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Assessing the association between the *Escherichia coli* counts in household drinking water and other risk factors with the occurrence of diarrhea in children under-five across four countries.

By

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Master of Public Health

Epidemiology

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Vishnu Ravi Kumar

B.D.S., Manipal University, 2015

Emory University, 2019

Faculty Thesis Advisor: Matthew C. Freeman, Ph.D., MPH

An abstract of

A thesis submitted to the Faculty of the

Rollins School of Public Health of Emory University

in partial fulfilment of the requirements for the degree of
Master of Public Health
in Epidemiology

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Abstract

Assessing the association between the *Escherichia coli* counts in household drinking water and other risk factors with the occurrence of diarrhea in children under-five across four countries.

By Vishnu Ravi Kumar

Background: Diarrhoeal diseases remain an important cause of mortality and morbidity among children, particularly in low- and middle-income countries. Diarrheal disease leads to around 525,000 deaths in the children under the age of five and is the second leading cause of under-five childhood deaths. Water quality is an important determinant of diarrheal disease but is captured in only a limited number of large routine population-based surveys. The aim of this study was to assess the association between *E.coli* counts in drinking water and the occurrence of diarrhea among children aged 0-5 years.

Methods: Multiple Indicator Cluster Surveys (MICS), a cross-sectional household survey, was conducted in Bangladesh, Nepal, Republic of Congo and Paraguay. The survey included an indicator for diarrhoea episodes in the two weeks preceding the survey, as recalled by the primary caregiver, and *E.coli* counts in drinking water. The sample size was 3609 children, yielding information on sociodemographic, environmental and hygiene related behavioural factors of the household. Mixed univariable and multivariable logistic regression analyses was used to identify the risk factors associated with the occurrence of diarrhoea in children under-five.

Results: The reported prevalence of diarrhoea among children under the age of five during the 2 weeks preceding the survey was 12%. Univariable and the multivariable analysis showed there to be no association between the incidence of diarrhea and the *E.coli* counts in the drinking water. Multivariable regression revealed an association between the incidence of diarrhea in children under-five and the source of drinking water, wealth index of the household and age (in years) of the child.

Conclusion: The results of this study reveal that there is no association between *E.coli*-based water quality measures on the day of the survey and the outcome of diarrhea. This may be because both exposure and outcome are imprecise; paternal recall is associated with bias and *E.coli* counts may not be a good predictor of the full extent of pathogen exposure in the drinking water or the overall environmental exposure.

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CHAPTER 1:

LITERATURE REVIEW

Introduction

Diarrhea is a leading cause of morbidity and is the second major cause of mortality among children under the age of five worldwide, especially in Low-and Middle-income countries (LMIC) (1, 2). Diarrhea is defined as the passage of three or more loose or liquid stools per day. In children, the frequent passing of well-formed stools is not diarrhea, neither is the passing of 'pasty' stools by breastfed children (3). It is a common sign of an intestinal infection that could be caused by bacteria, viruses and parasitic organisms (3). A major mode of transmission of these micro-organisms is through unclean water and food and is prevalent in areas with poor sanitation and reduced access to safe water.

Children under the age of five years in low- and middle-income countries are particularly vulnerable to the occurrence of diarrhea, leading to malnutrition, stunting and in some cases, death. Every year, approximately 1.7 billion cases of diarrhea with around 525,000 cases of death related to diarrhea are reported in children under the age of five (3). The World Health Organization (WHO) and United Nations Children's Fund (UNICEF), in the year 2013 initiated the Global Action Plan for Pneumonia and Diarrhea (GAPPD), as an outline to end preventable child deaths due to diarrhea and pneumonia by 2025 (4). GAPPD aims to reduce the number of deaths attributable to diarrhea to less than 1 per 1000 live births and to reduce the incidence of severe diarrhea by 75% compared to 2010 in the year 2015. A country's "GAPPD Score" measures the usage of interventions that protect against, prevent and treat pneumonia and diarrhea, the higher the score, more the usage of interventions and as of the 2018 estimates, the median score in the 15 high-burden countries is 36% (5). To meet these goals there has to be an increase in the access to improved water sources and sanitation facilities in all households by the year 2030 in addition to changes in socio-economic and behavioural determinants (4).

Water, sanitation and diarrhea

Infectious diarrhea can be caused due a range of pathogens that include viruses, bacteria and protozoa; Rotavirus, Shigella, enteropathogenic *Escherichia coli* (*ETEC*), salmonella and campylobacter are some of the causative agents of diarrhea in children under the age of five (6). These organisms are transmitted via faecal-oral route through the ingestion of contaminated water and food. Inadequate access to improved sources of water, improved sanitation facilities and unsafe disposal of human faecal waste are key risk factors for contracting these pathogens (7). Access to hand washing (with soap) facilities, improved sources of drinking water and sanitation facilities with safe disposal of human faecal waste have been associated with a 48%, 17% and 13%, reduction of diarrhea in children under the age of 5 years, respectively (7, 8). Some of the additional risk factors include socio-economic factors, maternal education, behavioural and environmental determinants such as, handwashing behaviours, treatment of water for drinking and distance to water source (9).

Globally, significant progress has been made in reducing child mortality over the past 25 years. There has been a 53% reduction in the number of deaths, from 91 deaths in 1000 live births to around 43 deaths per 1000 live births, hence saving around 48 million children since 2000. Presently, countries in Sub-Saharan Africa and South Asia have some of the highest rates of under 5 years child mortality (U5MR) (10). In the year 2000, the Millennium Development Goals (MDG) were adopted and aimed at reducing the severe gaps that existed between the rich and poor populations. Goal 4 of the MDG sought to reduce the mortality rate amongst children by two thirds (11). Even with a growing population, there has been a reduction by half of the mortality rates among children; still, this is short of the envisioned reduction of around two-thirds. The reduction in U5MR be attributed, in part, to access to improved drinking water sources, reduction in open defection, and access to improved sanitation (9). Given this knowledge, it is estimated that around 5.5% deaths among children under the age of 5 years can be prevented by increasing access to better WASH (Water, Sanitation and Hygiene) facilities (12).

'Improved drinking water sources' are those sources that, by nature of their construction or through active intervention, are protected from outside contamination, particularly faecal matter (13). Access to safe drinking water from improved sources in rural areas is decreasing, leading to people having to travel longer distances to get to sources of safe water (14). This leads to a reduction in the quantity and the quality of water stored in households. There is evidence to show that with improved water at point of source, there is a 73% reduction in the risk of diarrhea, and that the risk reduces by around 28% when water is treated at point of use and stored in the household (15).

