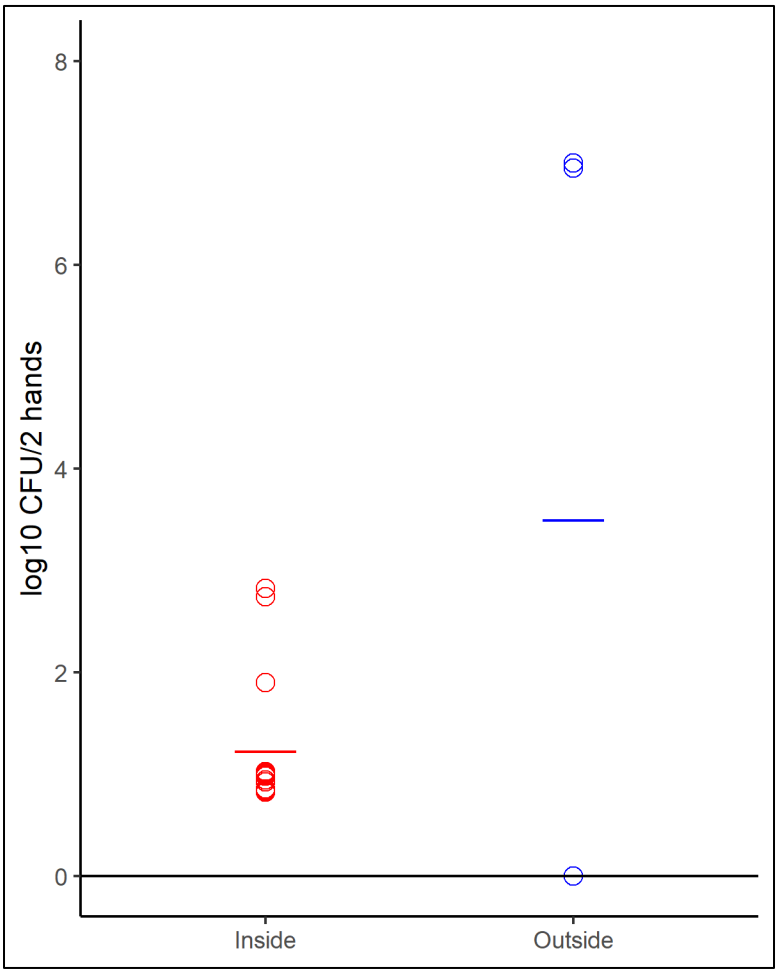

Figure 2. Dot plot of log10 concentration of the microbial loads on the hands of children and students (MPN and CFUs) as a factor of being sampled outside or inside. Each circle is a consensus measurement of the microbial load from a group of children and/or students. The red line indicates the mean concentration among *E. coli* samples taken indoors while the blue line indicates the mean of the concentration among *E. coli* samples taken outdoors.

Chapter 4. Discussion and Public Health implication

The goal of this project is to quantify microbial load on the hands of children and students and to conduct an exploratory analysis of factors contributing to the microbial load based on the available literature to date. The first of the main findings was that bacteria were the most commonly measured organism. The second finding was that concentrations of the microbial load on the hands of children and students varied greatly across many different microorganisms. The final takeaway from this analysis is that there was a statistically significant increase in the expected microbial load among hands contaminated with generic *E. coli* that were sampled outside or on the completion of outdoor activities compared to those sampled indoors.

4.1 Main Findings

Bacteria were a commonly quantified microbe

The first finding was that bacteria were the most commonly measured microorganism. One hypothesis to explain why bacteria were the most commonly quantified microbe is that the intrinsic biology of bacterial organisms may allow them to replicate outside of a human host in comparison to viruses. Because they are able to replicate outside of a human host they are more likely to be present in the environment and come into contact with hands, allowing them to be measured and quantified [7,48]. A second hypothesis may be that the ability of certain bacteria to persist in an environment may make them more likely to be measured. Persistence in an environment increases the likelihood of a bacteria surviving to the time in which they may be measured [36,48]. It is the combination of a microbe's ability to replicate and persist that ultimately results in a higher concentration of microbes on surfaces that may be touched by the hands of children and students [7,48].

