Cholera Case Control Studies: A Systematic Review and Analysis

A thesis submitted by

Mehar Kaur

In partial fulfillment of the requirements for the degree of

Master of Science in Civil and Environmental Engineering Tufts University

in

Environmental Health

Tufts University

May 2016

Thesis Committee Chair: Daniele Lantagne
Thesis Committee Members: Mark Woodin, David M. Gute, and Travis Yates

Abstract.

Cholera is an acute, diarrheal disease caused by *Vibrio cholerae* serogroup O1 or O139. Ingestion of fecally contaminated food and water causes the disease. Various exposures can lead to ingestion of contaminated food and water. A systematic review was performed to determine the exposures associated with cholera. Existing literature on cholera case control studies was examined for the following exposures: (1) Water supply, (2) Water Storage, (3) Water Treatment, (4) Sanitation, (5) Hygiene, (6) Knowledge of cholera prevention, (7) Food, (8) Socioeconomic status, and (9) Other. Exposures that were predicted risk factors were all, as expected, significantly associated with cholera outcome. Exposures predicted as protective were, as expected, protective against the disease but were not significant across all contexts. The results from this review explain exposures associated with cholera in different contexts and are expected to guide policy towards implementing context driven protective factors (interventions).

Acknowledgements.

I would first like to acknowledge the Chair of my committee, Daniele Lantagne, for her support and mentorship over the course of my graduate studies. I cannot thank her enough for her contribution to my personal, academic, and professional growth. I would also like to thank the rest of my committee: Mark Woodin for providing assistance with statistical data and for continuing support of my professional development in the field of epidemiology throughout my graduate years, David Gute for his insight on how to assess and synthesize data, and Travis Yates for sharing his knowledge about systematic reviews in the field of water, sanitation, and hygiene. I would not be able to complete this work without each of my committee members. I have truly enjoyed working with them and I value everything I have learnt in the process of producing this thesis.

Table of Contents

List of Figures and Tables

Chapter 1. Introduction

Cholera is an acute, diarrheal disease caused when toxigenic bacteria *Vibrio cholerae (V. cholerae)* serogroup O1 or O139 infects the intestine [1, 2]. In this chapter the historical emergence of the disease, the characterization of the bacteria, the infective dose for cholera, the case definition used by the World Health Organization (WHO), outline of the symptoms associated with the disease, different forms of treatment, and the fecal-oral transmission of cholera are described. The rationale for the use of case control study results as a means of assessing the importance of exposures of interest that increase the risk of cholera transmission is also described. The resulting systematic review allows for this thesis to determine the risk and protective factors for developing cholera in varying contexts.

Background

Emergence of the disease

Cholera emerged from endemic areas in Asia in 1817 [2]. Since then it has occurred in seven pandemic waves that have involved most of the world [3,4]. The first cholera pandemic was documented from 1817-24 [5]. The first six cholera pandemics originated in India or in the Ganges Delta and spread to different part of the world [5, 6, 7]. The seventh pandemic however, originated from Indonesia and spread to Bangladesh, India, Russia, to parts of North Africa and then to Italy. It was documented as occurring between 1961-1975 and is caused by the El Tor biotype of *V. cholerae* O1 [8, 9]. The seventh pandemic is thought to be still ongoing because the strain that re-emerged in 1991 in Latin America can be traced back to the seventh pandemic [10]. Studies of the Ganges Delta have shown that *V. cholerae* is an indigenous bacterium in water [11]. Areas where the bacteria is indigenous serve as environmental reservoirs capable of providing a basis for outbreaks [12].

Characterization of the bacteria: Vibrio cholerae

To determine the factors that may contribute to the transmission of the bacteria and propagation of the disease, it is important to first understand the characteristics of the bacteria. *V. cholerae* is a gram negative, comma shaped bacteria with a polar flagellum required for movement [13]. There are 34 *Vibrio* species, however only 11 are known human

pathogens [14]. Of all the 11 human pathogen *Vibrio* species, only *V. cholerae* is associated with cholera. Most *Vibrio* species require NaCl for growth however, *V. cholerae*, unlike other Vibrio species, can grow in the absence of NaCl [15].

Infective dose

According to the dose response literature, the infective dose for cholera is quite high, that is, an oral dose of more than 10^8 *V. cholerae* cells is required to induce infection and diarrhea. In a clinical study 10^8 *V. cholerae* cells in buffered saline were administered to volunteers [16]. In the study administration of sodium bicarbonate ($NaHCO_3$), a food additive used as a raising agent, also known as baking soda, neutralized the gastric acid and reduces the infectious dose to less than 10^4 cells [16, 17]. This suggests that the bacteria, *V. cholerae*, is acid sensitive and exposure to low pH in the gastric acid barrier kills most bacterial cells [16]. Alternatively, increasing the pH can create an environment favorable for bacterial growth.

Although the required dose to induce the disease is relatively large (10^8 *V. cholera* cells), people living in poor communities with unsanitary conditions are repeatedly exposed to the bacteria and regularly receive the required infective dose to develop the disease [18]. The majority of the cases occur in Africa and southern Asia in communities with poor living conditions [19]. Cholera is therefore a public health threat that disproportionately affects the poor in developing nations [12].

Transmission of the disease

Cholera is spread through the fecal oral route, which can occur through ingestion of fecally contaminated water and food [2, 20]. Drinking water or eating food contaminated with cholera bacterium transmits the disease [21]. Poor public health infrastructure that does not provide clean drinking water and separation of fecal waste from food and water facilitates the spread of cholera [20]. Factors contributing to the spread of cholera include: inadequate supply of safe water, poor sanitation, and improper disposal of solid waste, which are frequently observed in overcrowded areas such as slums, informal and often illegal settlements and Internally Displaced Persons Camps (IDP). Cholera epidemics have been caused by contamination of water and food supplies [21]. Contaminated water containing free-living,

virulent strains are considered the main sources of the epidemics, followed by contaminated food such as seafood [22, 23].

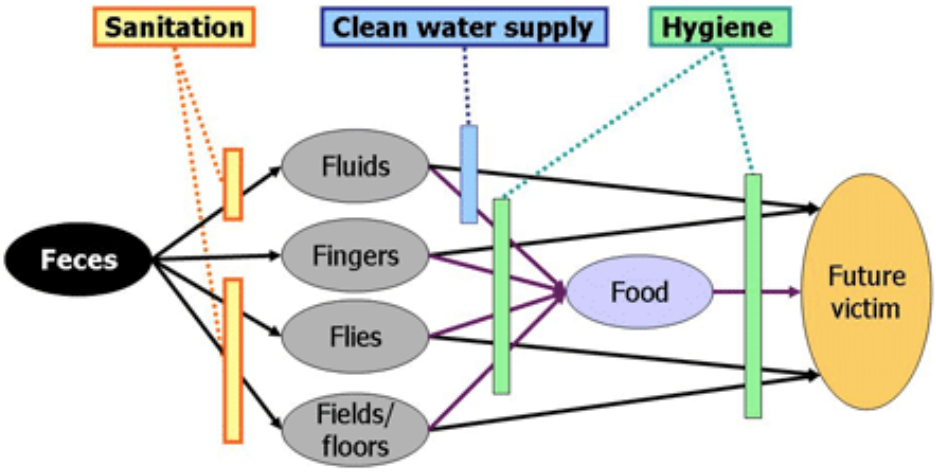
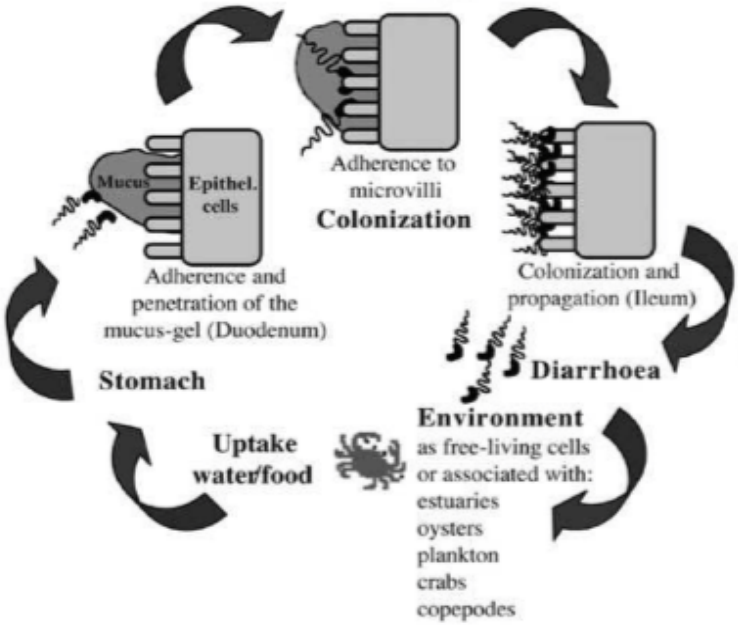


Figure 1. Fecal Oral Diagram [24]

As can be seen in Figure 1, the Fecal Oral diagram (F-diagram) is a framework describing various potential contamination routes from feces of an infected patient to future victims. The bacteria present in human or animal excreta require a medium to enter, which can be in the form of dry sanitation (where urine and feces are recovered and reused for various purposes such as compost generation), unclean hands (practicing improper hand wash), untreated sewage, and non-recycling latrines [24]. If practicing open defecation, bacteria can directly enter the environment. The environment depicted in Figure 1 is broadly defined and consists of flies, field/ floors, and fluids. Once in the environment, the bacteria may be transmitted to a susceptible host through the consumption of contaminated food and drinking water.

Mild infections, which are the most common form of cholera, play a role in reintroducing the agent into the environment and continuing the transmission of the disease [20]. This is because a person with a mild infection still excretes the bacteria in their feces, which can infect other people through F-O transmission



As seen in Figure 2, upon oral ingestion through F-O route, the bacteria must pass through the gastric acid barrier in the stomach and penetrate the mucus lining which coats the intestinal epithelia [25]. Surviving bacteria then adhere to and colonize the intestinal epithelial cells, primarily within the small intestine. This is where the bacteria produce cholera toxin, which causes the symptoms associated with the clinical presentation of cholera [26].

Figure 2. Life cycle of *V.cholerae* [25]

In the environment, *V. cholerae,* including pathogenic strains serogroups O1 and O139, has adapted to allow for long-term survival in surface waters, living symbiotically with zooplankton, plants, and crustaceans [27]. These organisms provide surfaces for the bacterium to attach to by forming multicellular structures known as biofilms [25, 28]. *V. cholerae* biofilms may also form on other solid surfaces such as sediments found in the aquatic environment [29]. Biofilm formation and conversion to dormant, viable but to the non-culturable (VNC) state are survival strategies that are important to the bacteria's life cycle. Such persistence facilitates survival during inter-epidemic periods and also in response to nutrient deprivation [25, 30, 31]. This allows the bacteria to survive extreme changes in temperature, acidity, and osmolarity as well as exposure to growth inhibitory substances such as bile salts and organic acids as it transitions from the aquatic system into the 'human intestine environment' [25, 32].

Seasonal variability is also an environmental risk factor contributing to the transmission of cholera [33]. Cholera may be present as an endemic disease process or can occur in discrete epidemics. Epidemics occur on top of endemic rates of disease. For example, in Bangladesh cholera occurs endemically with seasonal peaks but also in epidemics following floods and other natural disasters due to seasonal variability [34].

With increasing levels of globalization, transmission of cholera through international travel can also occur and lead to devastating results. A single *V. cholerae* strain can be transported from one country to another [12]. This was seen in the 2010 Haiti Cholera epidemic. Additional imported cases have been reported in Canada, USA, Australia and four European countries including France, Germany, Russian Federation and the United Kingdom [12].

Cholera symptoms

Once transmitted the symptoms start showing almost immediately since cholera has a short incubation period between two hours to five days. Infection symptoms of cholera include nausea, vomiting, profuse watery diarrhea and leg cramps [9, 20]. Infection from the disease can be asymptomatic, mild, or severe [2]. The mild form of cholera leads to diarrhea that is difficult to distinguish from diarrhea caused by other diseases. Only about 5-10% of patients will develop a severe form of cholera characterized by extremely rapid loss of body fluids, up to 2 liters an hour, leading to dehydration, electrolyte disturbances and hypovolemic shock [21, 2, 35]. Rapid dehydration leads to loss of skin turgor and results in sunken eyes [20]. If untreated, death can occur within hours [13, 27].

The risk factors of the disease for individuals with severe diarrhea are the same as those for individuals with mild or asymptomatic cholera. These factors include exposure to fecally contaminated food and water, however the severity of the disease may depend on pre-existing risk factors such as poor personal hygiene, malnutrition and availability of health care facilities. Other factors contributing to the severity of cholera include the age of the patient, gastric acidity and other barriers, intestinal motility, enteric microflora, immunity, and intestinal receptors [36].

Case definition

To diagnose the disease, the presence of *V. cholerae* is confirmed through fecal culture performed on patients with diarrhea [36]. According to the WHO Global Task Force on Cholera Control (GTFCC), a case is confirmed when *V. cholerae* O1 or O139 is isolated from any patient with diarrhea [37]. Laboratory confirmation of the first 10–20 cases is essential to establish a cholera outbreak. Once the outbreak has been established, WHO case definition to determine a suspected cholera case can be followed. According to the WHO case definition, a cholera case should be suspected when:

1) For a region where cholera is not known to be present, a patient ≥ 5 years develops severe dehydration and dies from acute watery diarrhea
2) For a region where there is a cholera epidemic, a patient ≥ 5 years develops acute watery diarrhea, with or without vomiting.

To maintain specificity, children below the age of 5 years are not tested for cholera [37]. This is because of the frequent occurrence of other causes of acute diarrhea is children, which is less common in older children and adults. Therefore there is higher specificity of diagnosis in children over five and adults [79].

Different forms of treatment for cholera

Cholera can be effectively treated if fluids and salts lost through diarrhea are replaced immediately [38]. Proper treatment of cholera includes oral rehydration therapy (ORT), which is inexpensive, simple and very effective in preventing mortality [20]. It is administered to patients displaying any of the cholera symptoms. Rehydration, if administered correctly and promptly, can maintain case fatality percent as low as 1% [37]. ORT, the main form of cholera treatment, the first order of treatment [40]. It uses oral rehydration salts (WHO/UNICEF ORS standard sachet) to replace salts lost during diarrhea. The ingredients in ORS include: sodium chloride (12.68%), glucose, anhydrous (65.85%), potassium chloride (7.32%), and trisodium citrate, dehydrate, also known as sodium citrate (14.15%) [39]. ORS prepackaged sachet is the recommended form of ORS and is prepared by diluting 1 sachet of ORS in 1L of safe water however, if not available ORS may be self-prepared using half a small spoon of salt and six level small spoons of sugar dissolved in 1L of safe water.

The second order of treatment is through intravenous (IV) rehydration [21]. Other forms of treatment, which is case-based, that is dependent on the severity of cholera and age of patient, includes antibiotic treatment and treatment with zinc supplementation [21]. Zinc supplementation is recommended for pediatric diarrhea as it reduces the period diarrheal illness [41]. In a randomized control trial in Bangladesh, children with cholera who received Zinc had on average 8 fewer hours of diarrheal illness than those not receiving Zinc [42]. Although the mechanism of action of Zinc is not fully understood, it has been shown to inhibit cAMP-induced, chloride-dependent fluid secretion in *in-vitro* studies conducted on the on rat ileum [43]. cAMP is a cyclic adenosine monophosphate, a derivative of adenosine triphosphate. Reduction in cAMP concentration leads to increased ion absorption and reduced production of cholera

toxin produced [44]. In addition to improved ion and water absorption, Zinc also regenerates the intestinal epithelium, increases the levels of brush border enzymes, and enhances the immune response. This allows for efficient clearing of the pathogens and better recovery [22].