Countries

A majority of the cases of diarrhea are reported to occur in Low- and Middle- income (LMIC) countries; five of these countries account for over half of all the cases of diarrhea; India, Pakistan, Nigeria, Afghanistan, and Ethiopia (16). Diarrhea in these LMIC is a result of the absence of sanitation, hygiene, safe drinking water, socio-economic and behavioural factors that lead to spreading of preventable diseases, and a lack of funding of appropriate treatment methods such as oral Rehydration Solution, with over 60% of the diarrheal disease burden being among children under the age of 5 years (17). Globally, 780 million people do not have access to safe drinking water, and around 2.5 billion people lack access to safe sanitation and hygiene , with 7 out of 10 of those having poor access to sanitation living in rural settings (18).

In the Federal Democratic Republic of Nepal, a county in South Asia with a population of 27.47 million people, in 2012 had around 80% of the population living in rural areas. The average life expectancy at birth is about 68 years (2). The prevalence of diarrhea in the under-five children in Nepal is around 14% with most of the cases have been associated poor sanitation and hygiene (2). As per the national government estimates, 85% of the population has access to the basic water supply, and 62% of the population has access to basic sanitation facilities and (19). Some of the major challenges faced in Nepal include; a lack of adequate financial and human resources and lack of affordable technology (20).

The People's Republic of Bangladesh, a country in South-Asia, has a population of around 162.95 million people. As of 2015, 98% of the population gets its water from a technologically improved source, and only around 3% of the population now practices open defecation, all of this due to significant changes in behaviour and building many new toilets over the past years. Even with such impressive improvement, there is a long way to go for Bangladesh to meet the Sustainable Development Goals (SDG) of providing universal access to safe water and sustainable sanitation by the year 2030. Bangladesh is one of the 15 high burden countries with a GAPPD for diarrhea of around 50% (5). Around 40% of the water provided by these improved sources of water are contaminated with *E. coli* (21).

The Republic of Paraguay, a landlocked country in South America with a population of 6.69 million people and has an expected life expectancy at birth of around 75 years. In 2012, around

147 deaths of children below the age of five were attributed to poor WASH facilities. 80% of the population has access to improved sanitation facilities and 94% had access to drinking water from improved sources. This was possible, in part, due to recognition by law that access to adequate and quality water is a human right and also, in part, due to the efforts of the government to provide piped water to both the rural and urban areas (22).

The Republic of Congo, a country in Sub-Saharan Africa has a population of 5 million people and a life expectancy at birth of about 60 years. Research on topics related to incidence of diarrhea in children under the age of 5 is scarce from the Republic of Congo, with most of the research and programs focused on the neighbouring country of the Democratic Republic of Congo.

Microbiological indicators and *Escherichia Coli*

Drinking water has been established as a mode of transmission for diarrheal pathogens and in most countries of the developed world the central water distribution systems test and treat the water for microbiological contaminants; this has led to an almost total elimination of waterborne diseases in the western world. But it is not feasible to test the water for all know entheogenic pathogens; instead there is a heavy reliance on the indicator organisms, of bacterial origin, as a proxy to measure the water quality for fecal contamination. (23–25). As per the current WHO guidelines, *Escherichia coli* are used as indicators of fecal contamination and as an index organism for the presence of contamination and waterborne pathogens (26, 27).*E.coli* is a common gut bacterium found in the lower intestines of warm-blooded organisms. Most strains are harmless, but certain strains such as the Shiga toxin-producing Escherichia coli (STEC) and the *eae* gene-positive strains belonging to the

Enteropathogenic Escherichia coli (EPEC) serotypes are associated with the diarrhea. The most common mode of transmission of *E.coli* is through consumption of faecal contamination of water, and undercooked and contaminated food items. Person to person spread via the faecal-oral route is a common mode of spread of *E. coli* and can be carried asymptomatically by carriers for up to 1 week or less in adults and more than 1 week in children (28). Since *E.coli* is found in all mammal faeces and is not known to multiply in the environment, it has been chosen as the biological indicator for any faecal contamination and hence of water potability (29). WHO has defined acceptable bacterial limits for *E.coli* in drinking water at 0 CFU in a 100ml sample (30).

Risk factors

Demographic Factors: There exists a strong association between demographic factors and the occurrence of diarrhea in children. The prevalence of diarrhea is higher among younger children and in the under 5 category the incidence of diarrhea is more common among children who are in the age group of 6 months to 11 months and remain high for the following year and then decreases as the child ages (31–33). The prevalence of diarrhea is also known to be higher in the cases of boys and in households where the mother or caretaker has low level education (2,32–34). The association between the incidence of diarrhea among children under-five and their mother's education level are inverse, as the level of education increases there is a reduction in the prevalence and risk of diarrhea (33, 35).

Socio-economic factors: Factors such as income range and the area where the household is located are known to be associated with the occurrence of diarrhea. Children in rural households tend to have a lower number of cases of diarrhea in children as compared to urban households (36), and as the wealth index rises there is a reduction in the prevalence and risk of diarrhea in children under-five (36). This can be attributed to easier access to quality medical care and better knowledge about hygiene and sanitation practices. Households that have a higher number of children under the age of five tend to have a higher risk of diarrhea incidence (31, 32). Similarly, houses with a higher number of members tended to have a higher incidence and risk of diarrhea among the children under the age of five, mainly in the rural and in households that are lower down on the wealth index (36, 39, 40).

Evincing the association between the incidence of diarrhea in children under-five and poor WASH facilities in LMIC, studies have shown that increased distance between the water source and household leads to greater odds of diarrhea (33, 34). Also, a strong association between the occurrence of diarrhea and the type of water source, has been shown. Households that derived water from an improved water source have been shown to have a lower risk of diarrhea compared to households that derived from un-improved water sources (14, 43).

Research question:

- What is the association between diarrhea in children under the age of five and *E. coli* in drinking water?
  - How is diarrhea in children under the age of five associated with environmental, behavioral and socio-economic risk factors?

Objectives:

- Quantify the association between *E. coli* in the drinking water and prevalence of diarrhea in children under the age of five.
- To determine if the *E. coli* associated bacterial water quality tests conducted in large population-based surveys are a good estimator for the prevalence of diarrhea in populations.
- Quantify the association between prevalence of diarrhea in children under-five and other risk factors such a socio-economic (wealth index, mother's literacy, age and sex of the child, region of household and number of members in household),

environmental (type of source of drinking water, distance to water source, presence of animals in the household) and behavioral factors (method of water treatment for drinking).

Inclusion criteria:

- Presence of at least one child younger than the age of 5 years;
- Households selected for *E. coli* testing of drinking water.

CHAPTER II:

MANUSCRIPT

Introduction

Over a billion people around the world do not have access to safe WASH (Water, Sanitation and Hygiene) facilities and are prone to various communicable disease, many of them preventable diseases. Diarrheal diseases are the second leading cause of child death, accounting for 525,000 deaths and 9% of the postnatal deaths in children under the age of five (44). Diarrhea is defined as three or more loose stools in a period of 24 hours, watery or unusual stools exceeding the child's normal stool, with or without vomiting (45).