Concentrations of microbial loads varied across many organisms

We found that the concentrations of the microbial load on the hands of children and students varied greatly across many different microorganisms. One hypothesis to explain this variation in the concentrations of microbial load is that hand hygiene practices may vary across populations. The evidence to support this hypothesis includes that hand hygiene practices can vary with age and access to handwashing facilities [20]. For example, small children and students may be less likely to practice proper hand hygiene compared to older students working in a clinical setting [21-23]. A disparity may also exist in which people with barriers to accessing proper hand washing facilities compared to those with access, may not actively maintain regular hand hygiene behaviors [20].

Concentrations sampled in outside, compared to inside, settings had an average higher microbial load

The final takeaway was drawn from an analysis examining the relationship between whether samples were taken outside or on the completion of outdoor activities compared to those sampled in indoor settings among people with *E coli*. There results of the analysis yielded a statistically significant increase in the expected microbial load. To explain this result, one hypothesis is that higher concentrations of microbes on the hands of children and students sampled in outside, compared to indoor, settings may be attributable to activities the participants are engaging in while outside, compared to inside. For children and young students, outside exposures may occur on playgrounds—where physical activity occurs—presenting opportunities for hands to come into contact with the ground, dirt, and unclean surfaces such as balls and playground equipment [49]. This is in contrast to indoor activities in which, while contacts with shared surfaces may still occur, students are more often restricted to working at their own desk and using their own school supplies

as applicable. Alternatively, this increase in the microbial load may also result from perceptions surrounding hand hygiene practices outdoors compared to indoors. For example, a socially accepted and encouraged habit such as washing hands upon returning inside as opposed to washing one's hands before going outside could result in lower microbial loads being recorded indoors [50].

Ultimately, this significant finding provides a justification for the investigation of further measurements of the microbial load on a broader scale to clarify the true relationship between setting and concentration of microbes found on the hands of children and students. Other variables for which data were collected, such as age, structural setting, and urbanicity could be taken into account in future analyses in countries for which there was not much available data such as in the United States [44,51].

4.2 Strengths and Limitations

There were several strengths to this systematic review with meta-analysis. A key strength was the screening method of the articles obtained across multiple databases: PubMed, Embase, and Web of Science. Our search yielded over 1000 papers for which it was discerned that titles and abstracts could not be accurately relied on to obtain the measurements of interest. This meant that a lot of time was allocated to scrutinizing the methods and data tables reported in each of these studies to ensure they did provide a numeric measure of the microbial load on the hands of children and students. This wide search provides a global picture of the existing literature surrounding this research topic. Another strength of this study was that it appeared to provide the most comprehensive picture of the setting for which a variety of pathogens were sampled from hands among this population. This allowed for the identification of potential understudied populations

across settings and various age groups that can be filled through the tailoring of a future intervention using the information found within Table 2. Another strength of this review was its assemblance of a dataset with numeric measures of microbial loads rather than using prevalence measures which typically collapse concentrations into “low” and “high” categories that fall above or below an arbitrary threshold. For the analysis portion, one strength was that adjustments were made to increase the error bounds of the confidence interval to account for the small sample size of twenty measurements. Another strength of our analysis was that we were able to estimate a quantifiable expected change in the microbial load dependent upon sample setting rather than simply indicating whether or not two measures were statistically different from one another.

There were also some limitations to this study. Only one search string was used across all three databases. Had we recognized the scarcity in existing literature surrounding viruses, multiple strings may have been crafted to identify journal articles by specific pathogen types. This may have contributed to the small sample size yielded from this search despite the considerable number of papers that were identified using the initial search string. This limitation prevented us from drawing any conclusions about the microbial load of viruses on the hands of children and students. Additionally, it was difficult to discern or provide an intelligible uniform measure of error for the measures of concentration as almost every study reported different values (e.g., quartile ranges, medians, standard error). Given that the concentrations reported in our results were converted to a log scale when applicable for clarity, these error measurements could not intelligibly be converted on the same scale. The small sample size also served as our greatest limitation for the linear regression of concentration of the microbial load on our independent binary “outside” variable. If the sample size were larger, we may have been able to adjust for multiple covariates and interaction

terms that may also impact the microbial load. Another limitation would be how we collapsed some of the variables into various age groups (e.g., including measurements from 10-12 year-olds in the same category as measurements from 6-8 year-olds).