Case control studies

Case control studies can be used to, relatively quickly and inexpensively, determine exposures resulting in the outcome of interest. Case control studies are analytical, observational studies that test a causal hypothesis concerning exposure and outcome by contrasting the experience of persons with the disease (cases) and those without the disease (controls) [45]. These studies are retrospective in design [45]. If an exposure is more common among cases than controls, then it can be identified as a "risk factor" for cholera [46]. Conversely, if an exposure is more common among controls than cases, then it may be determined to exert a "protective factor" for cholera. If there is no association between an exposure and the outcome, then the distribution of the exposure among the cases and controls should be the same [46]. The odds ratio is the measure of association used in case control studies. Since the underlying risk of developing the disease in the populations is unknown, we are not able to determine risk or rate ratios. Case control studies are the most appropriate study type given the nature of the infection, which most commonly presents in a mild form. Most case-control studies feature a cross-sectional design in which cases and controls are selected at the same point in time however, with the cross-sectional design, primarily prevalent cases, that is cases with the longest survival rate, are identified [47]. Selection of primarily prevalent cases does not pose a problem because the most common form of infection from cholera is mild, and most cases have a long survival rate.

The two major limitations of case control studies are selection and recall bias. Given the nature of the disease however, these biases may not pose a large problem. Recall bias for cholera is not as significant an issue as in other diseases because the disease has a short incubation period (symptoms can start anywhere between a few hours to 5 days). Selection bias occurs when cases are not from the same population as the controls or when losses in one group (cases or controls) is more than the losses in the other group [47]. However, case control studies can address the problem of selection bias either in the design phase by matching cases and controls and adjusting results in the analytical phase [47].

Current epidemiologic data

The number of cholera outbreaks reported to the WHO remains high. This underscores the importance of determining factors affecting the outcome in individuals [48]. Ali et al. 2012 estimated the global burden of cholera to be between 1.4-4.3 million cases and 28,000-142,000 deaths per year worldwide [19]. Case fatality rate (CFRs) from the disease can range from 0.01% to 25.71% [12].

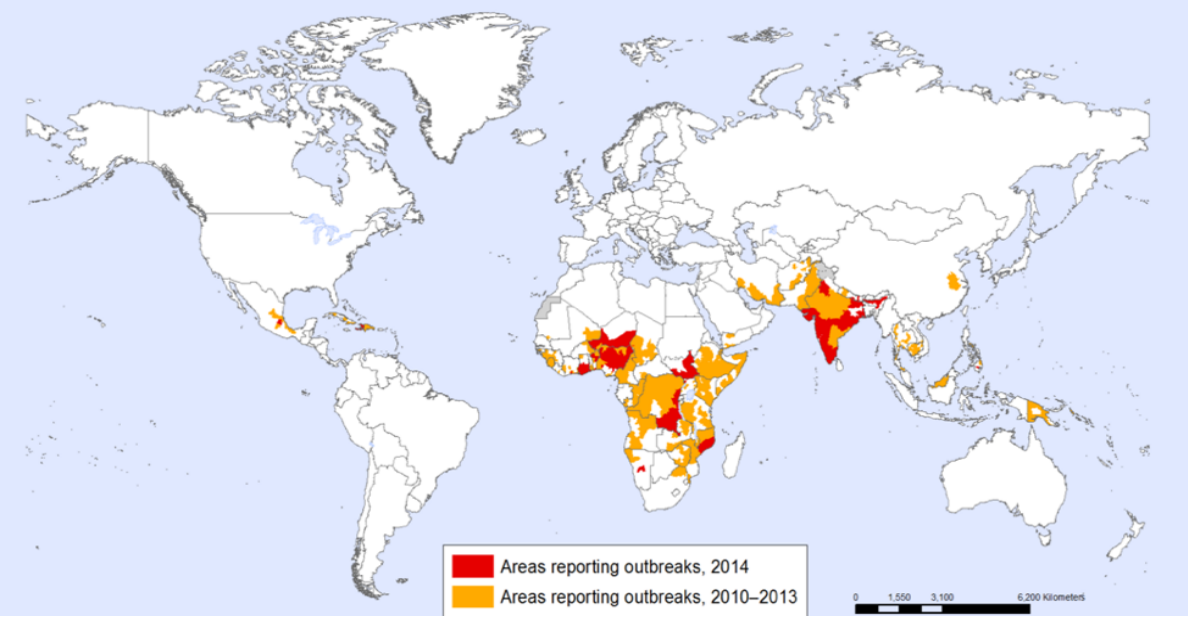
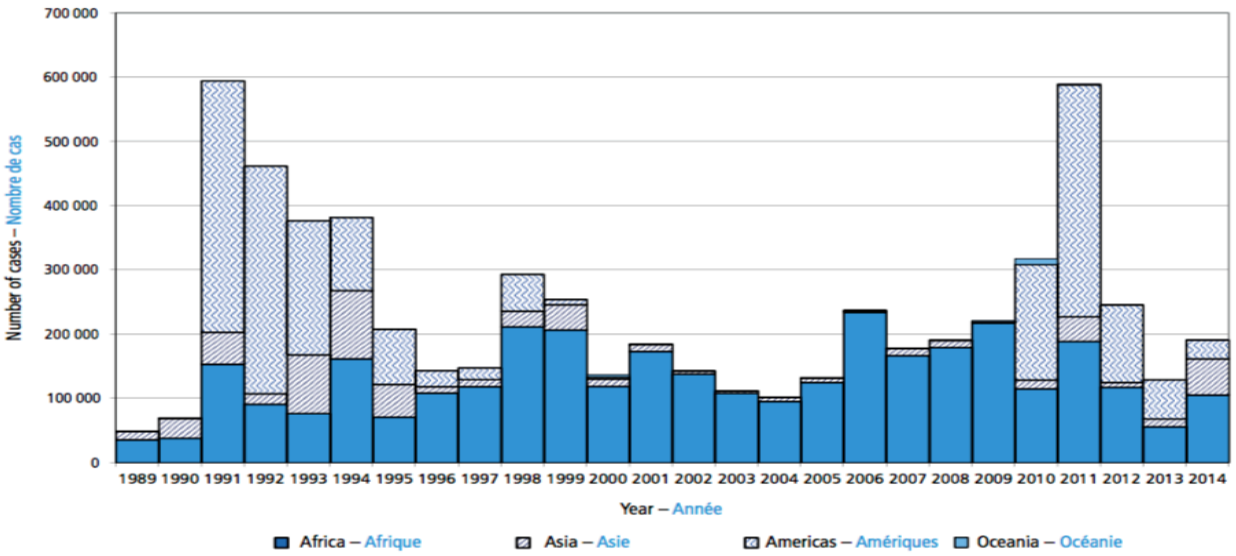


Figure 3. Areas reporting cholera outbreak 2010-2014 (WHO 2015, Health Statistics and Information Systems)

Areas reporting cholera outbreaks are displayed in Figure 3. The number of cases reported in 2012 and 2013 declined. In 2013, 47 countries reported 129,064 cholera cases and 2,102 deaths, which yields a CFR of 1.63% [49]. In 2014, the number of cases increased and 190,549 cholera cases were reported by 42 countries with an overall CFR of 1.17% [12].

As seen in Table 1 below, the number of cases reported from the Americas decreased after 1998, but increased in 2010 following the 2010 Haiti cholera outbreak. The number of cases reported from Africa and Asia increased in 2014 while those reported from the Americas and Oceana decreased. In 2014 the percentage of cases reported in Africa increased by 87% [12]. Number of cases reported from Asia increased from 11,576 cases in 2013 to 56,787 cases in 2014. The CFRs reported from Asia, Americas and Africa were 0.07%, 1.08% and 1.79% respectively.



Graph 1. Cholera cases reported yearly from 1989-2014 (source: http://www.who.int/wer/2015/wer9040.pdf)

As can be seen in Figure 3 and Graph 1, regions in Asia, Africa, and Americas have increased numbers of cases and rising CFRs, which suggests that the exposures causing the disease are still continuing and interventions to curb the spread of disease should be focused on these regions. The re-emergence of cholera has occurred alongside an increase in the number of populations residing in unsanitary conditions. Presence of cholera therefore raises additional concerns, "about the high proportion of people living in unsanitary conditions" [12].

The construction of proper infrastructure requires large investments and although it is a long-term goal, it is not always feasible. Even if water and sanitations systems are present, they may be disrupted following a disaster [48]. Climate change may further exacerbate cholera outbreaks. It can lead to changes in river discharge and since rivers transport the bacteria, changes in river discharge have shown to increase cholera cases [1]. Unusually high temperatures and periods of torrential rainfall favor the growth of bacteria [50]. Dry conditions, when river levels decrease, allow bacteria to accumulate in high concentrations and flooding allows bacteria to infect places that have not previously experienced cholera.

Protective factors that are expected to prevent the spread of cholera include universal access to clean and safe drinking water and adequate sanitation [48]. Presence of sewage disposal and latrine use constitute good sanitation practices. Hand washing with soap and water, cooking food and safely storing it, breastfeeding, and knowledge of the above

hygienic practices are protective factors against the disease. Even if public health systems fail, access to protective factors may prevent the morbidity related to cholera. It is therefore important to determine which factors are protective against cholera in all contexts and which risk factors potentiate marked increases in the disease. As a result, in this thesis both, the risk and protective factors for cholera, are addressed by consulting the case control study literature on cholera.

Purpose of the Thesis

To quantify risk and protective factors (exposures) for developing cholera

A systematic review of cholera case control studies was performed. The purpose of this systematic review is to quantify the exposures affecting the outcome of developing the disease [20,45]. The thesis describes exposures associated with individuals who develop all forms of cholera ranging from mild (most common form) to severe life threatening illness in all contexts including varying target populations and study locations.

This systematic review provides a platform of evidence based results that are reviewed for guidance in the control of cholera and to support implementation of evidence based interventions. Such a process is congruent with the aim of the WHO GTFCC, which is, "to support increased implementation of evidence based strategies to control cholera, through strengthened international collaboration and improved coordination among stakeholders active in cholera-related activities" [12].

Chapter 2. Methodology

To fulfill the purpose of this thesis, a systematic review was conducted. The methodology used to perform this review is described in detail in this chapter. A detailed outline of the study design, search methods used to identify the target case control studies on cholera, the criteria used for selection of manuscripts, and the filtering process used in the excluding of studies are presented here. Quality appraisal criteria used for determining study quality and specifics on the analysis of the collected data including how missing data were handled and synthesized are also described in this chapter.

Outline of study design

A systematic review of cholera case control studies was performed by following the principles of understanding the context and anticipating heterogeneity, as outlined by Howard White (2009) [51]. The other principles including- mapping the causal chain, rigorous factual analysis, rigorous counterfactual analysis, and use of mixed methods - are not applicable to this review.

Understanding context

Data extracted from the case control studies focused on contextual factors that affect exposure including: target population, study location, and study period [52]. To determine the range of explanatory power of specific exposures on a global scale, understanding context is crucial for understanding the effect of exposure on developing cholera.

Anticipating heterogeneity

Heterogeneity of exposures, contextual factors and affected populations (geographical location) is expected to be high. The primary source of data for this review included all case controlled studies from peer reviewed journals indexed in Web of Science (WOS) and PubMed, and reference list tracing. This formed the data set. The contextual factors that is target population, study location, study period, in which the study is conducted is expected to be high. The quality of the included papers is also expected to vary considerably. Sub-group analysis and stratification of data is conducted to allow for more homogeneous comparisons.

Search methods for identification of case controlled studies

A comprehensive search strategy was used to identify all cholera case control studies existing in the published literature. Sources were searched using the following keywords: "case control" OR "case-control" AND "cholera." The databases searched for relevant studies included WOS and Medline (Pubmed). Reference list tracing, that is reviewing references from the down-selected papers from WOS and PubMed, was used to find additional studies. This formed the data set.

Selection of manuscripts

For the systematic review, manuscripts were selected based on the modified Populations, Interventions, Comparisons, Outcome and Study type (PICOS) protocol for inclusion. Criteria formulated for inclusion of studies is described below. The identified studies were then selected for final inclusion following a filtering process outlined in Figure 5.

Criteria for including studies in the review

The inclusion of studies is based on the PICOS criteria for study selection. This criterion was first used in evidence-based medicine, which recommended that clinical questions be formulated in terms of the problem/population, intervention, comparison, and outcome, also known as PICO [53]. PICOS criteria for study selection increases transparency of how and why studies are included. PICOS criteria is used to identify the information needed to answer a research questions and to develop a structured search approach that clearly defines inclusion and exclusion criteria [54]. For this thesis, PICOS criteria was modified, as interventions are not included. In the review, case control studies which evaluate different exposures are included. Therefore, only the PCOS criteria was used and includes:

Populations: All populations in a cholera-affected area were considered, which included all age, gender, and socio-economic demographics. Both, cases and controls in the population will be considered.

Comparisons: Studies that compare exposures for cholera in different contextual factors

Outcomes: A study was included if cases and controls, and different exposures were present in the study

Study: Only case control studies with the correct measures of association (odds ratio) were included.

Selection of case controlled studies

The selection of studies for the systematic review adhered to the principles outlined in Figure 5. These principles are based on the standards set by the Cochrane Intervention Reviews (CIR), which is a four stage filtering process [55]. For this thesis the filtering CIR process was modified to have only two filters and was screened only once.

Filter 1: All titles/abstracts were numbered in sequence for identification. Filter 1 was governed by a liberal selection process that included: all case control studies on cholera. Any questionable title of an/abstract was also included.

Exclusions: studies that were not cholera case control studies.

Filter 2: The down selected titles/abstracts from Filter 1 were reviewed more rigorously with the following criteria: 1) clearly defined population of interest, 2) contextual factors such as target population, study location, and study period 3) risk and protective factors, and 4) reported outcome with appropriate measure of association (odds ratio) used. A detail list of the criteria used is listed in Table 1 and coded in a Microsoft Excel spreadsheet. The full manuscripts were read and coded in the spreadsheet. Study design, bias, and confounding were assessed using the risk of bias tools adapted from Waddington et al.(2012) protocol as outlined in Tables 2, 3.1, 3.2, and 3.3 [56].

Exclusions: studies not following the above criteria and /or not focusing on water, sanitation and hygiene

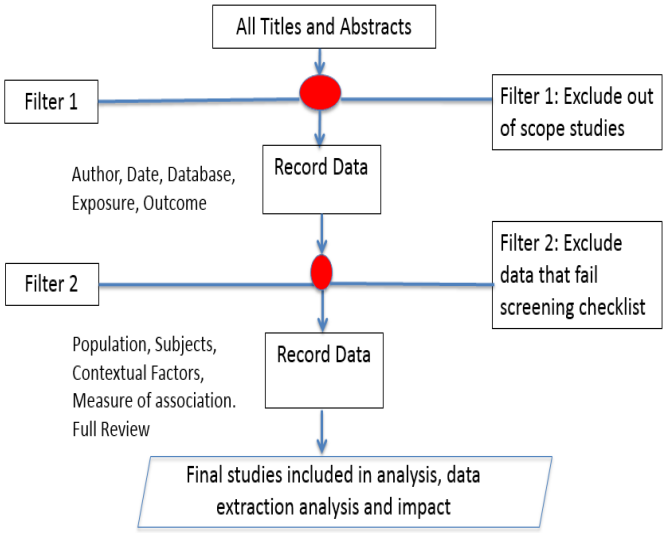


Figure 4: Flow diagram for study selection process [52]

Data extraction and processing

Once the manuscripts were selected, data was gathered for data extraction and comparison. Information recorded from each manuscript is based on the Waddington et al. (2012) protocol [56]. The Waddington protocol was modified to collect information on risk and protective factors for case control studies on cholera. The information recorded included: author and publication details, type of risk or protective factors, context for the factors, study design (were cases matched and/or results adjusted), study quality, impact estimates, case and control definition, number of cases and controls, and qualitative information. Measures of treatment effect collected from the selected studies included: sample size, 95% confidence intervals, and impact estimates. Impact will be described as an odds ratio for dichotomous variables. Ratios less than 1.0 represented a protective factor, while ratios greater than 1.0 represented an increased risk.

Detailed criteria from all included studies were extracted to produce a master list in Microsoft Excel (2010). The criteria used for data collection, including measures of treatment effect is given below and the data variables collected are listed in Table 1 below**.** Factors associated with cholera were categorized as predictive protective factors and predictive risk factors and grouped into sub-categories (as displayed in Table 1) under the following exposures: 1) Water supply, 2) Water treatment, 3) Water storage, 4) Sanitation, 5) Hygiene, 6) Knowledge of cholera, 7) Food, 8)

Socioeconomic status (SES), and 9) Other. This comprehensive list of criteria was used to establish the underpinnings for comparisons and for an appreciation of the heterogeneity contained within the selected studies.