In the 2017 Sustainable Development Goals (SDG) report, unsafe drinking water, unsafe sanitation and lack of hygiene are still reported to be major contributors to global mortality and diarrhea as still being very prevalent in the low- and middle-income countries, accounting for around 4 out of 5 under-five deaths (41, 42). 60% of the diarrheal disease burden amongst children under-five is due to a lack of safe WASH facilities (17). 780 million people do not have access to safe drinking water, and around 2.5 billion people lack access to safe sanitation and hygiene , with seven out of ten of those having poor access to sanitation living in rural settings (18). As such, approximately 5.5% deaths among children under the age of 5 years can be prevented by improving the access to improved drinking water, sanitation, and hygiene (12). Access to safe drinking water in rural areas is decreasing, leading to people having to travel longer distances to get to safe sources of water (14). When households have water from improved sources, there is a 73% reduction in the risk of diarrhea, and that the risk reduces by around 28% when water is treated at point of use and stored in the household (15). Past studies have shown there to be a 10-fold increase in the *E.coli* counts leading to a 16% increase in diarrhea (48).

Escherichia coli (*E. coli*), is a common gut bacterium found in the lower intestines of warm-blooded organisms. Most strains are harmless, but certain strains such as the Shiga toxin-producing *Escherichia coli (STEC*) and the *eae* gene-positive strains belonging to the Enteropathogenic *Escherichia coli (EPEC)* serotypes are associated with the diarrhea. The most common mode of transmission of *E.coli* is through consumption of faecal contamination of water, and undercooked and contaminated food items. Person to person spread via the faecal-oral route is a common mode of spread of *E. coli* and can be carried asymptomatically by carriers for up to one week or less in adults and more than one week in children (28). Since *E. coli* is found in all mammal faeces and is not known to multiply in the environment, it has been chosen as the biological indicator for any faecal contamination and hence of water potability (29).

We conducted a study to quantify the association between the detectible *E.coli* in drinking water and reported diarrhea in the previous two weeks. The purpose of the study was to (i) estimate if the prevalence of diarrhea in children under the age of five varied with the dose of *E.coli* in the drinking water; (ii) to determine if *E.coli* based bacteriological water quality tests conducted in large population-based surveys were good estimators of risk of diarrhea (iii) estimate the association between prevalence of diarrhea in children under-five and other risk factors such a socio-economic (wealth index, mother's literacy, age and sex of the child, region of household and number of members in household), environmental (type of source of drinking water, distance to water source, presence of animals in the household) and hygiene related behavioural factors (method of water treatment for drinking). We used data from the Multiple Indicator Cluster Surveys from four countries, Bangladesh, Republic of

Congo, Nepal and Paraguay to assess this association, controlling for the pre-specified confounders.

Methods

Study Design: We analyzed data from the Multiple Indicator Cluster Surveys (MICS 5), conducted in various countries by the United Nations International Children's Emergency Fund (UNICEF) in collaboration with the National Statistical Institute of each of the countries. The MICS surveys are a descriptive, cross-sectional household survey. The surveys were conducted as part of face-to-face interviews in selected representative households that were selected to be nationally and sub-nationally representative (49).

Study Population: The study assessed the occurrence of diarrhea in children under the age of five years in the past two weeks, as reported by the mothers/caregivers. Our study used MICS data from four countries; Paraguay (2016), Bangladesh (2012-13), Nepal (2014), and in the Republic of Congo (2014-15) (50).

The People's Republic of Bangladesh, a South-Asian nation, had a population of around 156.60 million people and a life expectancy at birth of about 69 years. It has a high a burden of diarrhea in children with over 7,062 deaths due to diarrhea in children under-five reported in 2016 and a GAPPD score of 54 (5). Over the years the country has made noteworthy progress in providing increased access to improved WASH facilities. 98% of the population now gets its water from a technologically improved source, and only around 3% of the population now practices open defecation, all this due to significant changes in behaviour and building many new toilets (51). But even with such impressive improvement, there is a long way to go for Bangladesh to meet the SDG of providing universal access to safe water and sustainable sanitation by the year 2030. Around 40% of the water provided by these improved sources of water are contaminated with *E. coli* (21). Widespread poverty and illiteracy leads to reduced healthcare seeking behaviour, reduced handwashing and disparate access and use of health facilities (51).

In the Federal Democratic Republic of Nepal, another South Asian country with a population of 28.32 million people and a life expectancy at birth of about 69 years in 2014, reported around 3522 diarrheal deaths in children under 5 that were attributed to poor WASH facilities (20). The usage of improved sanitation facilities was around 37%, and the use of water from improved sources was around 88%. Some of the major challenges faced in Nepal are: Lack of adequate financial and human resources, lack of affordable technology, illiteracy, poverty and unequal access to WASH facilities. (20).

The Republic of Paraguay is a landlocked country in South America with a population of 6.75 million people and has an expected life expectancy at birth of around 75 years in 2014. 80% of the population had access to improved sanitation facilities and 94% had access to drinking water from improved sources. This was possible, in part, due to recognition by law that access to adequate and quality water is a human right and also, in part, due to the efforts of the government to provide piped water to both the rural and urban areas (22).

The Republic of Congo, a country in Sub-Saharan Africa has a population of 5 million people and a life expectancy at birth of about 60 years. Research on topics related to incidence of diarrhea in children under the age of 5 is scarce from the Republic of Congo, with most of the research and programs focused on the neighbouring country of the Democratic Republic of Congo.

Districts/sub-regions were identified as the main sampling strata, followed by the selection of census enumeration areas, and using the household listings, a subset of houses was selected to be tested for the *E. coli* levels in the household water. Household were considered eligible for inclusion in our research if the following criteria were met: (i) Presence of at least one child younger than the age of five years; (ii) Household selected for *E.coli* testing of drinking water.

Data Collection. The data collection was done by the MICS5 based as per their published protocol (52). Based on the time allocated to undertake the survey and the sample size in each country, the required number of fieldworkers were trained in administering the customized version of the questionnaires. The teams included a supervisor, interviewers, and a water quality measurer. The supervisor was tasked with monitoring and troubleshooting daily operations. Interviewers conducted the face-to-face interviews using a tablet, and the measurers are trained to conduct the water quality testing. The field teams were all provided with guidelines, manuals, and detailed instructions. The MICS program required a minimum of four weeks training with the support of a regional expert, this includes the training for the interviewers and the measurers. The measurer was responsible for the water sample collection from the field and were trained in aseptic water collection techniques to prevent any contamination. To collect the household drinking water samples, the measurer was trained to ask the survey respondent for a "glass of water that a member of the household would drink" as per the instructions provided in the guidelines. Sterile Whirl-Pak bags were used to collect the water (53).