4.3 Implications

The results of this review would be helpful in informing researchers aiming to design interventions to fill the existing gap in the quantification of the microbial load on the hands of children. The identification of gaps relating to the lack of diversity in the groups of children and students identified within this review could help set guidelines as to how public health experts might define their populations of interest in future studies. This study also serves to identify multiple settings to recruit participants from to obtain a snapshot of the microbial load across a diverse group of children and students. Industries with a stake in hand hygiene behaviors may also use these results to better inform the testing of their products against the specific microbes that have been documented to be found on the hands of children and students. Lastly, this analysis provides a foundation for future research quantifying the microbial load on hands to be done with the intent to incorporate a more robust model using a greater and more diverse sample population.

4.4 Conclusion

This review provides an introductory examination of the characteristics of the quantified measurements of the microbial load available in the existing literature. Importantly, several gaps in the existing literature were identified. Specifically, concentrations of the viral load on the hands

of children and among students were scarce. Inconsistencies in data reporting through differences in units of measurement used to report results have demonstrated an opportunity for consideration in future cross-sectional study designs. Furthermore, our metanalysis indicated that more information could be gleaned from future investigation into the impact of setting on the microbial load.

Appendix A. Summary of Selected Documents Sampling Methods, & Enumeration Processes

Table A-1. Article Titles, Authors, Countries, Sampling Methods, & Enumeration Processes

Title	Authors	Country	Sampling Method	Enumeration Process
Animal Feces Contribute to Domestic Fecal Contamination: Evidence from E. coli Measured in Water, Hands, Food, Flies, and Soil in Bangladesh	Ercumen, A. et al[41]	Bangladesh, Central Region	To sample child hands, field workers asked the respondent to place both hands of the youngest child <5 years, one at a time, into a Whirlpak prefilled with 250 mL of distilled water. Each hand was massaged from outside the bag for 15 s, followed by 15 s of shaking, and the rinsewater was preserved in the Whirlpak.	Samples were analyzed using IDEXX Quantitray with Colilert-18 media (IDEXX Laboratories, Maine, U.S.A.) and incubated at 44.5 °C for 18 h to enumerate E. coli with the most probable number (MPN) method. The Quantitray-2000 system with a wide detection range of 1−2419 MPN per tray was selected
Child hand contamination is associated with subsequent pediatric diarrhea in rural Democratic Republic of the Congo (REDUCE Program)	George, CM et al [42]	Democratic Republic of the Congo, Walungu Territory, South Kivu	The caregiver or child was asked to place first one hand, and then the other hand, into the same 500 ml Whirl-Pak bag containing 350 ml of PBS. Each participant dipped his or her hand into the PBS solution for one minute, which included 30 s of shaking followed by 30s of research staff massaging the participant's hand through the Whirl-Pak bag.	For analysis, 100 ml of the sample was processed. Bacterial counts were multiplied by 3.5 (to account for the initial water volume) and reported as MPN/both hands
Co-Infection by Waterborne Enteric Viruses in Children with Gastroenteritis in Nepal	Tandukar, S. et al [39]	Nepal, Kathmandu	The hand swabs were collected using a BM Fukitool A kit (GSI Creos, Tokyo, Japan) containing 10 mL of phosphate-buffered saline and a cotton swab. The swab was dipped into the buffer.	Hand swab samples collected were first analyzed for fecal indicator bacteria (total coliforms and E. coli) by the most probable number (MPN) method using a Colilert reagent (IDEXX Laboratories, Westbrook, CA, USA).
Comparative Efficacy of Hand Disinfection Potential of Hand Sanitizer and Liquid Soap among Dental Students: A Randomized Controlled Trial	Khairnar, MR et al [40]	India, Pune	A swab of each participant was taken by rotating the swab 360° once on middle three fingertips of left hand and palm.	Swabs were cultured on agar plates to determine a maximum spectrum of microbes present [number of colony-forming units (CFU)]
Detection of pathogenic micro-organisms on children's hands and toys during play	Martinez-Basidez, T. et al [43]	Mexico, Culiacan, Sinaloa	The sampling of the CS was extended for 2 months on a weekly basis. Children washed their hands with Escudo antibacterial soap (sodium oleate, sodium palmitate, water, sodium laurate, Zea mays (Corn) starch, glycerin, fragrance, triclocarban, sodium chloride, titanium dioxide, sodium citrate, citric acid, tetrasodium EDTA), rinsed their hands in a sterile plastic bag containing 50 ml of PBS and vigorously washed for 2 min, this rinsing water was analyzed to determine the absence of microorganisms. After hand washing, children spent 1h playing with toys at either public parks or sidewalks. After that time, both children's hands and toys were introduced in a sterile plastic bag containing 200 ml of PBS and vigorously washed for 2 min to allow the release of microorganisms.	e identification and quantification of bacteria were performed by diluting and extending 0.1 ml of the sample in selective and differential culture media dependent upon the bacterial species. To test for Giardia, Samples contained in sterile plastic bags were manually agitated, and 50 ml of sample were centrifuged at 1000 g for 10 min. Supernatant was removed and 3⁄4 parts of sugar solution (SG 1.27) were added to the sediment and centrifuged again at 250 g for 5 min. Samples were allowed to stand for 5 min and then 1.8 ml was loaded in a McMaster chamber for microscopic observation using the 10X objective