Table 1: Data collection variables

General Information	
First Author	Surname
Year of Publication	(YYYY)
Publication Type	Journal Article Abstract only
Protective and Risk factor Design	
Population (Age + gender)	Refugee Camp Men Women Children (<5) Elderly General Population - All Not Reported
Type of predictive protective factor(s)	1) Water Supply - Clean drinking water - Sufficient water - Improved water source - Protected water source/ not improved - No contact with other water sources - Water for washing utensils 2) Water Treatment - Community based water treatment - Household – Point of Use – Cl tablets - Household Cl drinking water > 0.5mg/l - Household Cl drinking water > 0.2mg/l - Water treated before outbreak, during outbreak, or before illness - Chlorination bottle observed 3) Water Storage - Safe - Water storage container with lid and insulated 4) Sanitation - Latrine present at home - Access to latrine - Use toilet paper - Latrine w\ soap + water - Shared Latrine - Safe disposal 5) Hygiene - Proper hand washing technique - Clorox in the house - Hand washing designated area 6) Knowledge of cholera/diarrhea prevention/transmission

	- Has knowledge - Has access to knowledge - Received care - Home treatment antibiotics/ ORS 7) Food - Cooked - No seafood - Milk - Eating at home - Fruits, vegetables, meat fish/ poultry - Alcoholic beverage 8) SES - High income - Employed - 2nd education and above - Family size - House hold has appliances + vehicle + electricity - Formal residence - Family size small (4-5) 9) Other - No contact with cholera patient - Religion - Marital status - Attending function - Gender - Age - Nation - Breast feeding
Type of predictive risk factor(s)	1) Water Supply - Unclean drinking water - Insufficient water - Not improved water source - Contact with other water source - Water for washing utensils - Swallowing water while bathing/ washing mouth - Water for cooking - Mixed drinking water - Any use of water source (rainwater, other sources) 2) Water Treatment - No water treatment - Household Cl drinking water < 0.2mg/l - Household point of use - untreated 3) Water Storage - Unsafe - Lack of safe storage - Container to transport or store water – without lid

	4) Sanitation - Latrine not present - Open defecation - Shared Latrine - Latrine overflowing - No drainage/ pit latrines 5) Hygiene - No proper hand washing technique - No soap for hand washing 6) Knowledge of cholera/diarrhea prevention/transmission - Has no knowledge - No access to knowledge - Did not receive care 7) Food - Uncooked - Seafood - Milk - Eating or drinking out - Unwashed or uncooked fruits, vegetables, meat, fish, poultry - Cold food - Alcoholic beverage 8) SES - Low income - Unemployed - 2nd education and below - Family Size - No appliances + vehicle - Displaced/ informal residence - Large Family size > (6-8) 9) Other - Contact with cholera patient - Religion - Marital status - Age - Gender - Attending function - Nation - Travel - Using same bowl - Infection	
Availability of Protective Factors	Yes/No/ Unclear	If Yes: 1) Chlorine tablets 2) Soap and bucket 3) Water filter 4) Personal hygiene items 5) Other
Timing		

Start date of Cholera outbreak	
Context	
Country	Specific country N/A
Region	Sub-Saharan Africa Middle East and North Africa Central Asia South Asia East Asia and Pacific Latin America Caribbean and South America non-LMIC
Region (conti.)	Rural / urban
Study Design	
Method of Selecting Cases and Controls	Systematic / Descriptive
Odds ratio matched (mOR) [control for confounding]	Yes/ No
Confounding factors	Age, Gender, Socioeconomic background
Study Quality	
Selection Bias and Confounding	Yes / No / Unclear / Not Applicable
Selective Reporting	Yes / No / Unclear / Not Applicable
Other Biases	Yes / No / Unclear / Not Applicable
Outcomes	
Effect estimation	
Odds Ratio	Adjusted / Unadjusted

Missing data

Primary authors were contacted for missing data. Where no additional data was retrieved, only the given risk and protective factors were used. Twelve authors were contacted out which three responded, but could not provide the complete information of their data.

Quality of appraisal for quasi-experimental studies

The quality appraisal process employed in this thesis determined study quality (internal validity), recall biases, and selection biases to identify the potential for bias and confounding in each manuscript. Studies with low risk of bias identified risk and protective factors, and controlled for confounding by matching on age, sex, neighborhood, or on another contextual factor or by determining the statistical difference between two groups [52, 56]. Reported bias, selection bias, and recall bias was minimal in low risk of bias studies. Studies with

medium risk of bias were taken to be studies with unclear methodology that had poorly defined control groups that did not control for confounding, displayed recall bias [52,56]. High risk studies had clear flaws such as, not defining control group, numbers of controls used, using proxies to substitute for deceased cases, including children under five years of age and patients with bloody diarrhea in design or implementation, and had a high likelihood of the above described biases or did not control for known confounders [52, 56].

Case control studies are a quasi-experimental study design. Participant selection, confidence in the exposure/screening of risk and protective factors, and the appropriate use of matching are the foundation for quasi-experimental validity. The quasi-experimental assessment tool as applied here was adapted from Cochrane and the Critical Appraisal Skills Program (CASP) assessment tools for case control designs [57, 58]. The case control studies followed the screening checklists outlined in Tables 2, 3.1, 3.2, and 3.3 below.

Screening Checklists

Screening checklists are used to identify key aspects of a study without a full review. Screening checklists were used at the second of three filters during the abstract assessment (Figure 5). Quasi-experimental questions for the screening checklist were adapted from the Cochrane and CASP evaluation tools for case control studies [57, 58].

Table 2. Screening checklists [57, 58]

Quantitative Study Design Questions	Yes / No / Maybe
1. Clear Study objective/question?	
2. Was the selection of participants clear and appropriate with explicit inclusion and exclusion criteria?	
3. Were populations matched or results adjusted for confounding factors?	

Quality Appraisal Checklists

The appropriate assessment of studies requires frameworks specific to the case control design. The assessment tools listed below were intended to aid the conducting of a review that objectively assessed the candidate

manuscripts for common biases and internal validity. The quality assessment checklist, selective reporting, and other biases checklists specific to the case control study design, are presented in tables below.

Quality assessment checklists:

- (3.1) Quasi-experimental appraisal

Selective reporting

- (3.2) Selective reporting appraisal

Other biases:

- (3.3) Other bias appraisal (including selection bias appraisal)

Table 3.1: Quasi-experimental study appraisal.

Adapted from Cochrane and CASP evaluation tools for cohort and case-control studies [57, 58].

Appraisal Questions	Guiding Questions	Yes / No / Maybe
1. Was the selection of groups appropriate?	· Were controls clearly selected from the same underlying population as the cases and equally at risk of exposure? · Were exposed and unexposed drawn for same administrative data base of patients presenting at same points of care over the same timeframe?	
2. Was matching between groups appropriate?	· Were statistical tests run to assess difference in groups? · Were confounding factors appropriately controlled for?	

Table 3.2: Selective reporting appraisal

Adapted from Waddington et al. (2012) [56].

Appraisal Questions	Guiding Questions	Yes / No / Maybe
Is there potential for selective reporting?	· Are all relevant outcomes in the methods section reported in the results section? · Are common (and appropriately robust) methods used for data analysis and reporting? Any 'exploratory' methods?	

Table 3.3: Other bias appraisal

Adapted from Waddington et al. (2012) [56].

Appraisal Questions	Guiding Questions	Yes / No / Maybe
Is there potential for other biases?	· Was the term 'self-reported' or similar words used to describe data collection? · Are there other critical questions (recall period) addressed in the design or implementation of the project?	

Heterogeneity assessment

Sub-group analysis was conducted as part of this thesis. Data was stratified by predicted risk/protective factor within the sub-categories of different exposures. Forest plot analysis was conducted to determine the overall association and heterogeneity within studies.

Heterogeneity was determined by two tests χ^2 (Pearson Chi-square test) and I^2 tests. χ^2 test is a statistical test indicating variability within studies [59]. High p-value (p-value > 0.05) suggest studies are homogeneous and a low p-value (p-value <0.05) suggest studies are heterogeneous. The I^2 test determines the percentage of variation due to variability between studies and is interpreted as follows: $I^2 = 0\%$ (no heterogeneity), $I^2 = 25\%$ (low heterogeneity), $I^2 =$ % (moderate heterogeneity), $I^2 = 75\%$ (high heterogeneity) [59].

Method of synthesis

To answer the research question, predicted exposures across studies, were synthesized in a results summary table in Microsoft excel (2010). The results summary table for each sub-category under the 9 main exposures (water supply, water treatment, water storage, sanitation, hygiene, knowledge of cholera prevention, food, socioeconomic status (SES), and other) is given in the results section. The information in the results summary tables include: 1) Number of studies 2) Papers that include the studies 3) Predicted risk factors 4) Predicted protective factors 5) Bias (L/M/H) associated with the study 6) Impact estimates, and 7) 95% CI. Analysis conducted in the original studies to determine the association (odds ratio) (that is univariate, bivariate and/or multivariate analysis) is also listed in the results summary tables in the results section.

Forest plot analysis, a technique used in meta-analysis, was conducted on studies listed in the results summary tables using the statistical software, STATA 14. Forest plot analysis was limited to sub-categories with 4 or more studies, and analysis was conducted for separate predicted risk and protective factors, to quantify the overall association between an exposure and the outcome of cholera and the heterogeneity between studies.

In the discussion chapter, exposures determined as risk and protective from the overall estimates presented in forest plots are compiled and discussed in detail. Heterogeneity of the results and possible sources of heterogeneity are also discussed. Finally, limitations of the study are also described in the discussion section.

Chapter 3. Results

The results section includes study selection, exposure results, and sensitivity analysis. The results consist of the number of included studies, the analysis performed on the included studies, and the bias of the included studies, which is described in study selection below. Under the exposure results section, the exposures, predicted risk and protective factors for cholera, obtained from the included studies are compiled in a summary of results table arrayed by the specific factor. Data from the table is analyzed using forest plot analysis to determine the overall association between an exposure and cholera and the heterogeneity found to be present in the included studies. In the sensitivity analysis section studies forest plot analysis was re-run by excluding studies with high bias and stratifying existing data to allow for more homogenous comparisons.

Study selection

In Figure 6, the number of papers obtained from the initial search, the papers included after reviewing the title and abstract, papers excluded, and the associated exclusion criteria are outlined. The analysis conducted on the studies (univariate or multivariate analysis) is also included. The number of studies assessed to have high, medium, or low bias, and the associated bias criteria used are described in Figure 7. The criteria used to assign high, medium, or low bias is also outlined in the figure below.

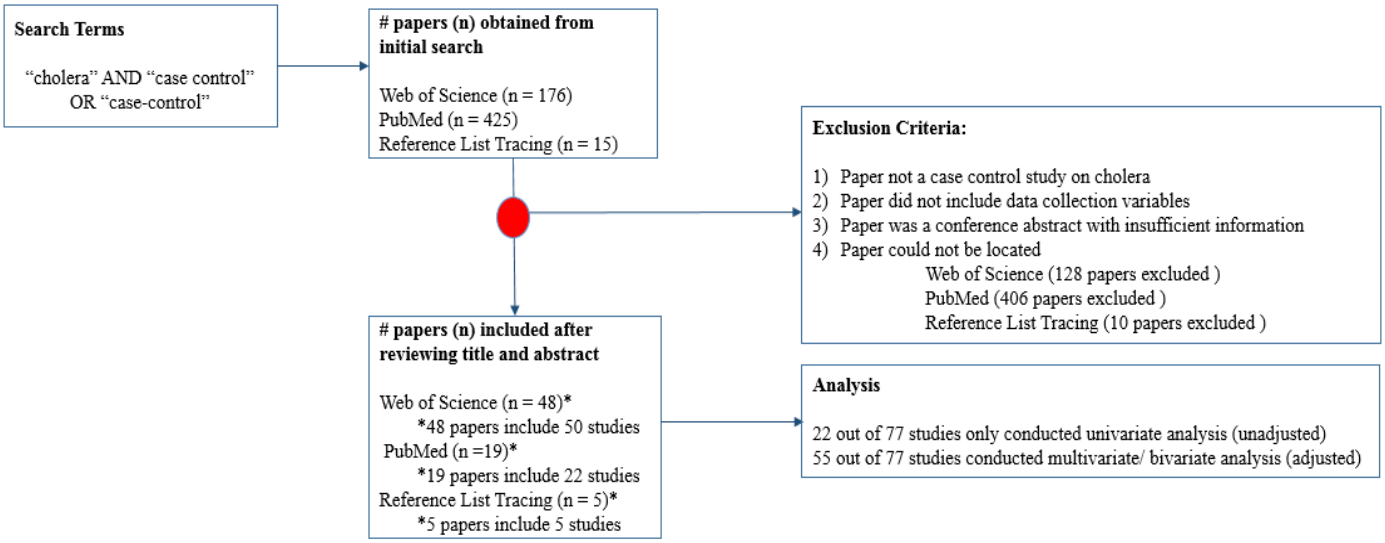


Figure 5. Results of the number of studies selected and analysis conducted [Note: the number of studies are more than the number of papers included, this is because a paper can include more than one case-control study]

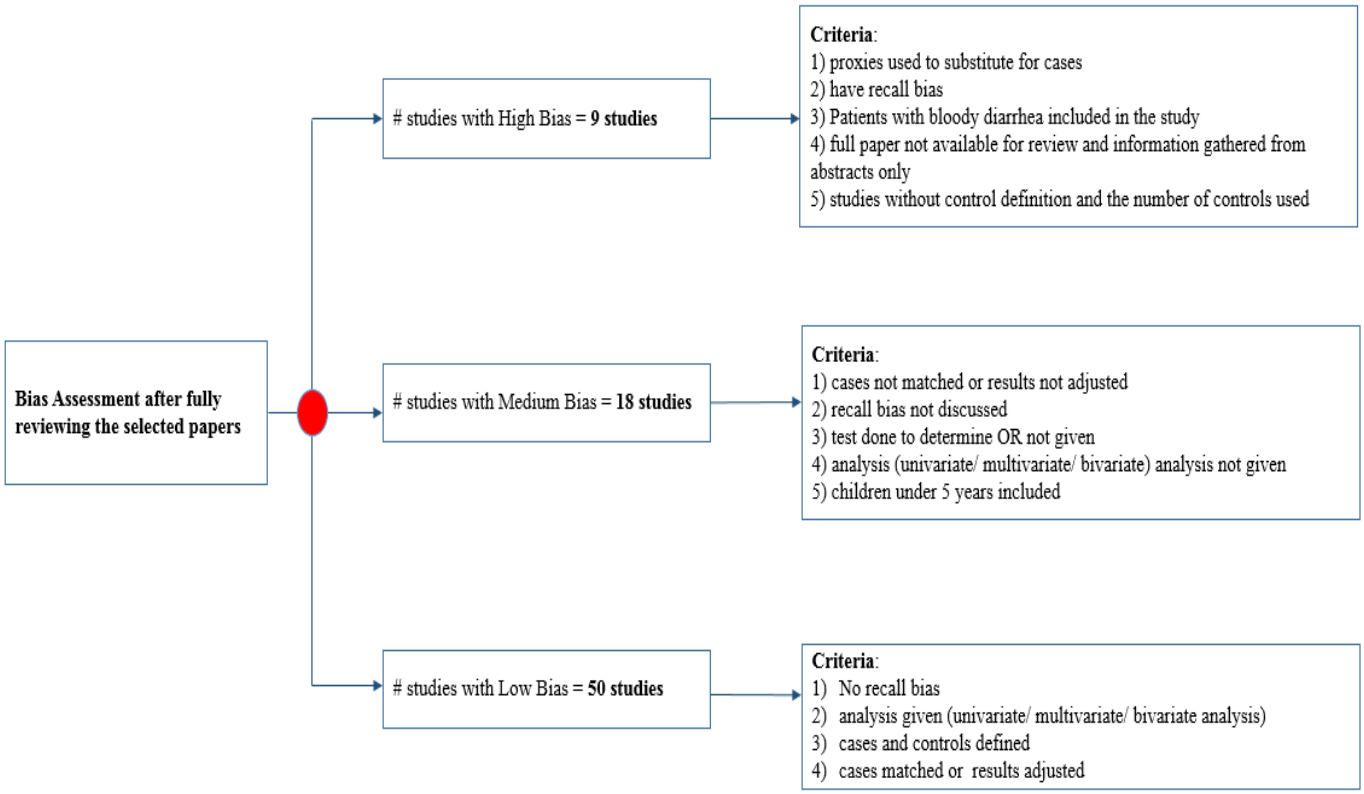


Figure 6. Bias assessment of the included studies and the criteria used to assign bias

Exposures results

In this section, sub-categories under each of the 9 main exposures [water supply, water treatment, water storage, sanitation, hygiene, knowledge of cholera prevention, food, socioeconomic status (SES), and other] are described in detail. A summary results table is presented for each sub-category, which contains information from the included papers that is the predicted risk and protective factor, the actual association of the exposure and outcome of cholera given by the odds ratio and 95% CI, and the associated bias of the paper as characterized by high (H)/ medium (M) /low (L). Univariate, bivariate and/or multivariate analysis conducted in the original studies to determine the association (odds ratio) is also listed in the tables below. For each sub-category under the main exposure, forest plot analysis was conducted to determine the overall association of between an exposure and outcome of cholera and the heterogeneity among studies.