Variables. The outcome variable was the period prevalence of diarrhea (as recalled by the primary caregiver) during the 2-week period prior to the survey interview. The primary predictor of interest was presence and severity of contamination of *E.coli* in household drinking water. We categorized detectible *E. coli* into four categories per established cut-offs: <1 *E.coli* (CFU/100ml) was '*Low Risk*', 1-10 *E.coli* (CFU/100ml) was '*Medium Risk*', 11-100 *E.coli* (CFU/100ml) was '*High risk*', and >100 *E.coli* (CFU/100ml) was '*Very High risk* ' (30). Other confounding variables included (i) Socio-demographic information (age of the child in years, sex of the child, region of the household (urban/rural), number of children below the age of 5 in the household, number of household members and wealth index quintiles); (ii) Mother's level of education; (iii) Region of the household *(urban/rural)*; (iv) Presence of animals in the household *(Yes/No)*; (v) Method of water treatment to make it safe for drinking, as reported, was classified as appropriate *(boiling, adding bleach, solar disinfection or ceramic filters)* or inappropriate *(sari cloth filtration, let standing, no purification)* (54); (vi) Time to source of water (self-reported), classified as : *<10 minutes, 10 to 30 minutes, and > 30 minutes* ; and (vii) Source of drinking water (self-reported) classified as Improved *(Piped water, tube well, Borehole, protected well, protected spring),* Unimproved *(Unprotected well or spring, rain water collection, tanker truck, surface water and others)* (52,55–58).

Statistical Analysis. Statistical analyses were performed in SAS Version 9.4. The data for each of the countries is open sourced (50). Descriptive statistics were used to summarize the study variables, individually for each country, and for the pooled data. Mixed univariable and multivariable logistic regression models were performed to understand the association between the outcome and the exposure variables and was expressed by their odds ratios (OR) and 95% confidence intervals (CI). The regression models were used with random intercepts to account for the country level clustering. To improve the precision of the estimates of the final models, we only considered variables that had a *P*-value below 0.2 in the univariable analysis for the Multivariable analysis. The statistical significance was defined at α=0.05.

Results

Descriptive Analysis: A total of 3609 children under the age of five years were included in the study (49.6% female) from the four countries. Demographic and socio-economic characteristics of children and respective households are summarized in Table 1. The overall period prevalence of diarrhea in the previous two weeks as reported by the primary caregiver was 11.7%. 57.9% and 23.8% of the households had *E.coli* counts in the *'High Risk*' and *'Very High Risk'* categories, respectively. A majority of the households in Bangladesh and The Republic of Congo (76.8% and 92.7%, respectively) had drinking water with *E.coli* counts in the *'High risk'* category ; whereas in Nepal and Paraguay, 57.2% and 54.8% of the households, respectively, had drinking water with *E.coli* counts in the '*Very High Risk'* category. The prevalence of diarrhea among children was found to be about the same across the four categories of *E.coli* associated risk category (Table 2).

Most of the households surveyed, in Bangladesh, Nepal, and The Republic of Congo, were classified as being in rural areas, whereas only 41.4% of the households in Paraguay were rural. Animals were found in 54.6% of the households; with 70.5% and 78.6% of the households in Bangladesh and Paraguay, respectively, having animals in the household. Over half of the mothers (58%) in the survey had some formal education, but both Nepal and The

Republic of Congo (44.4% and 60.9%, respectively) had a substantial proportion of mothers with no formal education, or had not completed their primary education.

77% of households had a primary drinking water source within 10 minutes of their home; only 42.6% of the households in the Republic of Congo were within 10 minutes from their primary source of drinking water. A pooled average of 85% of the households used an improved source of water for drinking purposes, whereas overall, 11% of these households used appropriate procedures to treat the water prior to drinking.

Multivariable Analyses: We did not find any association between *E. coli* and diarrhea. In households with *E.coli* counts in the *'Medium Risk', 'High Risk'* and *'Very High Risk'* categories, the aOR of diarrhea among children under the age of five was 1.14, 0.93, 0.96 respectively, when compared to the odds of diarrhea in children exposed to bacterial counts in the *'Low Risk'* category. Results from the univariable and multivariable analysis are presented in Table 2 and Table 3.

The level of education of the mothers was inversely associated with the prevalence and odds of diarrhea in children under-five. Households with mothers who had completed primary education had 18% lesser odds of their children having diarrhea when compared to mothers who did not have any education. The odds were even lower for mothers who had completed their secondary education (aOR=0.77). The wealth index of the household was strongly associated with the prevalence of diarrhea in the child in both the univariable and multivariable analysis. When compared to the 'Poorest', the 'Richest' had an almost 50% lower odds of diarrhea. Neither the number of members in the household (5 to 8 members: aOR=1.0; more than 8 members: aOR=0.9) nor the number of children under-five in the household (aOR=1.0) had an association with the prevalence of diarrhea in children under the age of five. In rural households the adjusted odds ratio of diarrhea among children under the age of five years was 33% lesser than that of urban households.

The method of water treatment was associated with diarrhea; households practicing inappropriate water treatment practises had 20% greater odds of diarrhea compared to households practicing acceptable water purification practices. Distance to a primary drinking water source was associated with higher odds of diarrhea (10 to 30 minutes: aOR=1.21; >30 minutes : aOR=1.16). Un-improved sources of drinking water were associated with a higher odd (aOR=1.25) of diarrhea in children under-five when compared to water from improved sources.

Discussion

In this study, we quantified the association between the occurrence of diarrhea and its risk factors among the children younger than 5 years of age. We found no overall association between *E. coli* contamination and diarrhea when controlling for the predetermined confounders. Prior studies that have evaluated the relationship of bacterial counts in drinking water and the occurrence of subsequent diarrhea have produced conflicting reports, with some authors reporting that there is no association between indicator organisms, such as *E.coli* , and the occurrence of diarrhea whereas, some other studies have shown there to be a weak association (23,24,61–65). These findings indicate that other transmission routes of diarrheal disease are more important. This could be because the indicator organisms are not strong indicators of the presence of enteropathogenic bacteria in the test sample (65, 66). Another possible explanation is that widespread immunity in the population to the common entheogenic pathogens weakens the association between the dose of the exposure and the risk of diarrhea (40, 53, 57, 58). Importantly, the water quality at the time of testing may not be indicative of the contamination at the time of occurrence of diarrhea. Microbial indicators of drinking water have been shown to vary markedly over short durations and have been shown to be weak indicators of contamination weeks or months after the outcome (69, 70).These findings draw into question the validity in using *E.coli* as an indicator organism to measure the quality of drinking water by the UNICEF as part of the MICS and other organizations and stakeholders.