Table A-1. (Continued)

Effect of fecal contamination on diarrheal illness rates in day-care centers	Laborde, DJ et al [51]	USA, Cumberland County, North Carolina	Hands were rinsed in 1/2-gallon (1.89-liter) Dow freezer bags containing 200 ml of 0.1 percent sterile peptone water (28, 29). 3) Faucet handles were sampled using premoistened, sterile cotton swabs that were subsequently placed in vials containing 2 ml of 0.1 percent peptone water.	Samples were transported on ice and processed within 4 hours of collection. Inverted RODAC plates were incubated aerobically at 44°C for 24-^8 hours. Vials containing swab samples were vortexed, and serial 10-fold dilutions (up to 10~2) of the eluents were inoculated onto duplicate MacConkey agar plates. A 50-ml aliquot of each rinse bag was concentrated to 5 ml by centrifugation at 2,000 X g. Dilutions up to 10^-6 for hand samples were plated in duplicate onto MacConkey agar plates and incubated at 44°C for 24—48 hours. Colony-forming units (CFUs) were enumerated on duplicate plates and then averaged. Fecal coliforms were presumptively identified by their ability to grow at 44°C on MacConkey agar and their lack of indophenol oxidase. Identification was confirmed by the production of indole or by biochemical profile.
Effect of Neighborhood Sanitation Coverage on Fecal Contamination of the Household Environment in Rural Bangladesh	Huda, TMH et al [52]	Bangladesh, Mymensingh district	The field team rinsed both hands of the target child (aged 6–24 months) from each target household. Hands were rinsed for 30 seconds each, in a Whirl-Pak bag (19 × 38 cm) (Nasco, Fort Atkinson, WI) filled with 200 mL of Ringer's solution.	Fecal coliforms were enumerated using a membrane filtration technique with modified FC agar plates, within 24 hours of collection. The results were calculated as colony-forming units (CFUs) present per 200 mL of recovered media that bathed the hands.
Enterococcus spp on fomites and hands indicate increased risk of respiratory illness in childcare centers	Julian, TR et al [44]	USA, California	Hand rinse sampling was performed using the hand rinse method.14 Volunteers were asked to place first one hand and then the other into a Whirl-pak bag (Nasco, Fort Atkinson, WI) filled with 350 mL of distilled water. Hands were shaken vigorously by volunteers for 15 seconds before being massaged by researchers for an additional 15 seconds. After collection, the sample was placed on ice, returned to the laboratory, and processed within 6 hours.	The hand rinse method has a lower detection limit of detection of 2.5 colony-forming units (CFU) per hand based on the volume assayed for each FIB assay (65-80 mL). Median recovery of E coli from hands using the hand rinse method is approximately 52%
Fecal Indicator Bacteria along Multiple Environmental Transmission Pathways (Water, Hands, Food, Soil, Flies) and Subsequent Child Diarrhea in Rural Bangladesh	Pickering, AJ et al [45]	Bangladesh	To sample hands, field workers rinsed both of the child's hands in a Whirlpak bag filled with sterile water.	Hand rinse samples were diluted with distilled water as follows: 2-fold dilution (50 mL of sample diluted with 50 mL of distilled water). All samples were analyzed using the IDEXX Quanti-Tray system with Colilert-18 media (IDEXX Laboratories, Inc., Westbrook, ME) and incubated for 18 h at 44.5 °C.
Hand hygiene intervention to optimize soil-transmitted helminth infection control among primary school children: the Mikono Safi cluster randomized controlled trial in northwestern Tanzania	Makata, K et al [53]	Tanzania, Bakoba and Muleba districts	1 mL from each hand rinse water sample was taken using a sterile Pasteur pipette and placed into 9 mL of Brain Heart Infusion Broth (BHI; HI Media®, India). Samples were processed in the laboratory within 2–3 h after collection by inoculating them onto MacConkey agar w/0.15% bile salt, CV and NaCl using a calibrated 1 μL loop. The plates were incubated at 35°C to 37°C for 18 to 24 h. The absolute numbers of colonies detected on the MacConkey agar plates were multiplied by 1000 to get the corresponding number of coliforms CFU/ml. The resulting value was also multiplied by 10 taking into account the 1:10 sample to BHI dilution to get the final total coliforms CFU/ml. For eggs, light microscopy used a 10mL sample.	For the identification and quantification of helminth ova, 10 ml of the main hand rinse water sample was processed using the zinc sulfate centrifugal flotation method and examined by light microscopy for presence of STH eggs.