1) Association between water supply and cholera

The association between various water supply exposures and cholera was identified in 45 studies. The water supply exposures presented in the studies include 1.1. Clean drinking water, 1.2. Changing water source, 1.3. Water quantity, 1.4. Improved or unimproved water source, and 1.5. Water contact. The summary results tables and forest plot analysis for the sub-categories is given below.

1.1. Clean drinking water and cholera outcome

Drinking bottled water or sachet water is expected to be safe and characterized as predicted protective factor in Table 4.1 below [60]. Drinking vended water, water in which hands had been introduced, water obtained outside the home, or at a funeral is expected to be unclean and unsafe and characterized as predicted risk factors in Table 4.1 below. As seen in Table 4.1, in four out of 12 studies drinking water outside the home/ street vended water is a risk factor. In one study drinking water at a funeral is a risk factor and in another study drinking water in which hands had been introduced is also a risk factor. In three studies drinking bottled water is protective but in two other studies, both conducted on passengers on the same flight, bottled water obtained on a plane is a risk factor. Sachet water is protective in one study but a risk factor in the other study.

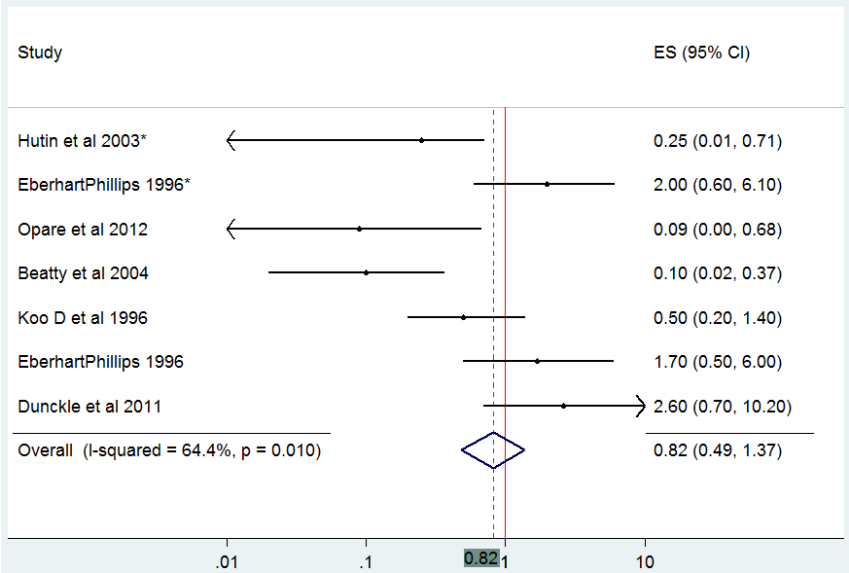
Table 4.1. Association between clean drinking water and cholera outcome[+]

Study	Papers	Predicted Risk Factor	Predicted Protective Factor	Bias	OR univariate	95% CI	OR multivariate	95% CI
1	Opare et al. 2012		Sachet water	M	0.09	0.00-0.68		
2	Beatty et al. 2004		Bottled water	L	0.10	0.02-0.37		
3	Koo D et al. 1996		Bottled water	L	0.50	0.2-1.4		
4	Hutin et al. 2003		Bottled water	L			0.25	0.01-0.71
5	Nguyen et al. 2014	Unsafe drinking Water		L			3.43	1.07-11.04
6	Nguyen et al. 2014	Vended water		L			9.70	2.01-43.72
7	Mugoya et al. 2008	Drinking water outside the home		L	4.10	1.7–9.8		
8	Gunnlaugsson 1998	Drank water at the funeral		M	3.00	0.7–12.3		
9	EberhartPhillips 1996		Bottled water (flight Buenos Aires to Lima)	L	1.70	0.5-6.0		

10	EberhartPhillips 1996		Bottled water (flight Lima to LA)	L			2.00	0.60-6.10
11	Quick et al. 1995	Drank water outside home		L	8.80	1.7-44.6		
12	Swerdlow et al. 1992	Drank water from container in which hands had been introduced into the water		L	4.20	1.2-14.9		
13	Hutin et al. 2003	drinking water sold by street vendors		L			4.10	1.7-9.5
14	Dunkle et al. 2011		purchased bags-sachet	L	2.60	0.7-10.2		

[+] Please note the exposure is highlighted in pink when it is not as expected [75, 83, 89, 100, 103,110, 112, 113, 119, 125, 132]

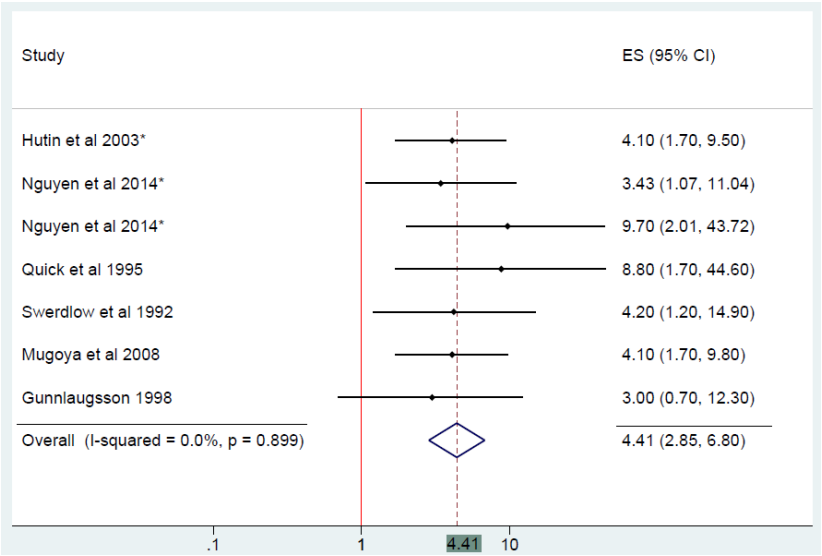
Based on the forest plot analysis conducted on seven studies, categorized as predicted protective factors in Table 4.1, the cumulative effect of consuming clean drinking water is 0.82 (0.49-1.37). As expected the factor is protective however, the overall association, odds ratio and 95% CI, is not significant. The χ^2 test has a low p-value < 0.05 (p-value = 0.010) and a high I^2 test value of 64.4%, which suggests that 64.4% of variation in studies is due to heterogeneity.



Graph 1.1.0. Forest plot analysis of the effect of clean drinking water (predicted protective factor) on the outcome of cholera [Note: Studies with * are multivariate analysis]

Based on the forest plot analysis conducted on seven studies, categorized as predicted risk factors in Table 4.1, the cumulative effect of consuming unclean drinking water is 4.41 (2.85 – 6.80). The overall association (odds ratio and

95% CI) is significant, which suggests that unclean drinking water is a risk factor for cholera. The χ^2 test p-value = 0.889 and a high I^2 test value of 0.0%, which suggests that none of variation in studies is due to heterogeneity.



Graph 1.1.1. Forest plot analysis of the effect of unclean drinking water (predicted risk factor) on the outcome of choler [Note: Studies with * are multivariate analysis]

1.2. Changing water source and cholera outcome

Changing main source of drinking water or access to temporary water source can be a risk factor but it may also be protective if the main water source is contaminated. Two studies determined the association between changing water source and the outcome of cholera. In one study changing main source of drinking water is a risk factor however, temporary water source for the other study is not a risk factor.

Table 4.2. Association between changing water source and cholera outcome **

Study	Papers	Predicted Risk Factor	Predicted Protective Factor	Bias	OR univariate	95% CI	OR multivariate	95% CI
1	Killewo et al. 989		Temporary water source	L	0.83	0.18-3.80		
2	Mugoya et al. 2008	Changing main source of drinking water		L	4.50	1.6–12		

** Forest plot analysis not conducted on sub-categories with <4 studies [100, 140]

1.3. Water quantity and cholera outcome

Insufficient water supply is expected to be a risk factor and characterized as a predicted risk factor in Table 4.3 below [61]. Two studies determine the association between insufficient water and cholera and in both studies water cuts and shortage of water in household were risk factors for cholera.

Table 4.3. Association between water quantity and cholera outcome**

Study	Papers	Predicted Risk Factor	Predicted Protective Factor	Bias	OR univariate	95% CI	OR multivariate	95% CI
1	Kone-Coulibaly et al. 2010	Always had water cut		M	2.00	1.18-3.40		
2	Cárdenas V et al. 1993	Shortage of water in household <l400 l/week		L			3.60	0.80-16.4

** Forest plot analysis not conducted on sub-categories with <4 studies [126, 135]

1.4. Improved/ unimproved water source and cholera outcome

Improved drinking water sources as defined by the World Health Organization includes piped water inside the user's home, public taps, standpipes, tube wells, boreholes, protected dug wells, protected springs, and rainwater collection [62]. Improved water sources are expected to be protective and characterized as predicted protective factors in Table 4.4 below [20]. Aqueducts, municipal water and water from reservoir are also expected to be protective factors. Water sources that are not improved such as unprotected, shallow or deep wells, water from lake or river, and water from tanker trucks, are expected to be risk factors and characterized as predicted risk factors in Table 4.4 below.

The relationship between varying water sources and cholera was determined in 69 studies. Thirty-two studies determined association between unimproved water sources and cholera, which included no tube well (1), wells (12), tanker (2), aqueducts (2), stream (2), river (6), lake (3), or pond water (2), and water from other sources such as no tap water, no tube well (5). In three studies drinking water from unimproved well is not a risk factor, aqueducts are protective in one study but not protective in another study.

In thirty-eight of the 69 studies, association between improved water sources and cholera was determined. In one study leaking tap or piped water is a risk factor. In nine studies improved water sources are not protective however, in 16 other studies improved sources were protective factors. In two studies reservoir water is a risk factor however, in one study it is protective. Public/municipal/military base water is an indicator of improved water supply and is expected to be protective [80].

In three studies public/ municipal water is protective however, in seven studies public, municipal water, or water from community, and a military base are risk factors. In one study public water source has no association with cholera.

Table 4.4. Association between improved water source and cholera outcome[+]

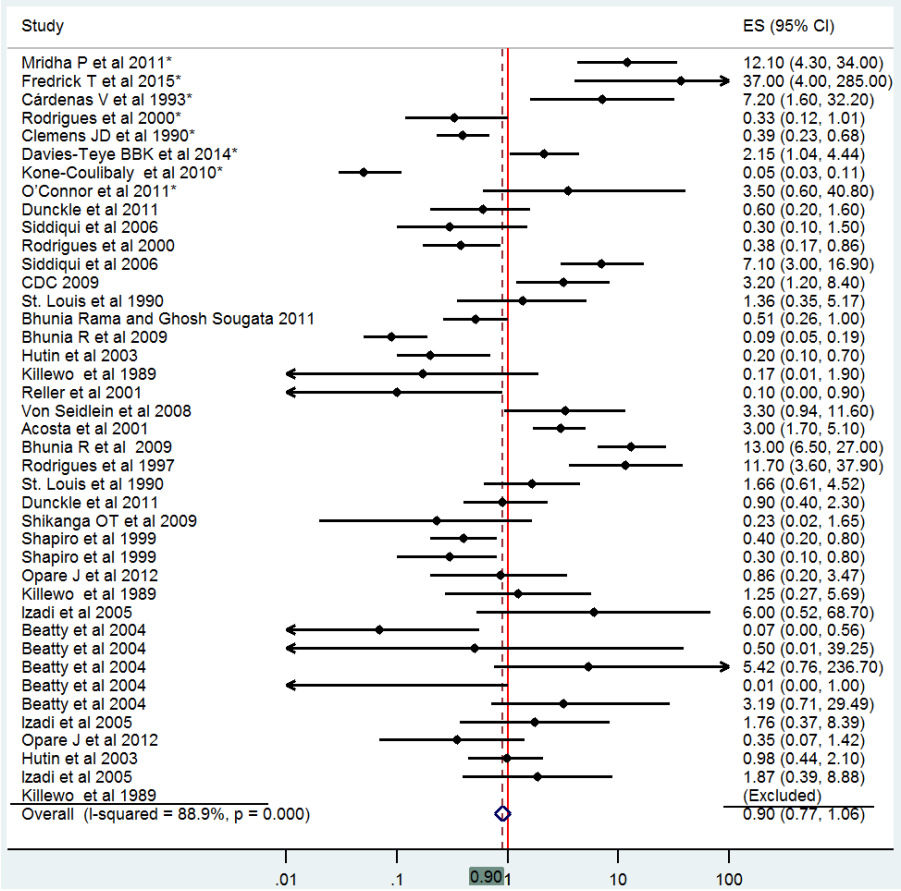
Study	Papers	Predicted Risk Factor	Predicted Protective Factor	Bias	OR univariate	95% CI	OR multivariate	95% CI
1	Dunkle et al. 2011		Piped (house, yard, public tap)	L	0.60	0.2-1.6		
2	Siddiqui et al. 2006		Source of water-reservoir water	L	0.30	0.1-1.5		
3	Rodrigues et al. 2000		Exclusive use of municipal water for drinking	L	0.38	0.17–0.86		
4	Mridha P et al. 2011		Reservoir within mill compound as the main source of drinking-water during work	L			12.10	4.3-34.0
5	Siddiqui et al. 2006		Source of water- reservoir water	L	7.10	3-16.9		
6	Fredrick T et al. 2015		Consumption of water from public drinking-water systen	M			37.00	4.0-285.0
7	Kur L et al. 2009		Drinking water source ≤ 20m from residence	L	3.20	1.2-8.4		
8	Cárdenas V et al. 1993		Drinking municipal water	L			7.20	1.6-32.2
9	St. Louis et al. 1990		Exclusively used piped water for drinking	L	1.36	0.35-5.17		
10	Kirk MD et al. 2005	Leaking pipe or tap		L	4.00	1.1-22.1		
11	Bhunia and Ghosh Sougata 2011		Tube well drinking water	L	0.51	0.26–1.0		
12	Rodrigues et al. 2000		City pump	L			0.33	0.12–1.01
13	Kone-Coulibaly al. 2010		Drink tap water	M			0.05	0.03-0.11
14	Bhunia R et al. 2009		Exclusive tube well water intake	M	0.09	0.05–0.19		
15	Hutin et al. 2003		Drinking tap water at home	L	0.20	0.1-0.70		
16	Clemens JD et al 1990		Tube well in residential compound	L			0.39	0.23-0.68
17	Killewo et al. 1989		Standpipe water source	L	0.17	0.01-1.90		
18	Reller et al. 2001		Water from home faucet	L	0.10	0.0-0.90		
19	Davies-Teye BBK et al. 2014		Community pipe-borne water	H			2.15	1.04-4.44
20	O'Connor et al. 2011		Improved water source	L			3.5	0.6–40.8
21	Von Seidlein et al. 2008		Access to tap water at home	M	3.30	0.94–11.6		
22	Acosta et al. 2001		Inside tap water	L	3.00	1.7-5.1		
23	Bhunia R et al. 2009		Exclusive municipal piped water intake	M	13.00	6.5–27		
24	Rodrigues et al. 1997		Tap water	L	11.70	3.6-37.9		
25	St. Louis et al. 1990		Exclusively used piped water for bathing	L	1.66	0.61-4.52		
26	Dunkle et al. 2011		Purchased bottle/filter	L	0.90	0.4-2.3		
27	Shikanga OT et al. 2009		Protected water source for drinking water (wells, rainwater, and tap water)	H	0.23	0.02–1.65		
28	Shapiro et al.		Drinking rain water	L	0.40	0.2, 0.8		