The prevalence of diarrhea among children under the age of five, in Bangladesh was similar to the results from other studies that indicated it to be around 1 % to 6% (31, 32). Whereas in Nepal, the estimated prevalence was higher than the 8% reported in other research studies (60). The occurrence of diarrhea in the two countries can be attributed to the occurrence of extreme poverty and high population density (61). The results showed there to be an inverse association between the age of the child, mother's level of education, wealth index and the prevalence of diarrhea among children, respectively. Girls under the age of five and children from rural households tended to have lower odds of diarrhea as compared to boys under the age of five and children from urban households, respectively. Access to improved WASH facilities (reduced distance to source of drinking water and improved sources of drinking water) and appropriate water treatment methods resulted in children under five having lower odds of diarrhea.

Our results showed an association between the occurrence of diarrhea and the mother's level of education, with there being a lower odd of diarrhea in households where the mother or the caregiver had attended formal education (primary education and above). These results are corroborated by previous results that show there to be an association mother's level of education and the occurrence of diarrhea (32, 33, 36, 71).Diarrhea was seen to be less prevalent in girls as compared to boys. This is consistent with results of prior studies that showed boys to have a higher odds of diarrhea as compared to girls (64, 72–74).

Prior studies evaluating the relationship between the socio-economic status of the household and the occurrence of diarrhea have shown households higher-up on wealth index as having a lower prevalence of diarrhea and a reduced aOR of diarrhea among the children in the under-five category. This concurs with the results of our study that show there to be a negative association between the prevalence of diarrhea and the wealth index. The higher the households are on the socio-economic scale, the lower the odds of under-five children having diarrhea (76–78). A plausible explanation for this observation could be that wealth and socio-economic status of the household is associated with better access to amenities, facilities and hygiene, thus leading to a reduction in diarrhea. Our unadjusted results also indicate that children in rural households were more likely to have diarrhea as compared to children in households located in urban areas, even when controlling for water type and distance. We found that the children living in rural households were at lower odds of contracting diarrhea than children in urban households, which is supported by other articles that compare the prevalence of diarrhea based on the region their households are located in (79).

The results of our univariable analysis show no association between the number of under-five children in a household and incidence of diarrhea. Previous studies show a positive association between the number of under-five children in the household and the incidence of diarrhea (32, 36, 79). Similarly, this study shows there to be no association between the association between the incidence of diarrhea and the presence of animals in the household. This is in contrast to other studies that have shown a positive association between presence of household animals and diarrhea in children under-five (36, 79, 80).

The results of the adjusted analysis showed that U5 children in households using unimproved water sources had 25% increased odds of contracting diarrhea. The finding is similar to that of a randomized controlled study done in Ghana, that showed that the use of drinking water from an improved source can lead to a reduction of cases of diarrhea by as much as 11% (82).

We found that that the prevalence of diarrhea in U5 children increased with an increase in the time taken to the source of water. When compared to time taken to water source being less than 10 minutes, there was around a 20% increase in the aOR of diarrhea among the children, this is similar to the findings of a study in Kenya (83). The results from our study and other studies show that the prevalence of diarrhea in households that use inadequate modes of water treatment is higher as compared to those that use adequate methods of water treatment. Also, there is a 20% increase in the odds of diarrhea in U5 children in households that use inadequate modes of water treatment (84).

One strength of the study is that data were obtained as part of the Multiple Indicator Cluster Surveys conducted by the UNICEF in the four countries, which is representative at the national and sub-national levels, and hence generalizable to the children under the age of 5 years. The quality of the data collected was ensured by the complex clustering and sampling strategies to reduce bias. A limitation associated with using data from the MICS is the reduced data quality as part of the surveying process, leading to a reduction in the validity of the results.

There were several limitations of this study. Since the household surveys the test for water quality were after the clinical manifestations of diarrhea, the results from the water sample tests may not be an accurate representation of the water quality at the time of diarrhea incidence in the child. The children in the households were not clinically assessed; rather, the illness was measured based on the primary caregivers' report of the child having the condition in the period 2 weeks prior to the survey. This could lead to an under- or over-reporting of the occurrence of diarrhea based on the socio-economic cultural perception of the families and households involved. Under-reporting could also occur due to recall bias, mothers may have forgotten the incidence of diarrhea over the past 2 weeks. Since the data is cross-sectional and based on a minor representation of the population from the four countries at a specific time, causal associations cannot be drawn between the outcome, diarrhea, and the various socio-economic and behavioural practises. The lack of certain variables in the data analysis, such as breast-feeding history, the nutritional status of the child and the hygiene and sanitation practices also limited our overall assessment.

TABLES

Table 1: Socio-demographic features of under-five children and households in the four countries that were surveyed.