Table A-1. (Continued)

Hygiene practices and faecal contamination of the hands of children attending primary school in Mauritius	Padaruth, SK et al [54]	Mauritius	A cotton swab moistened with sterile saline was rolled on the palm and fingers of the dominant hand of each participant. All samples were stored overnight at 48C before processing.	Under aseptic conditions, the specimens were streaked on blood agar, MacConkey agar and Deoxycholate citrate agar (all Hi-Media, Mumbai, India). After an incubation period at 378C for 24 h, the bacterial load was read as colony forming unit (CFU) counts per hands. The colonies were identified by morphological properties and standard biochemical tests.
Impact of Educational Intervention for Hand Hygiene on Dental Students' Knowledge, Attitude, and Bacterial Contamination Level on Hands	Lingawi, H. et al [55]	Saudi Arabia	Fingertip prints of the five fingers of the dominant hand from every participant were gently impressed on sheep blood agar plates (Saudi Prepared Media Laboratory Company Ltd.) for three times. Plates with fingertip prints were aerobically incubated at 37°C for 24 hours and after those total bacterial counts on each plate were recorded as the number of CFUs. Bacterial isolates were identified using standard microbiological procedures; colony morphology, Gram staining, and Vitek 2.	prints were impressed on blood agar. Plates with fingertip prints were aerobically incubated at 37°C for 24 hours and after those total bacterial counts on each plate were recorded as the number of CFUs. Bacterial isolates were identified using standard microbiological procedures; colony morphology, Gram staining, and further identification using Vitek 2 microbial identification system (Biomerieux, USA) was done.
Impact of regular soap provision to primary schools on hand washing and E. coli hand contamination among pupils in Nyanza Province, Kenya: a cluster-randomized trial	Saboori, S. et al [46]	Kenya, Nyanza Province	Enumerators asked each selected pupil to place one hand in a 500-mL Whirl-Pak (Nasco, Fort Atkinson, WI) bag containing 250 mL sterile phosphate-buffered saline solution and wiggle fingers around while counting to 10 slowly; then, the student repeated the procedure with the other hand in the same bag.	Hand rinse samples were analyzed for E. coli by the membrane filtration method using m-ColiBlue24 broth (Hach, Loveland, CO), and plates were incubated and counted
Microbiological contamination of young children's hands in rural Bangladesh: Associations with child age and observed hand cleanliness as proxy	Parvez, SM et al [47]	Bangladesh, Gazipur, Kishoregan, Mymensingh, and Tangail districts	Child hand rinse samples were taken after the child hand observation. Field workers sampled child hands by rinsing both hands, one at a time, in a single Whirlpak bag (Nasco Modesto, Salida, CA) pre-filled with 250 mL of distilled water. Each hand was massaged from the outside of the bag for 15 seconds, followed by 15 seconds of shaking, and the rinse water was preserved in the Whirlpak bag [27]. Samples were preserved on ice and transported to the field laboratory to be processed on the same day, typically within 12 hours of collection. Upon arrival at the laboratory, samples were kept on ice until they were processed. Hand rinse samples were diluted 1:2 with distilled water (50 mL of sample diluted with 50 mL of distilled water).	. Samples were analyzed using the IDEXX Quanti-Tray 2000 system with Colilert-18 media (IDEXX Laboratories, Inc., Westbrook, ME) and incubated for 18 hours at 44.5˚ C to enumerate the most probable number (MPN) of E. coli [28]. The E. coli concentration was expressed as MPN per two hands. To accommodate variability in hand contamination, the Quanti-Tray 2000 system with a wide detection range (1−2419 MPN) per tray was selected. For quality control, field workers collected 10% field blank samples by opening and shaking a Whirlpak bag pre-filled with distilled water in the household as if collecting a hand rinse sample. Each laboratory technician processed one laboratory blank sample per day.