	1999							
29	Shapiro et al. 1999		Drinking water from a borehole	L	0.30	0.1, 0.8		
30	Opare J et al. 2012		Drinking from borehole	M	0.86	0.2-3.47		
31	Killewo et al. 1989		Kamanzimeru water source	L	1.00	1.0-1.0		
32	Killewo et al. 1989		Kitanga water source	L	1.25	0.27-5.69		
33	Izadi et al. 2005		Tap water and deep well	H	6.00	0.52-68.7		
34	Beatty et al. 2004		Rainwater	L	0.07	0.00-0.56		
35	Beatty et al. 2004		Ebeye municipal system	L	0.50	0.01-39.25		
36	Beatty et al. 2004		Kwajalein military base	L	5.42	0.76-236		
37	Beatty et al. 2004		Rainwater	L	0.01	0.00-1.00		
38	Beatty et al. 2004		Kwajalein military base	L	3.19	0.71-29.49		
39	Izadi et al. 2006	Sources of drinking water (well, river, aqueducts, tankers)		L	2.83	1.12-7.19		
40	O'Connor et al. 2011	Unimproved well		L			0.3	0.10–2.50
41	Opare J et al. 2012	Well		M	0.35	0.07-1.42		
42	Hutin et al. 2003	Drinking water from a well		L	0.98	0.44-2.1		
43	Mahamud et al. 2012	Use water from sources other than tap		L	1.45	0.68 - 3.13		
44	Dunkle et al. 2011	Tanker		L	1.40	0.4-5.0		
45	MCLim-Quizon et al. 1994	No tap water at home		M			2.70	1.63-4.46
46	Moren et al. 1991	Shallow wells		L	4.50	1-20.8		
47	Rosewell, A. et al. 2012	River as drinking water source		M	2.50	1.2-5.2		
48	Shapiro et al. 1999	Drinking water from a stream		L			10.80	1.7-70.1
49	Kone-Coulibaly et al. 2010	Drank unprotected well water		M	16.98	8.58-33.62		
50	Das A et al. 2009	Drinking water from contaminated unprotected well		M	11.30	1.2-42.1		
51	Kur L et al. 2009	Drinking water from tanker truck vs other water sources		L	1.80	0.5-6.8		
52	Rodrigues et al. 1997	Well/ lake		L			2.98	1.49-5.94
53	Killewo et al. 1989	Small rivers		L	6.00	0.82-43.74		
54	Izadi et al. 2005	Deep well		H	1.87	0.39-8.88		
55	Shapiro et al. 1999	Drinking water from Lake Victoria		L			6.50	1.6-25.5
56	Das A et al. 2009	Drinking water from contaminated unprotected well		M	12.00	1.2-44.1		
57	Killewo et al. 1989	Small water sources		L	2.67	0.52-13.63		

58	Mukherjee et al. 2011	Drinking pond water		H	2.10	0.9-4.7		
59	Mukherjee et al. 2011	Drinking pond water		H	3.20	1.3-8.2		
60	Das A et al. 200	Drinking water from contaminated well		M	4.70	1.6-19		
61	Shultz A et al. 2009	Drinking river water		M	1.60	0.4-5.8		
62	Sasaki, S 2008	Drinking water from shallow well		L			7.25	1.49-35.3
63	Swerdlow et al. 1997	Drank any river water		L	2.20	0.8–6.3		
64	Swerdlow et al. 1997	Drank river water		L	3.00	1.4-6.4		
65	Birmingham et al. 1997	Drank lake water		L			2.80	1-7.5
66	Opare et al. 2012	Drink from stream/river		M	6.99	2.75-18		
67	Kone-Coulibaly al. 2010	Drank unprotected well water		M	16.98	8.58-33.62		
68	Izadi et al. 2005		Aqueducts	H	1.76	0.37-8.39		
69	Acosta et al. 200	Other sources of water		L	3.70	2 - 6.7		

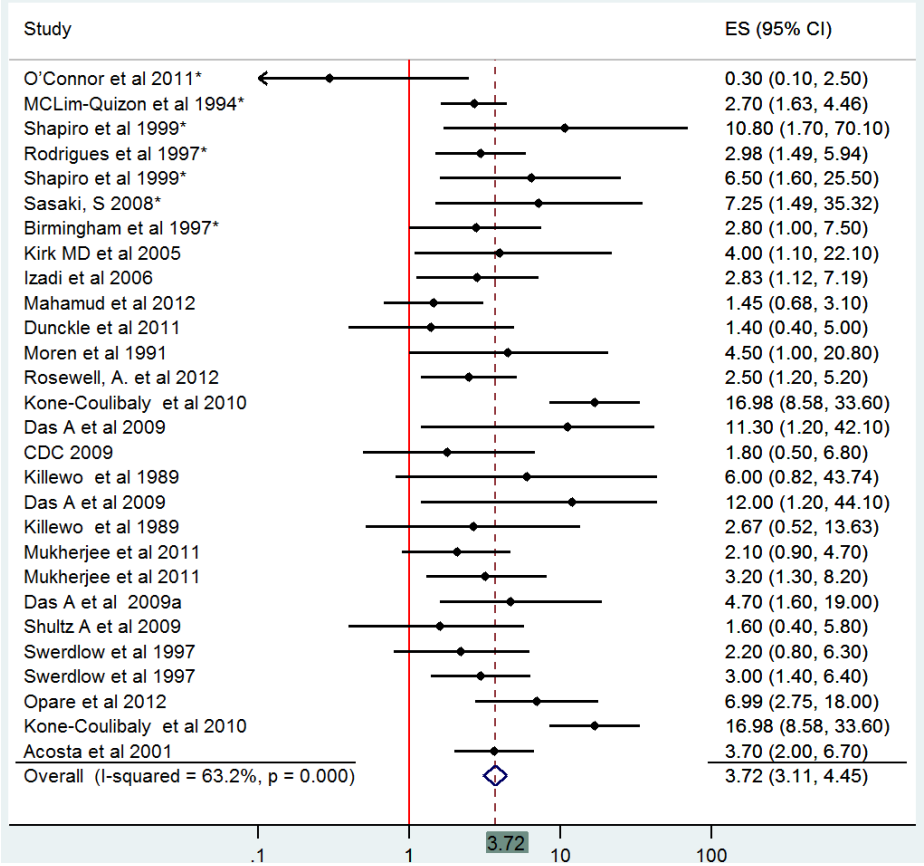
[+]Please note the exposure is highlighted in pink when it is not as expected, in orange when there is no association [82, 85, 87, 88, 89, 91, 92, 93, 94, 95, 97, 98, 99, 102, 103, 104, 105, 106, 108, 109, 117, 120, 123, 125, 126, 128, 129, 131, 132, 134, 135, 136, 137, 140, 142]

Based on the forest plot analysis conducted on 41 studies, categorized as predicted protective factors in Table 4.4, the cumulative effect of consuming water from improved water source is 0.90 (0.77 – 1.06). The overall estimate has no association and is not significant. The χ^2 test has a low p-value < 0.001 and an I^2 test value of 88.9%, which suggests that 88.9% of variation in studies is due to heterogeneity. The result of no association is as expected because improved water sources do not always indicate safe water quality and does not reliably predict microbial safety [65, 64].



Graph 1.4.0. Forest plot analysis of the effect of improved water source (predicted protective factor) on outcome of cholera [Note: Studies with * are multivariate analysis]

Based on the forest plot analysis of 28 studies, categorized as predicted risk factors in Table 4.4, the cumulative effect of consuming water from unimproved water source is 3.72 (3.11 – 4.45). The overall association (odds ratio and 95% CI) is significant, which suggests that unimproved water source is a risk factor for cholera. The χ^2 test has a p-value < 0.001 and an I^2 test value of 63.2%, which suggests that 63.2% of variation in studies is due to heterogeneity. Outliers including O'Connor et al. 2011 and Opare J et al. 1997 do not show differences in study design that could contribute to such a difference in results [88, 125].



Graph 1.4.1. Forest plot analysis of the effect of unimproved water source (predicted risk factor) on outcome of cholera [Note: Studies with * are multivariate analysis]

1.5. Water contact and cholera outcome

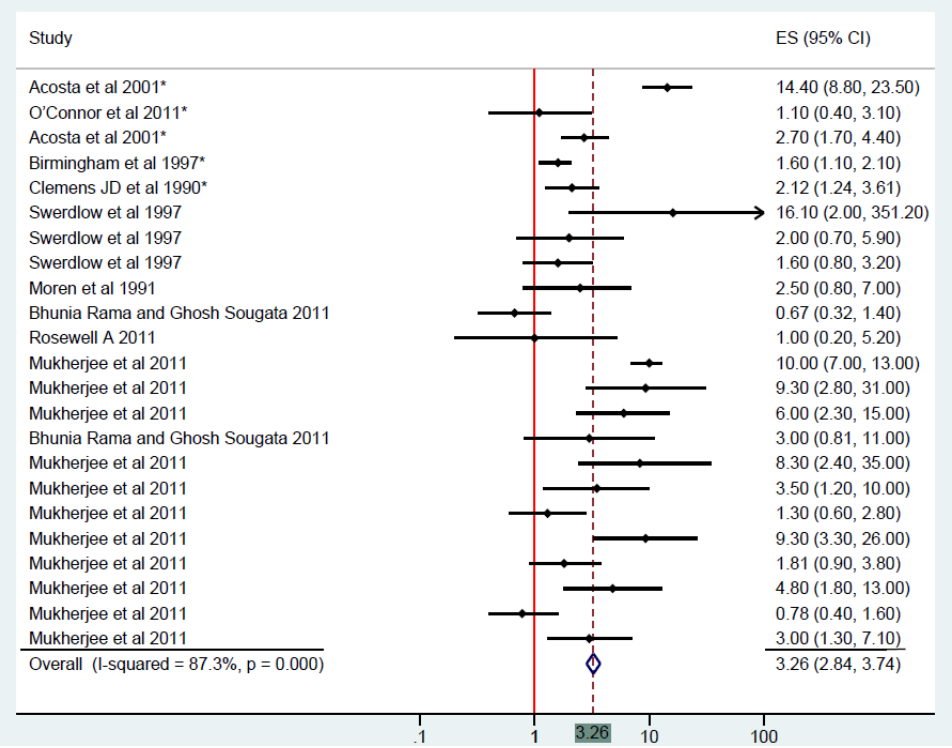
Vibrio cholerae can live in oceans, estuaries, brackish waters, rivers, ponds of coastal areas of the tropical world [57]. Contact with contaminated water sources such as pond (5), river (10), lake (1), ocean (1), other (1) water is expected to be a risk factor and is categorized as predicted risk factor in Table 4.5 below. Such contact may occur due to proximity of residence to a water body, bathing, swallowing, washing, and cooking. Twenty-three studies determined association between water contact and cholera. Cooking in pond water is protective for one study but a risk factor for another study. Washing utensils is a risk factor in three studies, a protective factor in one study, and has no association in one study.

Table 4.5. Association between contact with water source and cholera outcome[+]

Study	Papers	Predicted Risk Factors	Predicted Protective Factors	Bias	OR univariate	95% CI	OR multivariate	95% CI
1	Swerdlow et al. 1997	Among persons who went to river drank river water		L	16.10	2-351.2		
2	Acosta et al. 2001	River bathing		L			14.40	8.8-23.5
3	O'Connor et al. 2011	Contact with river water		L			1.1	0.4–3.1
4	Acosta et al. 2001	Distance >10 min to water source (river)		L			2.70	1.7-4.4
5	Swerdlow et al. 1997	Went to river		L	2.00	0.7-5.9		
6	Swerdlow et al. 1997	Went to river		L	1.60	0.8-3.2		
7	Birmingham et al. 1997	Bathed in lake (30 min increments)		L			1.60	1.1-2.1
8	Moren et al. 1991	river contact		L	2.50	0.8-7.0		
9	Clemens JD et al. 1990	residential proximity to river		L			2.12	1.24-3.61
10	B and Ghosh Sougata 2011	Utensil wash in pond water		L	0.67	0.32–1.4		
11	Rosewell A 2011	Washed utensils in the ocean		L	1.00	0.2–5.2		
12	Mukherjee et al. 2011	Washing utensils in pond water		H	10.00	7.0-13		
13	Mukherjee et al. 2011	Washing utensils in pond water		H	9.30	2.8-31		
14	Mukherjee et al. 2011	Washing utensils in pond water		H	6.00	2.3-15		
15	B and Ghosh Sougata 2011	Utensil wash in river water		L	3.00	0.81-11		
16	Mukherjee et al. 2011	Swallowing pond water while bathing		H	8.30	2.4-35		
17	Mukherjee et al. 2011	Swallowing pond water while bathing		H	3.50	1.2-10		
18	Mukherjee et al. 2011	Swallowing pond water while bathing		H	1.30	0.6-2.8		
19	Mukherjee et al. 2011	Swallowing pond water while bathing		H	9.30	3.3-26		
20	Mukherjee et al. 2011	Washing mouth with pond wate		H	1.81	0.9-3.8		
21	Mukherjee et al. 2011	Washing mouth with pond wate		H	4.80	1.8-13		
22	Mukherjee et al. 2011	Cooking with pond water		H	0.78	0.4-1.6		
23	Mukherjee et al. 2011	Cooking with pond water		H	3.00	1.3-7.1		

[+] Please note the exposure is highlighted in pink when it is not as expected and in orange when there is no association [91, 92, 104, 108, 109, 120, 137, 141]

Based on the forest plot analysis of 23 studies, categorized as risk factors in Table 4.5, the cumulative effect of water contact and cholera is 3.26 (2.84 – 3.74). The overall association (odds ratio and 95% CI) is significant, which suggests that extended water contact with river, lake, or pond is a risk factor for cholera. The χ^2 test has a low p-value of < 0.001 and an I^2 test value of 87.3%, which suggests that 87.3% of variation in studies is due to heterogeneity. As expected, water contact is a significant risk factor for cholera.



Graph 1.5.1. Forest plot analysis of the effect of water contact (predicted risk factor) on the outcome of cholera
[Note: Studies with * are multivariate analysis]

2) Association between water treatment and cholera

The association between various water treatment exposures and cholera outcome is identified in 51 studies. Treated water is expected to be a protective factor whereas untreated water is expected to be a risk factor for developing cholera [48]. Treated water includes boiled water (12), water with chlorine present (4), household with chlorine present (4), household with other water treatment products observed (1). Studies that do not mention the exact method used to treat water are also included (5). Untreated water includes not boiling (6) and not chlorinating water (6), untreated well (2), tap (2), piped (1), and rain water (1), and using mixed water (combination of treated and untreated water) (1).

In twenty six of the 51 studies, the association between treated water and cholera outcome is determined. In 24 of the 26 studies treating water is a protective factor however, in one study drinking boiled water is not protective. In another study bottle of chlorine water treatment solution observed is not protective. In twenty-five studies the association between untreated water and cholera is determined. In 23 of the 25 studies untreated water is a risk factor however, in two studies, drinking untreated water and drinking mixed drinking water (combination of chlorinated/tubewell and non-chlorinated water) is not a risk factor.