Characteristics	Total	Bangladesh	Republic of Congo	Nepal	Paraguay
Child Characteristics	*N=3609*	N=1019	N= 1070	N=622	N=898
Age of the Child (years)					
< 1	715 (19.8%)	181 (17.8%)	253 (23.6%)	116 (18.7%)	165 (18.4%)
1	694 (19.2%)	195 (19.1%)	181 (16.9%)	114 (18.3%)	204 (22.8%)
2	734 (20.3%)	205 (20.1%)	220 (20.6%)	129 (20.7%)	180 (20.0%)
3	740 (20.5%)	216 (21.2%)	207 (19.4%)	139 (22.4%)	178 (19.8%)
4	726 (20.2%)	222 (21.8%)	209 (19.5%)	124 (19.9%)	171 (19.0%)
Gender: Female	1791 (49.6%)	486 (47.7%)	569 (53.2%)	325 (52.2%)	411 (45.8%)
Diarrhea (2-week recall)	422 (11.7%)	37 (3.6%)	203 (19.0%)	93 (15.0%)	89 (9.9%)
Household Characteristics					
***E. coli* associated risk (for diarrhea)#**					
Low Risk	360 (10.0%)	119 (11.7%)	42 (3.9%)	74 (11.9%)	125 (13.9%)
Medium Risk	300 (8.31%)	113 (11.1%)	28 (2.6%)	43 (6.9%)	116 (12.9%)
High Risk	2089 (57.88%)	783 (76.8%)	992 (92.7%)	149 (24.0%)	165 (18.4%)
Very High Risk	852 (23.8%)	4 (0.4%)	8(0.8%)	356 (57.2%)	492 (54.8%)
Mother's Education					
None	682 (18.9%)	272 (26.7%)	129 (12.1%)	269 (43.2%)	12 (1.3%)
Primary Incomplete	846 (23.4%)	138 (13.5%)	346 (32.3%)	110 (17.7%)	252 (28.1%)
Primary Complete	890 (24.7%)	142 (14.0%)	470 (43.9%)	126 (20.3%)	152 (16.9%)
Secondary Complete	819 (22.7%)	359 (35.2%)	125 (11.7%)	117 (18.8%)	218 (24.3%)
Secondary education or higher	372 (10.3%)	108 (10.6%)	0 (0.0%)	0 (0.0%)	264 (29.4%)
Region					
Rural	2502 (69.3%)	860 (84.4%)	754 (70.5%)	516 (83.0%)	372 (41.4%)
Number of HH members					
Less than 5	1217 (33.7%)	377 (37%)	283 (26.4%)	195(31.4%)	362 (40.3%)
5 to 8	1990(55.1%)	570 (55.9%)	648 (60.6%)	333 (53.5%)	439 (48.9%)
More than 8	402 (11.14%)	72(7.1%)	139 (13.0%)	94 (15.1%)	97 (10.8%)
Number of U5 children in HH					
2 or less	3297 (91.4%)	993 (97.5%)	904 (84.5%)	563 (90.5%)	837 (93.2%)
3 or more	312 (8.6%)	26 (2.5%)	166 (15.5%)	59 (9.5%)	61 (6.8%)
Animals in HH					
Yes	1971 (54.6%)	718 (70.5%)	368 (34.4%)	489 (78.6%)	396 (44.1%)
Time to source of water					
< 10 mins	2767 (76.8%)	814 (79.9%)	456 (42.6%)	622 (100%)	875 (97.5%)
10 to 30 mins	614 (17.0%)	185 (18.1%)	407 (38.0%)	0 (0.0%)	22 (2.4%)
>30 mins	228 (6.2%)	20 (2.0%)	207 (19.4%)	0 (0.0%)	1 (0.1%)
Source of Drinking water					
Improved	3081 (85.4%)	981 (96.3%)	687 (64.2%)	551 (88.6%)	862 (96 %)
Treatment of Drinking water					
Appropriate	380 (10.5%)	50 (4.9%)	50 (4.7%)	105 (16.9%)	175 (19.5%)

Categorized detectible *E. coli* into four categories per established cut-offs: <1 E. coli (CFU/100ml) was '*Low Risk*', 1-10 E. coli (CFU/100ml) was '*Medium Risk*', 11-100 E. coli (CFU/100ml) was '*High risk*', and >100 E. coli (CFU/100ml) was '*Very High risk*

Table 2. Unadjusted Analysis of Risk Factors Associated with Diarrhea in under-five children

Characteristics	n (%)	OR (95% CI)	P-value
***E. coli* associated risk (for diarrhea)#**			
Low Risk	9.2	Ref.	
Medium Risk	10.3	1.2 (0.9 -1.6)	0.39
High Risk	12.1	1.1 (0.7- 1.5)	0.74
Very High Risk	12.2	1.1 (0.8- 1.7)	0.58
Age of the child (in years)			
< 1	13.6	Ref.	
1	18.7	1.6 (1.2-2.1)	<0.001
2	12.1	0.9 (0.7-1.3)	0.62
3	8.0	0.6 (2.4-0.9)	<0.001
4	6.5	0.5 (0.3-0.7)	<0.001
Gender			
Male	12.1	Ref.	
Female	11.3	0.9 (0.7-1.1)	0.22
Mother's Education			
None	12.6	Ref.	
Primary Incomplete	14.8	1.0 (0.7-1.3)	0.74
Primary Complete	12.7	0.7 (0.5-1.0)	0.01
Secondary Complete	9.4	0.8 (0.6-1.2)	0.04
Secondary education or higher	5.7	0.6(0.3-0.9)	<0.001
Region			
Urban	10.8	Ref.	
Rural	12.1	1.2 (1.0-1.6)	0.1
Number of HH members			
Less than 5	10.9	Ref.	
5 to 8	12.3	1.0 (0.8-1.3)	0.77
More than 8	11.2	0.9 (0.6-1.2)	0.32
Number of U5 children in HH			
2 or less	11.4	Ref.	
3 or more	15.1	1.0 (0.7-1.4)	0.99
Animals in HH			
Yes	10.9	Ref.	
No	12.7	0.9 (0.8-1.2)	0.25
Time to source of water			
< 10 mins	10.1	Ref.	
10 to 30 mins	16.3	1.4 (1.1-1.9)	0.01
>30 mins	19.3	1.4 (0.9-2.2)	<0.001
Source of Drinking Water			
Improved	10.3	Ref.	
Unimproved	20.1	1.5 (1.1-1.9)	0.01
Treatment of Drinking Water			
Appropriate	9.5	Ref.	
Not Improved	12.0	1.3 (1.1-1.5)	<.0001
Wealth Index			
Poorest	15.9	Ref.	
Second	11.6	0.8 (0.6-1.0)	<0.001
Middle	10.2	0.7 (0.5-0.9)	<0.001
Upper	8.7	0.6 (0.4-0.9)	<0.001
Richest	6.9	0.5 (0.4-0.8)	<0.001

Categorized detectible *E. coli* into four categories per established cut-offs: <1 E. coli (CFU/100ml) was '*Low Risk*', 1-10 E. coli (CFU/100ml) was '*Medium Risk*', 11-100 E. coli (CFU/100ml) was '*High risk*', and >100 E. coli (CFU/100ml) was '*Very High risk*

Table 3. Multivariable Analysis of risk factors associated with diarrhea in under-five children

Characteristics	aOR	P-value
E. coli associated risk (for diarrhea)#		
Low Risk	Ref.	
Medium Risk	1.14 (0.71,1.82)	0.59
High Risk	0.93 (0.63,1.35)	0.69
Very High Risk	0.96 (0.64,1.45)	0.86
Age of the Child (years)		
< 1	Ref.	
1	1.61 (1.2,2.16)	<0.001
2	0.91 (0.67,1.24)	0.57
3	0.58 (0.41,0.83)	<0.001
4	0.48 (0.32,0.71)	<0.001
Gender		
Male	Ref.	
Female	0.88 (0.72,1.08)	0.22
Mother's Education		
None	Ref.	
Primary Incomplete	1.00 (0.74,1.36)	0.99
Primary Complete	0.82 (0.59,1.14)	0.23
Secondary Complete	1.05 (0.75,1.48)	0.77
Secondary education or higher	0.77 (0.45,1.29)	0.32
Region		
Urban	Ref.	
Rural	0.88 (0.67,1.16)	0.37
Time to source of water		
< 10 mins	Ref.	
10 to 30 mins	1.21 (0.9,1.64)	0.20
>30 mins	1.16 (0.76,1.79)	0.49
Source of Drinking water		
Improved	Ref.	
Unimproved	1.5 (1.1,1.9)	<0.001
Method of Water Treatment		
Appropriate	Ref.	
Not Improved	1.25 (0.92,1.7)	0.15
Wealth Index		
Poorest	Ref.	
Second	0.8 (0.6,1)	<0.001
Middle	0.7 (0.5,0.9)	<0.001
Upper	0.6 (0.4,0.9)	<0.001
Richest	0.5 (0.4,0.8)	<0.001

categorized detectible *E. coli* into four categories per established cut-offs: <1 E. coli (CFU/100ml) was '*Low Risk*', 1-10 E. coli (CFU/100ml) was '*Medium Risk*', 11-100 E. coli (CFU/100ml) was '*High risk*', and >100 E. coli (CFU/100ml) was '*Very High risk*