Table A-1. (Continued)

Observation of everyday hand-washing behavior of Japanese, and effects of antibacterial soap	Toshima, Y et al [56]	Japan, Tokyo	each participant rubbed the palms together five times back and forth with 1 ml of either soap, interlaced the fingers and rubbed the fingers of each hand against the forks of the fingers of the other five times, switching the relative positions of the hands, rubbed each palm against the back of the other hand five times, scratched each palm with the nail tips of the opposing hand five times, ran each palm against the sides of the other hand, five times on each side, rubbed each wrist with the palm of the other hand five times back and forth, rinsed the hands by moving them under tap water in the same manner used for lathering, ran 100 ml of sterilized water over the hands without rubbing them together, and absorbed remaining water on the hands by lightly pressing a sterilized towel against the palms and backs of the hands.	The lower limits of aerobic mesophilic counts and viable counts for total coliforms were both 30 cfu/glove, and those for other kinds of bacteria, 300 cfu. For the latter microorganisms, i.e. Salmonella, V. parahaemolyticus, E. coli O157, and Campylobacter, sample solutions were also cultured in enrichment medium, according to the Guidelines on Food Hygiene Testing Japanese Ministry of Health Ž and Welfare, 1990, and then the selective plate. media were inoculated. In recovery tests with Salmonella, V. parahaemolyticus, E. coli O157, and S. aureus at 104 –105 cfu/glove, recovery was more than 98%. To confirm the efficacy of the neutralizing solution, antibacterial hand soap, in various quantities up to 1 ml, was added to gloves containing suspension of non-pathogenic E. coli and to gloves with suspension of Salmonella. This test recovered more than 99%; therefore, the neutralization was presumed satisfactory.
Parasite egg contamination of hands in a suburban area of Hanoi, Vietnam	Nguyen, TVH et al [38]	Vietnam, Hanoi	Parasite eggs on hands were collected at home or in school by soaking hands in a container containing 700ml of 0.5% Tween 20 solution for 2 minutes and rubbing the hands together for 3 minutes. Then, hands were washed with 200ml of new solution. All samples were taken to the laboratory.	Each hand-wash sample was transferred to a one-liter beaker, and 100 ml of solution used to wash the container was also transferred to the same beaker. Samples were left until the next day. After removing 900 ml of the supernatant, the remaining fluid was transferred to two 50 ml centrifuge tubes and centrifuged for 2,000rpm for 5 minutes. After removing the supernatant, 5ml of saturated NaNO3 solution was added to a level of three-quarters of the tube, and the tube was centrifuged at 2,000 rpm for 10 minutes. Saturated NaNO3 solution was added to the top of the centrifuge tube, and a grease-free slide glass was placed carefully on the top to contact the surface of the fluid. After 20 minutes, the slide glass was removed, and a cover glass was placed on the slide glass for observation under a microscope. Several drops of NANO3 solution were added again to the centrifuge tube, and a new slide glass was placed on top. After 5 minutes, the slide glass was removed, and a cover glass was placed for observation. Two cover glasses were observed under a microscope.
Ruminants Contribute Fecal Contamination to the Urban Household Environment in Dhaka, Bangladesh	Harris, AR et al [57]	Bangladesh, Murpur, Dhaka	Each hand was inserted into a single Whirl-Pak Bag (Nasco, Fork Atkinson, WI) filled with 250 mL of autoclaved (121 °C for 15 min) 1/4-strength Ringer's solution (Oxoid Ltd., Hampshire, UK), then massaged and agitated for 30 s per hand using established techniques.	To elute bacteria from the sponge, 250 mL of sterile 1/4- strength Ringer's solution was added in 3 incremental volumes (100, 100, and 50 mL).29 First, 100 mL of Ringer's solution was added to the sponge bag. The sponge was then agitated in the bag for 15 s and then massaged for 15s; next, the Ringer's solution was removed and added to a new Whirl-Pak bag. The next two volumes (100 and 50 mL) were processed in the same manner as the first volume, to create a total of 250 mL rinse of the sponge sample in the new sample bag. The sponge and hand rinse samples were processed for EC and ENT using Colilert-18 and Enterolert, respectively. For the hand rinse sample, 50 mL of the sample was added to 50 mL of distilled water to create the necessary 100 mL sample volume for the IDEXX assays. For the floor sponge eluent, 10 mL of the eluent was added to 90 mL of distilled water to create the 100 mL sample volume for the assays. The samples were processed following the same Colilert-18 and Enterolert methods as used for the fecal samples. Quanti-trays with zero positive wells were recorded as 0.5 most probable number (MPN) per volume tested.
Screening for faecal contamination in primary schools in Crete, Greece	Kyriacou, A. et al [58]	Greece, Heraklion, Crete	Each child was sampled six times over three consecutive days, as preliminary studies had shown wide variation per child and per day. In order to isolate faecal streptococci from hands, children were asked to place the fingertips of both hands onto Petri dishes containing Slanetz & Bartley medium (LAB-M LML166). Immediately after sampling, each child washed its hands with soap and water.	