Table 4.6. Association between water treatment and cholera outcome[+]

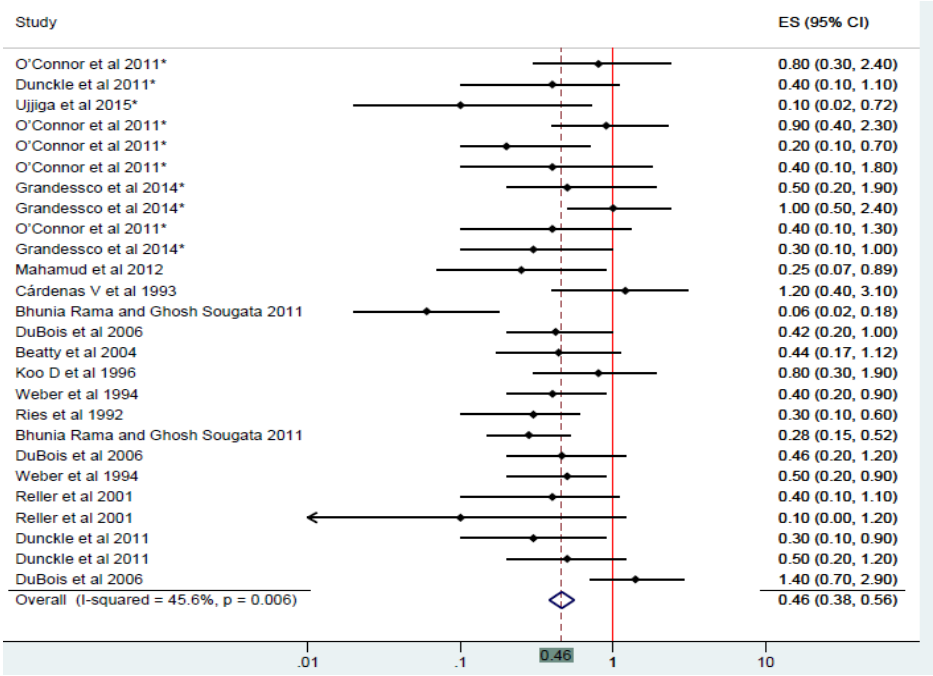
Study	Papers	Predicted Risk Factors	Predicted Protective Factors	Bias	OR univariate	95% CI	OR multivariate	95% CI
1	Mahamud et al. 2012		Treated water before drinking	L	0.25	0.07-0.89		
2	Bhunia and Ghosh 2011	Mixed drinking water		L	0.96	0.44-2.1		
3	Cárdenas V et al. 1993		Drinking water boiled	L	1.20	0.4-3.1		
4	O'Connor et al. 2011		Water treatment product in home	L			0.8	0.3–2.4
5	Dunkle et al. 2011		Boiling water or using chlorine	L			0.40	0.1-1.1
6	Bhunia and Ghosh 2011		Chlorine-treated drinking water	L	0.06	0.02-0.18		
7	DuBois et al. 2006		Regularly boil drinking water	L	0.42	0.2–1.0		
8	Beatty et al. 2004		Boiling	L	0.44	0.17-1.12		
9	Koo D et al. 1996		Always boil water	L	0.80	0.3-1.9		
10	Weber et al. 1994		Boiled water present home	L	0.40	0.2-0.9		
11	Ries et al. 1992		Always drink boiled water	L	0.30	0.1-0.6		
12	Ujjiga et al. 2015		Treated drinking water at home				0.10	0.02-0.72
13	B and Ghosh 2011		Boiled drinking water	L	0.28	0.15-0.52		
14	DuBois et al. 2006		Regularly use any Water treatment	L	0.46	0.2–1.2		
15	Weber et al. 1994		Always boiling drinking water at home	L	0.50	0.2-0.9		
16	Reller et al. 2001		Boiled water	L	0.40	0.1 -1.1		
17	Reller et al. 2001		Water treated with Sûr'Eau (0.5% sodium hypochlorite solution)	L	0.10	0.0 -1.2		
18	O'Connor et al. 2011		Treating drinking water before the outbreak	L			0.90	0.4– 2.3
19	Dunkle et al. 2011		Boiling water or usin chlorine before Nov 1, 2010	L	0.30	0.1-0.9		
20	Dunkle et al. 2011		Boiling water or usin chlorine ≤3d before illness	L	0.50	0.2-1.2		
21	O'Connor et al. 2011		Treating drinking water 3d before illness onset (during outbreak)	L			0.20	0.1–0.7
22	O'Connor et al. 2011		Residual chlorine presence in home drinking water >0.5mg/L	L			0.40	0.1–1.8
23	Grandesso et al. 2014		Chlorine level >0·2mg/l in drinking water stored at home	L			0.50	0.2- 1.9

24	Grandesso et al. 2014		Chlorine level >0·2n in drinking water stored at home	L			1.00	0·5–2·4
25	O'Connor et al. 2011		Residual chlorine presence in home drinking water >0.1mg/L	L			0.4	0.1–1.3
26	DuBois et al. 2006		Bottle of chlorine water treatment solution observed	L	1.40	0.7–2.9		
27	Grandesso et al. 2014		Always chlorinate water before drinking	L			0.30	0.1-1.0
28	Hoge et al. 1996	Consumption of untreated well water		L			13.98	2.56-76.5
29	Bhunia and Ghosh Sougata 2011	Drinking Non-chlorinated piped water		L			45.00	11-192
30	Hoge et al. 1996	Consumption of untreated well water		L			16.14	1.88-138.
31	Weber et al. 1994	Unboiled water		L	3.60	1.8-7.5		
32	Ries et al. 1992	Drank unboiled water		L	3.90	1.7-8.9		
33	Kirk MD et al. 2005	Untreated rainwater main source		L	8.00	1.1-355		
34	Reller et al. 2001	Untreated water, any source		L	5.00	1.3- 25.4		
35	Acosta et al. 2001	Unboiled drinking water		L	1.90	1.1-3.2		
36	Acosta et al. 2001	Unfiltered drinking water		L	1.70	0.9-3.0		
37	Mujica et al. 1994	Unboiled drinking water		L	2.10	0.9-4.8		
38	Kur L et al. 2009	Drinking untreated water vs. chlorinated water		L	0.20	0.1-0.7		
39	Swerdlow et al. 1992	Drank unboiled water		L	3.10	1.3-7.3		
40	Quick et al. 1995	Drank untreated water		L	1.90	0.5-7.2		
41	Mugoya et al. 2008	Not treating drinking water		L			4.90	1.6–15
42	Mugoya et al. 2008	Not treating drinking water		L	5.00	1.4–18		
43	DuBois et al. 2006	Drank any untreated water		L	1.90	0.9–3.9		
44	Koo D et al. 1996	Untreated water		L	2.70	0.9-8.2		
45	Hoge et al. 1996	Consumption of untreated tap water		L			3.60	1.69-7.67
46	Hoge et al. 1996	Consumption of untreated tap water		L			2.23	1.03-4.80
47	Fredrick T et al. 2015	Not boiling water		M			35.00	4.0-269.0
48	Grandesso et al. 2014	Using untreated water for washing dishes		L			3.20	1·4–7·3
49	Cummings et al. 2012	Does not use chlorine tablets to treat drinking		L			3.86	1.63-9.14

		water						
50	Shikanga OT et al. 2009	Chlorine absent in home water		H	2.83	0.86–10.8		
51	Sasaki S 2008	Not chlorinate drinking Water		L			2.23	1.01-4.94

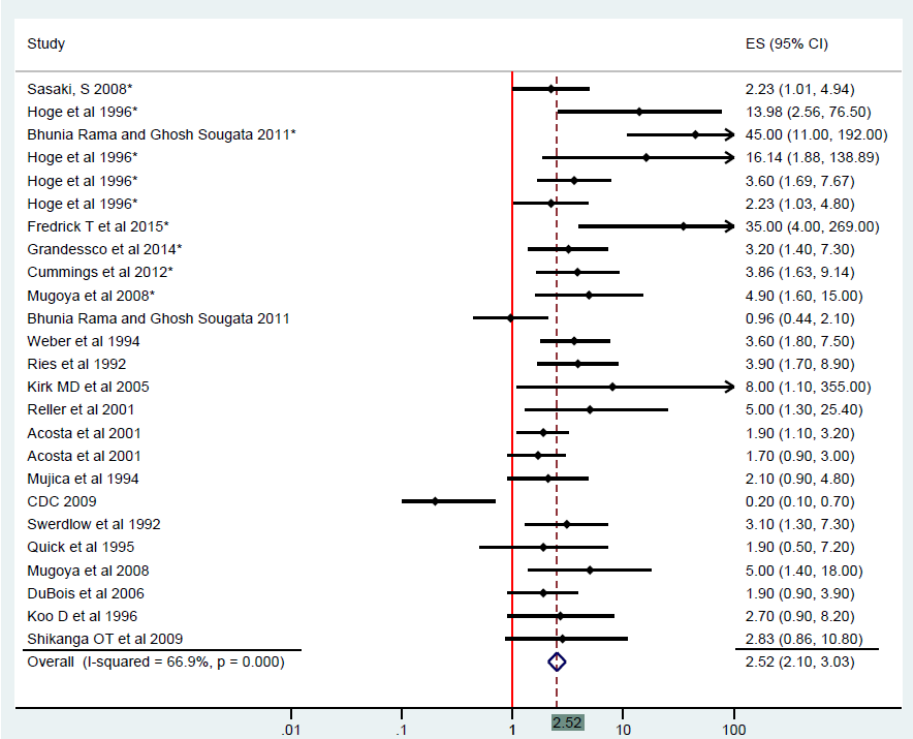
+ Please note the exposure is highlighted in pink when it is not as expected, in orange when there is no association [81, 86, 87, 88, 89, 92, 95, 98, 100, 101, 103, 104, 110, 111, 113, 115, 116, 119, 121, 122, 123, 129, 131, 135, 142]

Based on the forest plot analysis of 26 studies, categorized as predicted protective factors in Table 4.6, the cumulative effect of treated water on cholera outcome is 0.46 (0.38 – 0.56). The overall association, odds ratio and 95% CI, is significant, which suggests that treated water is protective against cholera. The χ^2 test has a low p-value < 0.05 (p-value = 0.006) and an I^2 test value of 45.6%, which suggests that 45.6% of variation in studies is due to heterogeneity.



Graph 2.0.0. Forest plot analysis of the effect of water treatment (predicted protective factor) on the outcome of cholera [Note: Studies with * are multivariate analysis]

Based on the forest plot analysis of 25 studies, categorized as predicted risk factors in Table 4.6, the cumulative effect of untreated water and cholera is 2.52 (2.10 – 3.03), which suggests that untreated water is a significant risk factor for cholera. The overall association, odds ratio and 95% CI, is as expected. The χ^2 test has a low p-value of < 0.001 and an I^2 test value of 66.9%, which suggests that 66.9% of variation in studies is due to heterogeneity. As expected untreated water is a risk factor.



Graph 2.0.1. Forest plot analysis of the effect of lack of water treatment (predicted risk factor) on outcome of cholera [Note: Studies with * are multivariate analysis]

3) Association between water storage and transfer and cholera

In 42 studies, the association between water storage and cholera was determined. Safe water storage is expected to be a protective factor [48]. Safe water storage can be in the form of 1) storage container used at home 2) storage container used by community and modified by an intervention program 3) commercial safe storage containers purchased by a program and distributed to users [65]. However, the water storage data collected for this study is comprised of storage containers used at home only. Safe water storage is characterized as a container that is narrow–mouthed and sealed and does not allow for contamination through hands or objects such as cups, or ladles [65]. Safe water storage includes plastic bottle (1), narrow mouth container (6), jerry can (2), bucket with lid (3), container that is covered/sealed (4), chlorine present in stored water (2), use of separate containers for drinking and washing (1), transporting and storing water via spout in vessel or pouring (2). Lack of safe water storage is considered a risk factor. Unsafe water storage includes bucket (4), open/unsealed container (1), presence of E.coli in stored water (2), container in which hands had been introduced (4), dirty container (1), shared water storage container with community (1), transporting and storing water via scooping water (1).

In twenty four studies the association between safe water storage and cholera outcome is determined. In eighteen out of 24 studies safe water storage is protective and in six studies safe storage is not protective. In one study the presence of free chlorine in stored water is not protective, in another study water stored in covered container is not protective, two studies determined jerry can is not protective, one study found narrow mouthed jug with/without lid to be not protective, pouring water for storage/drinking is also not protective. In eighteen studies the association between unsafe water storage and cholera outcome is determined. Unsafe water storage is characterized by presence of E.coli in water, hands introduced in the container, sharing water storage with neighbors, and a wide open-mouth container, such as a bucket without cover. In 16 out of 18 studies, unsafe water storage is a risk factor and in two studies unsafe storage is not a risk factor, scooping water to transfer for storage and using any container to store water is also not a risk factor.

Table 4.7. Association between water storage and transfer techniques and cholera outcome[+]

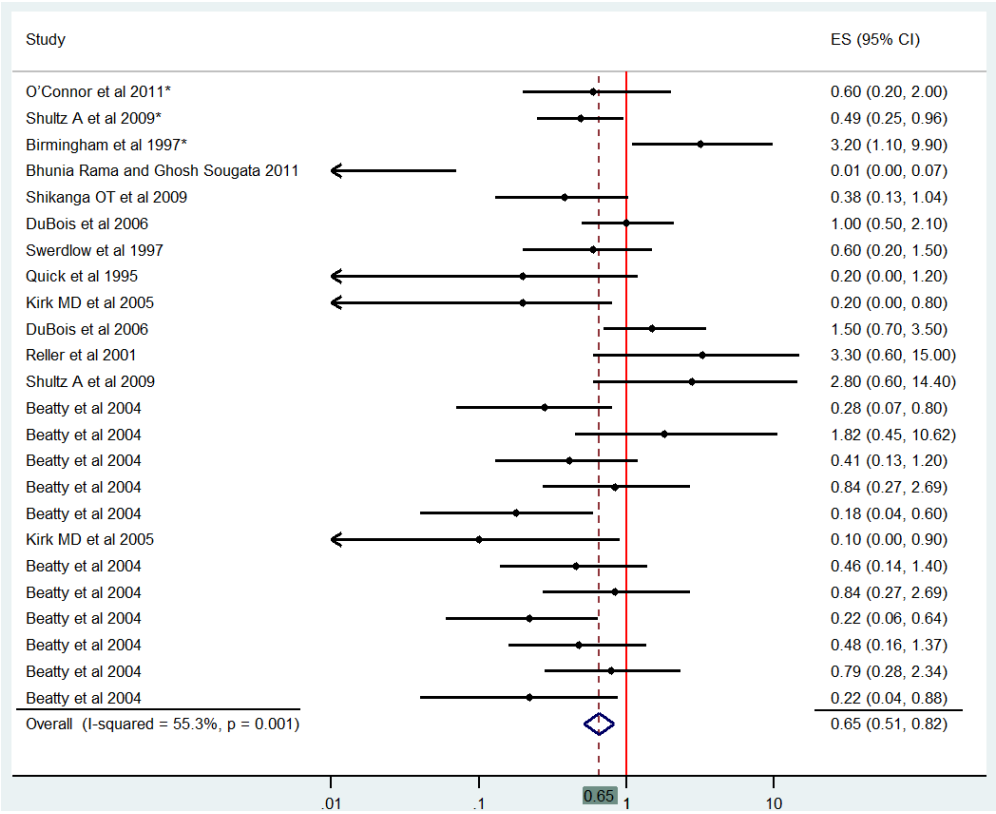
Study	Papers	Predicted Risk Factor	Predicted Protective Factor	Bias	OR univariate	95% CI	OR multivariate	95% CI
1	O'Connor et al. 2011		Plastic bottle- safe storage	L			0.6	0.2–2.0
2	Bhunia and Ghosh Sougata 2011		Narrow-mouthed container	L	0.01	0.001–0.07		
3	Shikanga OT et al. 2009		Stored water in narrow-mouthed container	H	0.38	0.13–1.04		
4	Shultz A et al. 2009		Water stored in house sealed/ covered	M			0.49	0.25-0.96
5	DuBois et al. 2006		Reported chlorination of stored water	L	1.00	0.5–2.1		
6	Swerdlow et al. 1997		Used separate container to drink or wash	L	0.60	0.2-1.5		
7	Quick et al. 1995		Covered drinking water vessel in home	L	0.20	0.0-1.2		
8	Hatch et al.l 1994	Any water containers		M			0.02	0.003-0.12
9	Kirk MD et al. 2005		Narrow-neck water Container	L	0.20	0.0-0.8		
10	DuBois et al. 2006		Free chlorine present in stored water	L	1.50	0.7–3.5		
11	Reller et al. 2001		Water stored in covered container	L	3.30	0.6-15.0		
12	Shultz A et al. 2009	Usually keep water stored in house		M	1.80	0.8-3.8		
13	Mugoya et al. 2008	Storing water in a bucket		L			3.80	1.2–12.0
14	Mugoya et al. 2008	Storing drinking water in open container		L			3.30	1.0–10.0
15	Rodrigues et al. 2000	Bucket/basin		L			4.73	1.95–11.4
16	Swerdlow et al. 1997	Put hands in water container		L	6.00	1.3-26.8		
17	Swerdlow et al. 1997	Put hands in water container		L	1.80	0.6-5.5		