CHAPTER III

This study does not evince an association between *E.coli* counts in the drinking water and the incidence of diarrhea in children under the age of five. The results of this study and various other prior studies, draw into question the validity of using *E.coli* as an indicator organism and hence have critical Public Health implications. This lack of positive correlation between E.coli counts and prevalence of diarrhea, provides uncertainty in how we measure safety of drinking water in the these LMIC and in the developed nations.

This study identifies various important socioeconomic, environmental and behavioural risk factors that lead to the occurrence of diarrhea in children under-five and these findings corroborate various national and global evidence of the epidemiology of diarrhea. This study does not evince an association between *E.coli* counts in the drinking water and increased incidence of diarrhea in children under the age of five. The study showed there to be an association between the occurrence of diarrhea and age of the children with children aged 1 having a higher odd of contracting diarrhea. Also, girls were noted to have lesser odds of having a bout of diarrhea. Children younger than five in households in rural areas and those that are lower down in the wealth index are at an increased risk of diarrheal illness, this is due to the scarcity of medical staff and the reduced access to quality medical healthcare. Factors such as access to improved source of water, distance to source of water and appropriateness of water treatment for drinking have been shown to be important factors the odds of occurrence of diarrhea in the children of the age category. The results validate the importance given to meet the goals set as part of the SDG and the Global Action Plan for Pneumonia and Diarrhea to reduce the incidence of diarrhea among the children under-five by provision of appropriate improved WASH facilities.

The results of this study have shown there to be no association between categories of *E.coli* based on the associated risk of diarrhea and the occurrence of diarrhea in children under the age of 5. This result is supported by various other studies that have shown that indicator organisms *E.coli* is not the optimal indicator organism to measure faecal contamination. Hence, to be able to better identify and manage the problem of unsafe drinking water there is a need to identify a more resilient indicator of faecal contamination.
The evaluation of diarrhea risk factors among under-five children is a fundamental step towards decreasing the higher prevalence of diarrhea among children. These findings will help public health officials, non-governmental organisations and the health departments in countries with high diarrhea burden to better understand the areas that need focus and will aid in coming up with evidence-based approaches to decrease the prevalence of diarrhea among under-five children. There is evidence to show that increases access to improved WASH facilities can reduce the burden of diarrhea in populations and this study shows that there is a higher incidence and prevalence of diarrhea in households that do not have access to improved sources of water and follow inadequate modes of water treatment for drinking purposes.

Further research is needed to understand the role of water in the transmission of disease to develop a cost effective and reliable mode of detection of entheogenic bacteria directly in water sources. This would significantly improve efforts to eliminate the prevalence of diarrhea in populations around the world. WASH interventions have been shown to be effective in preventing a large number of cases of diarrhea in children under the age of five by interrupting the transmission of the microbiological agents. Even though they are often difficult to implement in LMIC settings, a concentrated effort by the authorities in charge and the international community on a whole is needed to meet the goals set by the SDG.

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APPENDICES

Table 2a. Bangladesh Unadjusted Analysis of Risk Factors Associated with Diarrhea.

Characteristics	OR (95% CI)	P-value
***E. coli* associated risk (for diarrhea)**		
Low Risk	Ref.	
Medium Risk	1.90 (0.54,6.67)	0.98
High Risk	0.99 (0.34,2.88)	0.98
Very High Risk	<0.001	0.98
Age of the Child (years)		
< 1 year	Ref.	
1	2.37 (0.96,5.87)	<.0001
2	0.62 (0.19,1.99)	0.56
3	0.35 (0.09,1.37)	0.10
4	0.57 (0.18,1.84)	0.43
Gender		
Male	Ref.	
Female	1.30 (0.62,2.52)	0.43
Mother's Education		
None	Ref.	
Primary Incomplete	2.53 (1.02,6.26)	0.00
Primary complete	0.63 (0.17,2.37)	0.38
Secondary incomplete	1.01 (0.42,2.43)	0.91
Secondary education or higher	0.55 (0.12,2.59)	0.33
Region of the household		
Urban	Ref.	
Rural	0.95 (0.39,2.32)	0.92
Number of household members		
Less than 5	ref.	
5 to 8	0.88 (0.44,1.74)	0.90
More than 8	0.69 (0.15,3.08)	0.68
Number of U5 children		
2 or less	Ref.	
3 or more children	2.28 (0.52,10.03)	0.28
Animals in the household		
Yes	Ref.	
No	0.88 (0.42,1.84)	0.73
Treatment of Drinking water		
Appropriate	Ref.	
Unimproved	0.90 (0.21,3.85)	0.89
Time to source of water		
< 10 minutes	Ref.	
10 to 30 minutes	0.70 (0.27,1.83)	0.47
more than 30 minutes	1.33 (0.17,10.25	0.66
Source of Drinking water		
Improved	Ref.	
Not improved	1.50 (0.35,6.49)	0.59
Wealth Index		
Poorest	Ref.	
Second	0.69 (0.27,1.75)	0.71
Middle	0.78 (0.29,2.10)	0.99
Fourth	0.99 (0.39,2.53)	0.49
Richest	0.54 (0.17,1.68)	0.39

Categorized detectible *E. coli* into four categories per established cut-offs: <1 E. coli (CFU/100ml) was '*Low Risk*', 1-10 E. coli (CFU/100ml) was '*Medium Risk*', 11-100 E. coli (CFU/100ml) was '*High risk*', and >100 E. coli (CFU/100ml) was '*Very High risk*

Table 2b. Nepal Unadjusted Analysis of Risk Factors Associated with Diarrhea.