Table A-1. (Continued)

Transmission of Enterobius vermicularis eggs through hands of school children in rural South Africa	Cranston I. et al [59]	South Africa, Mutale Region, Limpop province	Hands were rinsed using a standard method (Jeandron et al., 2014): two zip lock bags (26.8 cm × 24.3 cm) were filled with 40 ml of non-ionic diluted 7× (1%) solution and placed on study participants hands and were massaged for approximately 30 s, ensuring as much dirt as possible was removed. Participant's hands were removed from the bags and rinsed with 5 ml of distilled water from a squeezy bottle ensuring that this water was 'caught' by the Ziplock bags. The contents of each Ziplock bag were then decanted into a separate 50 ml falcon tube- one for the left and one for the right hand.	The falcon tubes were centrifuged for 7 min at 2500 rpm. The supernatant removed, 4 ml sugar salt solution added (density 1.27), and the pellet homogenized. The homogenized pellet was transferred to McMaster slides (as described by Jeandron et al., 2014), which were then examined using a light microscope on 10× magnification for the presence of ova

Appendix B. List of Measured Organisms by Pathogenicity

Table B-1. List of Measured Organisms by Pathogenicity

Non-pathogenic

Aerobic Mesophilic
Bacteroidales (Genbac3)
Bacteroidetes (BacR)
Escherichia coli
Enterococci (ENT)
Enterococcus spp
Fecal coliform
Faecal streptococci
Staphylococcus
*

Pathogenic

Ancylostoma duodenale, Enterobius Vermicularis, Toxocara canis
Ascardia galli
Ascaris lumbricoides
Ascaris lumbricoides, Enterobius vermicularis
Ascaris lumbricoides, Enterobius vermicularis, Trichuris trichiura, Taenia sp.
Enterobius vermicularis
Enterobius vermicularis, Trichuris trichiura
Giardia lambia
Klebsiella pneumoniae
Staphylococcus aureus
Staphylococcus, Micrococcus, Klebsiella pneumonia (3)
Streptococcus pyogenes
Taenia sp.
Trichuris trichiura

* Pathogenicity could not be discerned as the organisms in a sample were not stated

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