18	Birmingham et al. 1997		Used 20 L jerry-can to store drinking wate (narrow mouthed jerry can)	L			3.20	1.1-9.9
19	Ries et al. 1992	Put hands in drinking water storage vessel		L	2.60	1.2-5.9		
20	Rodrigues et al. 1997	Bucket /basin		L			2.81	1.28-6.19
21	Reller et al. 2001	Stored water touched by hand		L	1.80	0.7-5.4		
22	Grandesso et al. 2014	Presence of E. coli in drinking water stored at home		L			3.50	1.2- 10
23	Grandesso et al. 2014	Presence of E. coli in drinking water stored at home		L			1.50	0·5–4·3
24	Cummings et al. 2012	Does not store water in sealed container		L	3.30	1.7-6.7		
25	Mahamud et al. 2012	Dirty water storage containers		L			4.39	1.12-17.14
26	O'Connor et al. 2011	Bucket-unsafe storage		L			1.1	0.4–2.8
27	Shultz A et al. 2009		Storing water in jerry can	M	2.80	0.6-14.4		
28	Swerdlow et al. 1997	Shared water containe with neighbors		L	2.30	0.7-7.3		
29	O'Connor et al. 2011	Lacked safe water storage		L			1.3	0.5–4.0
30	Beatty et al. 2004		Modes of transferring water for storage/ drinking by removal v spout in vessel	L	0.28	0.07-0.80		
31	Beatty et al. 2004	Modes of transferring water for storage/ drinking by scooping		L	0.38	0.10-1.34		
32	Beatty et al. 2004		Modes of transferring water for storage/ drinking by pouring	L	1.82	0.45-10.62		
33	Beatty et al. 2004		Bucket with lid	L	0.41	0.13-1.20		
34	Beatty et al. 2004		Narrow mouthed jug with/ without lid	L	0.84	0.27-2.69		
35	Beatty et al. 2004		Insulated water cooler	L	0.18	0.04-0.60		
36	Kirk MD et al. 2005		Container to store water safely	L	0.10	0.0-0.9		
37	Beatty et al. 2004		Bucket with lid	L	0.46	0.14-1.40		
38	Beatty et al. 2004		Narrow mouth jug with/ without lid	L	0.84	0.27-2.69		
39	Beatty et al. 2004		Insulated water cooler	L	0.22	0.06-0.64		
40	Beatty et al. 2004		Bucket with lid	L	0.48	0.16-1.37		
41	Beatty et al. 2004		Narrow mouth jug with/ without lid	L	0.79	0.28-2.34		
42	Beatty et al. 2004		Insulated water cooler	L	0.22	0.04-0.88		

[+] Please note the exposure is highlighted in pink when it is not as expected, in orange when there is no association [81, 86, 87, 88, 92, 95, 97, 100, 101, 103, 105, 108, 109, 113, 114, 121, 131, 134, 142]

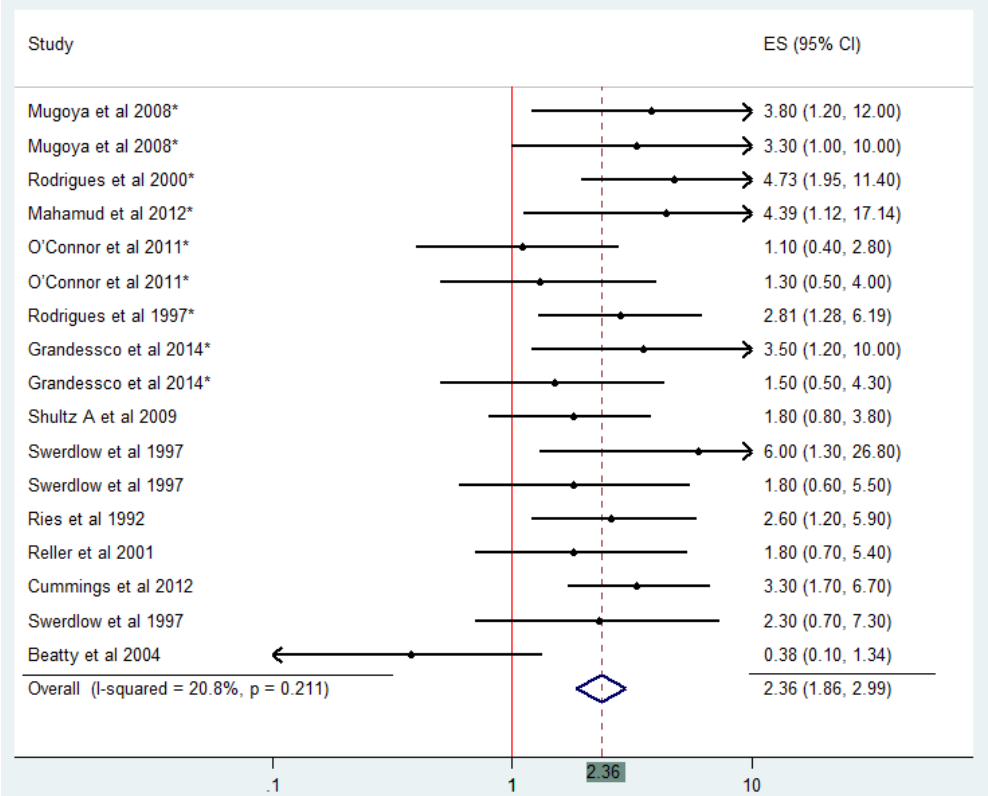
Based on the forest plot analysis of 24 studies, categorized as predicted protective factors in Table 4.7, the cumulative effect of safe water storage and transfer and cholera outcome is 0.65 (0.51– 0.82). The overall association, odds ratio and 95% CI, is significant, which as expected suggests that safe water storage is protective against cholera. The χ^2 test

has a low p-value of < 0.05 (p-value = 0.001) and an I^2 test value of 55.3%, which suggests that 55.3% of variation in studies is due to heterogeneity.



Graph 3.0.0. Forest plot analysis of the effect of safe water storage (predicted protective factors) on outcome of cholera [Note: Studies with * are multivariate analysis]

Based on the forest plot analysis of 17 studies, categorized as predicted risk factors in Table 4.7, the cumulative effect of unsafe water storage and cholera is 2.36 (1.86 – 2.99). The overall association, odds ratio and 95% CI, is significant, which suggests that unsafe water storage is a risk factor for cholera. The χ^2 test has a high p-value of > 0.05 (p = 0.211) and an I^2 test value of 20.8%, which suggests that 20.8% of variation in studies is due to heterogeneity. As expected, unsafe water storage is a significant risk factor for the study.



Graph 3.0.1. Forest plot analysis of the effect of unsafe water storage (predicted risk factor) on outcome of cholera
[Note: Studies with * are multivariate analysis]

4) Association between sanitation and cholera

The association between sanitation and cholera is determined in 25 papers. Adequate sanitation is expected to be a protective factor and inadequate sanitation is expected to be a risk factor for cholera [66]. Sanitation exposures include: 4.1. Latrine 4.2. Open air defecation 4.3 Drainage 4.4 Sanitary practices. The exposures in these sub-categories are described below.

4.1. Availability of latrine facility and cholera outcome

Presence of latrine is expected to be a protective factor and categorized as predicted protective factor in Table 4.8 below. Fully sanitary toilets which are exclusively used by the household are protective whereas unsanitary toilets, and toilets shared by the community are expected to be risk factors. In 16 studies the exposure of latrine on cholera outcome is determined. In three studies presence of latrine is not a protective factor and in two studies presence of unsanitary toilet (without soap) is not a risk factor. Five studies determine the association between lack of toilet facility

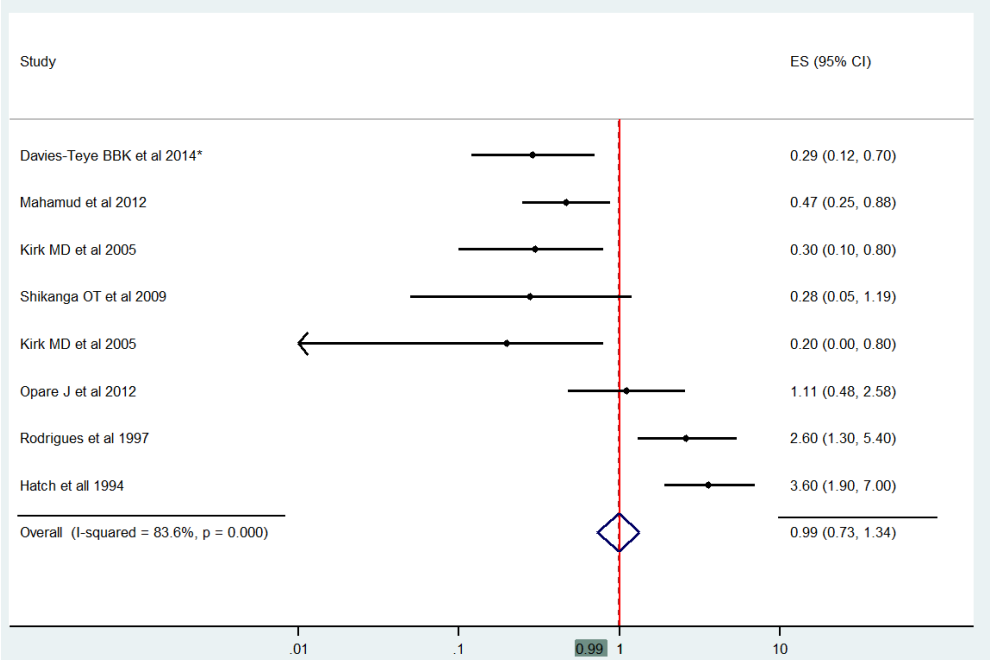
and cholera. In four out of five studies, the absence of toilet facility is a risk factor, however, in one study lack of toilet is not a risk factor.

Table 4.8. Association between latrine facility and usage and cholera outcome[+]

Study	Papers	Predicted Risk Factor	Predicted Protective Factor	Bias	OR univariate	95% CI	OR multivariate	95% CI
1	Davies-Teye BBK et al. 2014		Exclusive toilet facility	H			0.29	0.12-0.70
2	Mahamud et al. 2012		Latrine in compound	L	0.47	0.25-0.88		
3	Kirk MD et al. 2005		Toilet inside house	L	0.30	0.1-0.8		
4	Hatch et al.l 1994		Latrine	M	3.60	1.9-7.0		
5	Izadi et al. 2005	Not fully sanitary toilet		H	0.56	0.08-4.00		
6	Izadi et al. 2005	Unsanitary toilet		H	0.55	0.10-2.99		
7	Shikanga OT et al. 2009		Safe stool disposal (latrine, dug pit, or toilet)	H	0.28	0.05–1.19		
8	Kirk MD et al. 2005		Flush toilet	L	0.20	0.0-0.8		
9	Opare J et al. 2012		Availability of latrine In house	M	1.11	0.48-2.58		
10	Rodrigues et al. 1997		Latrine	L	2.60	1.3-5.4		
11	Mugoya et al. 2008	Unsound latrine superstructure		L	4.00	1.1–14		
12	Rodrigues et al. 1997	No toilet facility		L	16.00	0.5-35.8		
13	Izadi et al. 2005	Without toilet		H	0.76	0.14-4.19		
14	Cummings et al. 2012	No latrine present in household		L	4.80	1.3 -17.5		
15	Sasaki, S 2008	Not have a latrine inside premises		L			2.00	0.7-5.70
16	Acosta et al. 2001	No latrine at home		L	11.40	6.3-20.5		

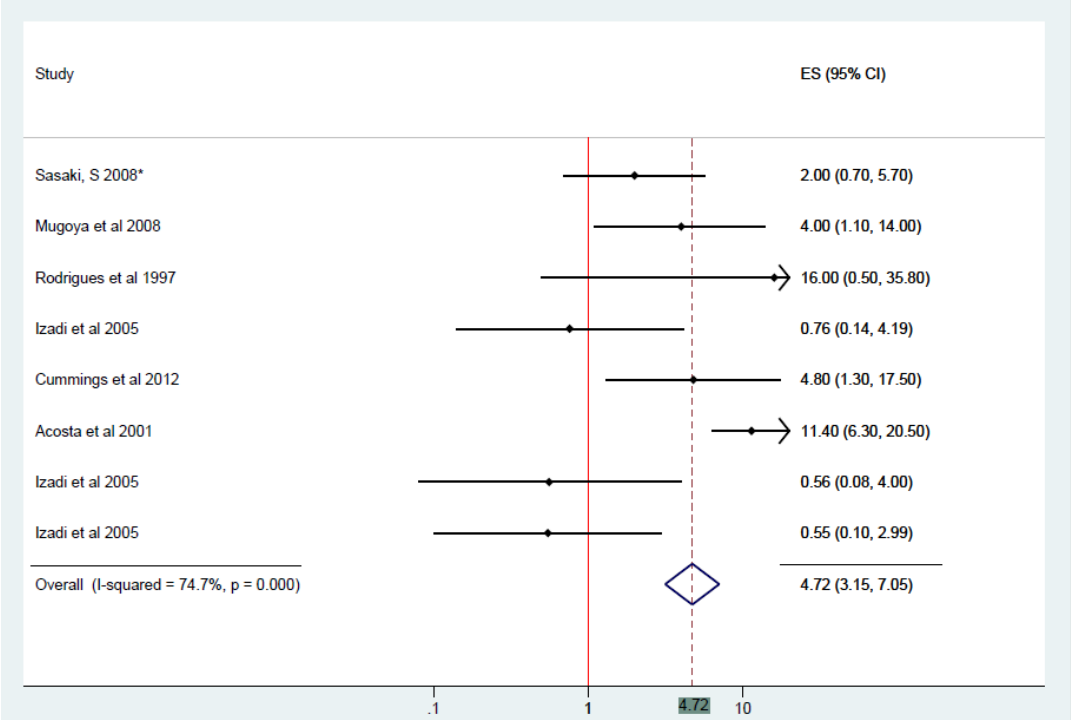
[+]Please note the exposure is highlighted in pink when it is not as expected [82, 86, 87, 95, 98, 104, 114, 125, 131, 134, 143]

Based on the forest plot analysis of eight studies, categorized as predicted protective factors in Table 4.8, the cumulative effect of latrine facility and cholera is 0.99 (0.73 – 1.34). Availability of latrine facility can be expected to be a protective factor if it separates feces from human contact. The overall association, OR and 95% CI, is not significant. The χ^2 test has a p-value < 0.001 and an I^2 test value of 83.6%, which suggests that 83.6% of variation in studies is due to heterogeneity. This results is as expected because although latrines might be present in some cities in developing countries, they lack adequate sanitation and such ineffective toilets are breeding grounds for bacteria [67].



Graph 4.1.0. Forest plot analysis of the effect of presence of latrine facility (predicted protective factors) on outcome of cholera [Note: Studies with * are multivariate analysis]

Based on the forest plot analysis of eight studies, categorized as predicted risk factors in Table 4.8, the cumulative effect of no facility or inadequate latrine facility and cholera is 4.72 (3.15 - 7.05). The overall association, odds ratio and 95% CI, is significant, which suggests that inadequate latrine facility is a risk factor for cholera. The χ^2 test has a p-value <0.001 and an I^2 test value of 74.7%, which suggests that 74.7% of variation in studies is due to heterogeneity. As expected, inadequate latrine facility is a risk factor for cholera outcome.