Characteristics	OR (95% CI)	P-value
E. coli associated risk (for diarrhea)		
Low Risk	Ref.	
Medium Risk	1.78 (0.71,4.47)	0.05
High Risk	0.71 (0.32,1.57)	0.10
Very High Risk	0.88 (0.45,1.75)	0.39
Age of the Child (years)		
< 1 year	Ref.	
1	1.02 (0.53,1.97)	0.10
2	0.65 (0.33,1.29)	0.67
3	0.88 (0.45,1.61)	0.43
4	0.33(0.15,0.76)	0.01
Gender		
Male	Ref.	
Female	0.83(0.54,1.3)	0.42
Mother's Education		
None	Ref.	
Primary Incomplete	0.77(0.4,1.47)	0.51
Primary complete	0.82(0.45,1.5)	0.69
Secondary incomplete	1.02(0.57,1.84)	0.55
Secondary education or higher	0	0.00
Region of the household		
Urban	Ref.	
Rural	1.19(0.65,2.19)	0.58
Number of household members		
Less than 5	Ref.	
5 to 8	1.20(0.73,1.99)	0.33
More than 8	0.91(0.44,1.89)	0.58
Number of U5 children		
2 or less	Ref.	
3 or more children	0.75(0.33,1.7)	0.49
Animals in the household		
Yes		
No	0.73(0.41,1.3)	0.29
Treatment of Drinking water		
Appropriate		
Unimproved	1.17 (0.64,2.16)	0.61
Source of Drinking water		
Improved	Ref.	
Not improved	1.62(0.88,3.01)	0.12
Wealth Index		
Poorest	Ref.	
Second	0.66 (0.37,1.18)	0.91
Middle	0.50 (0.23,1.08)	0.41
Fourth	0.59 (0.29,1.18)	0.75
Richest	0.56(0.27,1.19)	0.66

Categorized detectible *E. coli* into four categories per established cut-offs: <1 E. coli (CFU/100ml) was '*Low Risk*', 1-10 E. coli (CFU/100ml) was '*Medium Risk*', 11-100 E. coli (CFU/100ml) was '*High risk*', and >100 E. coli (CFU/100ml) was '*Very High risk*

Table 2c. Paraguay: Unadjusted Analysis of Risk Factors Associated with Diarrhea

Characteristics	OR (95% CI)	P-value
E. coli associated risk (for diarrhea)		
Low Risk	Ref.	
Medium Risk	0.83 (0.3,2.3)	0.20
High Risk	1.88 (0.83,4.26)	0.06
Very High Risk	1.52 (0.73,3.18)	0.26
Age of the Child (years)		
< 1 year	Ref.	
1	1.30 (0.71,2.38)	0.00
2	0.71 (0.35,1.42)	0.86
3	0.48 (0.22,1.03)	0.11
4	0.50 (0.23,1.08)	0.15
Gender		
Male	Ref.	
Female	0.87 (0.56,1.36)	0.54
Mother's Education		
None	Ref.	
Primary Incomplete	0.60 (0.13,2.88)	0.89
Primary complete	0.67 (0.14,3.31)	0.77
Secondary incomplete	0.59 (0.12,2.86)	0.84
Secondary education or higher	0.39 (0.08,1.9)	0.07
Region		
Urban	Ref.	
Rural	1.12 (0.72,1.73)	0.63
Number of household members		
Less than 5	Ref.	
5 to 8	1.20 (0.75,1.91)	0.50
More than 8	1.02 (0.47,2.21)	0.85
Number of U5 children		
2 or less	Ref.	
3 or more children	0.99 (0.41,2.37)	0.98
Animals in the household		
Yes	Ref.	
No	1.12 (0.72,1.75)	0.61
Treatment of Drinking water		
Appropriate		
Unimproved	1.33 (0.73,2.42)	0.35
Time to source of water		
< 10 minutes	Ref.	
10 to 30 minutes	3.58 (1.36,9.39)	0.99
more than 30 minutes	<0.001	0.99
Source of Drinking water		
Improved	Ref.	
Not improved	2.76 (1.22,6.25)	0.02
Wealth Index		
Poorest	Ref.	
Second	0.72 (0.38,1.38)	0.70
Middle	0.73 (0.38,1.4)	0.64
Fourth	0.52 (0.26,1.06)	0.31
Richest	0.47 (0.21,1.04)	0.21

Categorized detectible *E. coli* into four categories per established cut-offs: <1 E. coli (CFU/100ml) was '*Low Risk*', 1-10 E. coli (CFU/100ml) was '*Medium Risk*', 11-100 E. coli (CFU/100ml) was '*High risk*', and >100 E. coli (CFU/100ml) was '*Very High risk*

Table 2d. The Republic of Congo: Unadjusted Analysis of Risk Factors Associated with Diarrhea

Characteristics	OR (95% CI)	P-value
E. coli associated risk (for diarrhea)#		
Low Risk	Ref.	
Medium Risk	1.16 (0.35,3.8)	0.60
High Risk	0.99 (0.45,2.18)	0.79
Very High Risk	0.61 (0.07,5.66)	0.62
Age of the Child (years)		
< 1 year	Ref.	
1	2.12 (1.36,3.29)	<.0001
2	1.29 (0.83,2.01)	0.03
3	0.51 (0.3,0.87)	0.00
4	0.50 (0.29,0.86)	0.00
Gender		
Male	Ref.	
Female	0.82 (0.61,1.12)	0.21
Mother's Education		
None	Ref.	
Primary Incomplete	0.81 (0.5,1.3)	0.56
Primary complete	0.58 (0.36,0.92)	0.03
Secondary incomplete	0.68 (0.37,1.25)	0.61
Secondary education or higher	0.00	0.00
Region of the household		
Urban	Ref.	
Rural	1.35 (0.95,1.92)	0.09
Number of household members		
Less than 5	Ref.	
5 to 8	0.91 (0.64,1.29)	0.72
More than 8	0.73 (0.43,1.25)	0.28
Number of U5 children		
2 or less	Ref.	
3 or more children	1.02 (0.67,1.56)	0.91
Animals in the household		
Yes	Ref.	
No	0.92 (0.67,1.26)	0.60
Treatment of Drinking water		
Appropriate	Ref.	
Unimproved	1.76 (0.74,4.18)	0.20
Time to source of water		
< 10 minutes	Ref.	
10 to 30 minutes	1.52 (1.07,2.14)	0.13
more than 30 minutes	1.42 (0.93,2.17)	0.46
Source of Drinking water		
Improved	Ref.	
Not improved	1.24 (0.91,1.7)	0.18
Wealth Index		
Poorest	Ref.	
Second	0.83 (0.57,1.22)	0.25
Middle	0.71 (0.44,1.14)	0.88
Fourth	0.57 (0.31,1.07)	0.48
Richest	0.45 (0.21,0.98)	0.18

Categorized detectible *E. coli* into four categories per established cut-offs: <1 E. coli (CFU/100ml) was '*Low Risk*', 1-10 E. coli (CFU/100ml) was '*Medium Risk*', 11-100 E. coli (CFU/100ml) was '*High risk*', and >100 E. coli (CFU/100ml) was '*Very High risk*