Graph 4.1.1. Forest plot analysis of the effect of lack of latrine facility (predicted risk factors) on outcome of cholera [Note: Studies with * are multivariate analysis]

4.2. Practicing open air defecation and cholera outcome

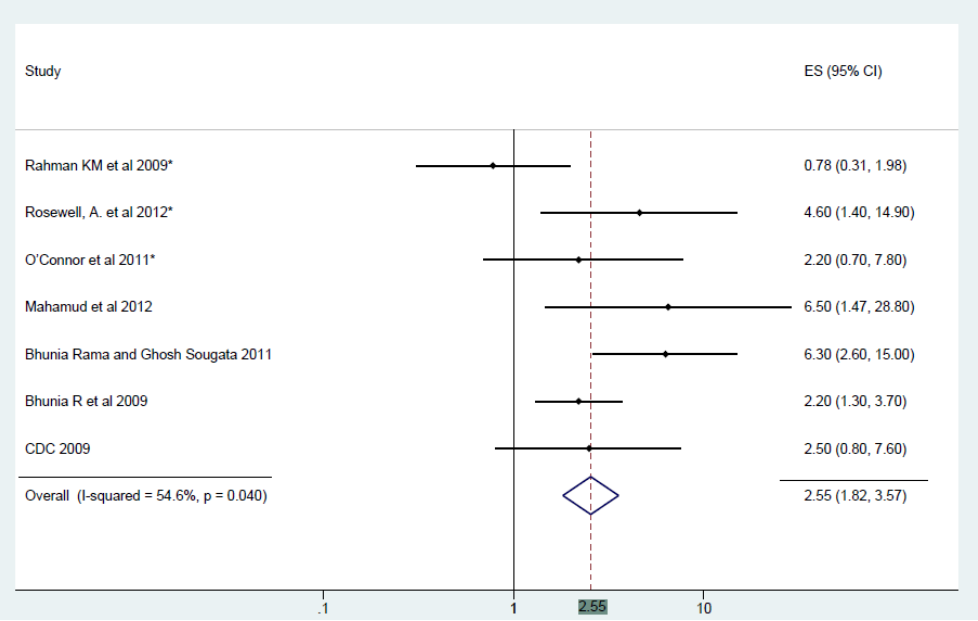
Open air defecation is expected to be a risk factor for cholera as it directly introduces the bacteria to the environment. In seven studies the association between open air defecation and cholera outcome is determined. In six out of seven studies, defecting in the open or observing feces on the ground is a risk factor for cholera. In one study however, child defecting in the open is not a risk factor.

Table 4.9. Association between defecating in the open and cholera outcome[+]

Study	Papers	Predicted Risk Factors	Predicted Protective Factor	Bias	OR univariate	95% CI	OR multivariate	95% CI
1	Rahman KM et al. 2009	Child defecating in open place		M			0.78	0.31-1.9
2	Rosewell, A. et al. 2012	Defecates in open air (or river)		M			4.60	1.4-14.9
3	Mahamud et al. 2012	Observed faeces on ground		L	6.50	1.47-28.8		
4	O'Connor et al. 2011	Open defecation		L			2.2	0.7–7.8
5	Bhunia Rama and Ghosh Sougata 2011	Open-air defecation practice		L	6.30	2.6-15		
6	Bhunia R et al. 2009	Open air defecation		M	2.20	1.3–3.7		
7	Kur L et al. 2009	defecating in an open field		L	2.50	0.8-7.6		

[+]Please note the exposure is highlighted in pink when it is not as expected [88, 35, 87, 92, 96, 128, 124]

Based on the forest plot analysis of seven studies, characterized as predicted risk factors in Table 4.9, the cumulative effect of open air defecation and cholera is 2.55 (1.82 – 3.57). The overall association, odds ratio and 95% CI, is significant, which suggests that open air defecation is a risk factor for cholera. The χ^2 test has a low p-value < 0.05 (p-value = 0.040) and an I^2 test value of 54.6%, which suggests that 54.6% of variation in studies is due to heterogeneity. As expected open air defecation is a significant risk factor for cholera outcome.



Graph 4.2.0. Forest plot analysis of the effect of open air defecation (predicted risk factors) on outcome of cholera
[Note: Studies with * are multivariate analysis]

4.3. Lack of drainage and cholera outcome

Lack of drainage is expected to be a risk factor, as feces may not be disposed properly, which may introduce bacteria to the environment. Lack of drainage includes using toilet without flush system such as pit latrines. Seven studies determine the association between lack of drainage and cholera, and in all seven studies lack of drainage as a risk factor for cholera.

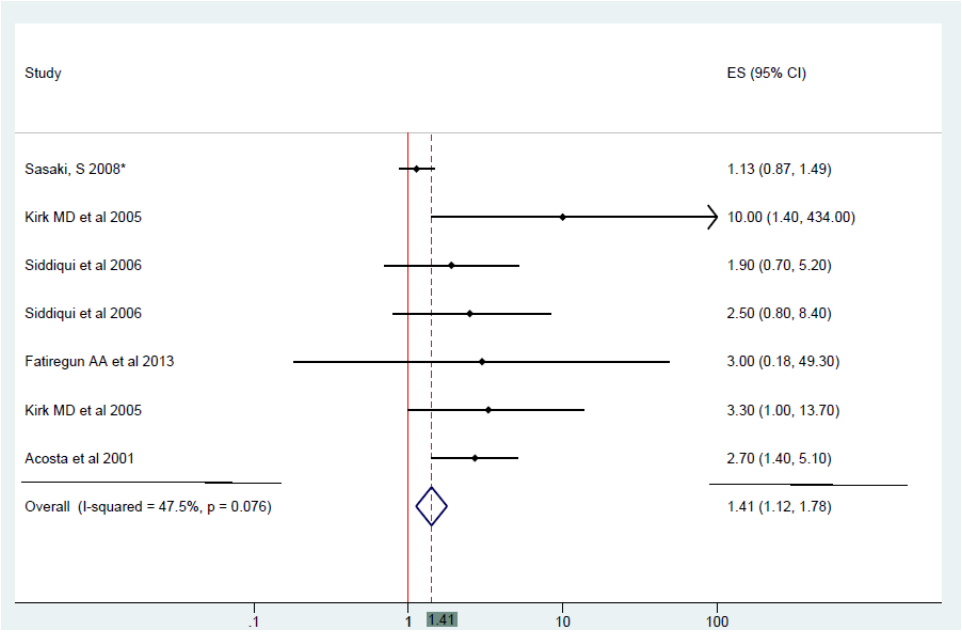
Table 5.0. Association between drainage facility and cholera outcome

Study	Papers	Predicted Risk Factors	Predicted Protective Factors	Bias	OR univariate	95% CI	OR multivariate	95% CI
1	Kirk MD et al. 2005	holes around pit latrine		L	10.00	1.4-434		
2	Siddiqui et al. 2006	type of toilet other than flush system		L	1.90	0.7-5.2		
3	Siddiqui et al. 2006	type of toilet other than flush system		L	2.50	0.8-8.4		
4	Fatiregun AA et al.	pit latrine		L	3.00	0.18-49.3		

	2013							
5	Kirk MD et al. 2005	pit latrine		L	3.30	1-13.7		
6	Acosta et al. 2001	simple pit latrine compared with ventilated improved pit latrines.		L	2.70	1.4-5.1		
7	Sasaki, S 2008	Households without drainage		L			1.13	0.86–1.49

[98, 102, 104, 124, 131]

Based on the forest plot analysis of seven studies, characterized as predicted risk factors in Table 5.0, the cumulative effect of lack of drainage and cholera is 1.41(1.12 - 1.78). The overall association, odds ratio and 95% CI, is significant, which suggests that lack of drainage is a risk factor for cholera. The χ^2 test has a p-value > 0.05 (p-value = 0.076) and an I^2 test value of 47.5%, which suggests that 47.5% of the variation in studies is due to heterogeneity.



Graph 4.3.0. Forest plot analysis of lack of drainage facility (predicted risk factors) and outcome of cholera [Note: Studies with * are multivariate analysis]

4.4. Sanitary practices and cholera outcome

Sanitary practices such as sharing toilet facility with community (3), and not using toilet are expected to be risk factors for developing cholera. Using toilet (1), access to toilet (1), and using toilet paper (1) are expected to be protective factors. Seven studies determine the association between sanitary practices and cholera. In three studies, communal toilet facilities are risk factors. In four studies, using latrine, toilet paper and access to toilet are protective factors but not using toilet to dispose children's feces is a risk factor.

Table 5.1. Association between varying sanitary practices and cholera outcome**

Study	Papers	Predicted Risk Factors	Predicted Protective Factors	Bias	OR univariate	95% CI	OR multivariate	95% CI
1	Shultz A et al. 2009	Three or more households sharing same latrine		M			2.17	1.01-4.68
2	Sasaki, S 2008	Share a latrine with more than two households		L			4.25	1.01-17.86
3	Von Seidlein et al. 2008	Use a communal toilet		M	1.49	0.47–7.73		
4	Dunkle et al. 2011		Access to toilet/ latrine	L	0.50	0.1-1.7		
5	Cummings et al. 2012	Does not use latrine to dispose of children's feces		L			15. 76	1.54–161.25
6	Shultz A et al. 2009		Uses latrine	M	0.90	0.5- 1.6		
7	Cárdenas V et al. 1993		Toilet paper used	L	0.00	0.0 - 3.6		

** Forest plot analysis not conducted on sub-categories with <4 studies [86, 89 97, 98, 99, 135]

5) Association between hygiene and cholera

In 56 studies the association between hygiene practices and cholera was determined. Hygiene practices can be characterized as safe and unsafe. Safe practices include hand washing with soap and water (9), hand wash after visiting toilet, before eating meals, and before preparing food (16), presence of hand washing designated area (1), has soap (13), has hand washing buckets (1), Clorox present in the house (2) and are expected to be protective against cholera [66]. Unsafe practices include no soap (2), no hand washing (10) and are expected to be risk factors for cholera. In nine studies, lack of hand washing is a risk factor for cholera however, in one study no hand washing before meals has no association. Absence of soap in one study is a risk factor and in another study has no association. Presence of soap used by men is not protective in one study. Hand washing has no association in two studies and is not protective in two other studies. In 39 studies safe hygienic practices are protective against cholera.

Table 5. 2. Association between hygiene practices and cholera outcome+

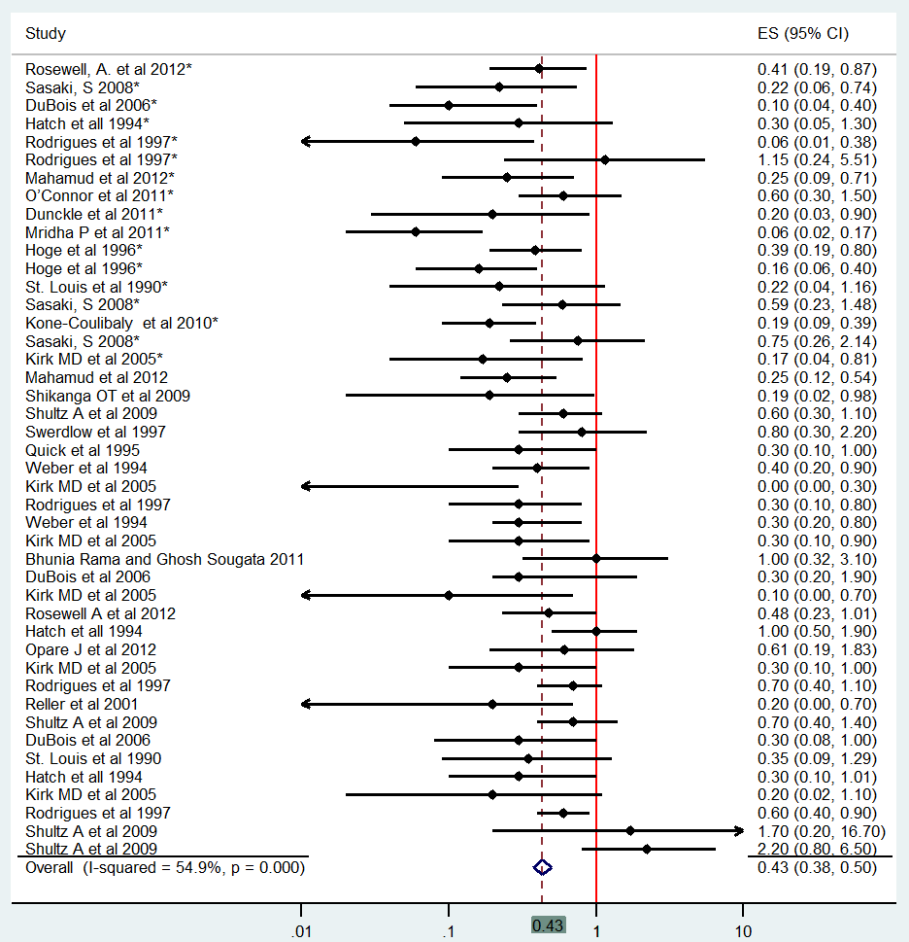
Study	Papers	Predicted Risk Factors	Predicted Protective Factors	Bias	OR univariate	95% CI	OR multivariate	95% CI
1	Rosewell, A. et a 2012		Has soap for hand washing at home	M			0.41	0.19-0.87
2	Mahamud et al. 2012		Soap at home	L	0.25	0.12-0.54		
3	Shikanga OT et a 2009		Soap for hand washing observed in home	H	0.19	0.02–0.98		
4	Shultz A et al. 2009		Washes hands with soap	M	0.60	0.3-1.1		
5	Sasaki, S 2008		Wash hands with pouring water and soap	L			0.22	0.06–0.74
6	DuBois et al. 200		presence of hand soap	L			0.10	

			in the home					0.04–0.4
7	Swerdlow et al. 1997		Owned soap	L	0.80	0.3–2.2		
8	Quick et al. 1995		Used soap always or almost always to wash hands	L	0.30	0.1-1		
9	Hatch et al.l 199		Any soap in household	M			0.30	0.05-1.3
10	Weber et al. 199		Soap in bathroom	L	0.40	0.2-0.9		
11	Kirk MD et al. 2005		Soap present in kitchen	L	0.00	0.0-0.3		
12	Rodrigues et al. 1997		Have soap in the house	L	0.30	0.1-0.8		
13	Izadi et al. 2005	Soap in hand washing place abse		H			4.70	1.28-17.30
14	Weber et al. 199		Soap in kitchen	L	0.30	0.2-0.8		
15	Kirk MD et al. 2005		Soap present in bathroom	L	0.30	0.1-0.9		
16	Rodrigues et al. 1997		Soap in the house-women	L			0.06	0.01-0.38
17	Bhunia Rama an Ghosh 2011		Water hand-wash before food	L	1.00	0.32–3.1		
18	Rodrigues et al. 1997		Soap in the house – men	L			1.15	0.24-5.51
19	DuBois et al. 20		Designated hand-washing area	L	0.30	0.2–1.9		
20	Kirk MD et al. 2005		≥ 2 handwashing buckets	L	0.10	0.0-0.7		
21	Rosewell, A. et a 2012		Washes hands before eating	M	0.48	0.23-1.01		
22	Mahamud et al. 2012		Used soap to wash hands	L			0.25	0.09-0.71
23	O'Connor et al. 2011		Handwashing with soap and lather	L			0.6	0.3–1.5
24	Dunkle et al. 2011		Proper handwashing	L			0.20	0.03-0.9
25	Mridha P et al. 2011		Regular washing of hands before food and after defecation	L			0.06	0.02-0.17
26	Hoge et al. 1996		Washes hands before eating	L			0.39	0.19-0.80
27	Hoge et al. 1996		Washes hands before eating	L			0.16	0.06-0.4
28	Hatch et al.l 199		Mother washes hands before preparing food	M	1.00	0.5-1.9		
29	Opare J et al. 20		Washing of hands with soap before meals	M	0.61	0.19-1.83		
30	Kirk MD et al. 2005		Bucket with water and Clorox	L	0.30	0.1-1.0		
31	Rodrigues et al. 1997		Women wash hands before cooking	L	0.70	0.4-1.1		
32	St. Louis et al. 1990		Routine hand washing with soap before meals	L			0.22	0.04-1.16
33	Reller et al. 200		Using soap to wash hands	L	0.20	0.0-0.7		
34	Shultz A et al. 2009		Washes hands after visiting toilet	M	0.70	0.4-1.4		
35	Sasaki, S 2008		Wash hands after defecating	L			0.59	0.23-1.48

36	DuBois et al. 200		Reports hand washing after defecating	L	0.30	0.08–1.0		
37	Kone-Coulibaly et al. 2010		Washed hand after using toilet	M			0.19	0.09-0.39
38	St. Louis et al. 1990		routine handwahsing with soap after defecation	L	0.35	0.09-1.29		
39	Sasaki, S 2008		Wash hands before eating	L			0.75	0.26-2.14
40	Hatch et al. 1994		Children wash hands before eating	M	0.30	0.1-1.01		
41	Kirk MD et al. 2005		washes hands before eating	L	0.20	0.02-1.1		
42	Rodrigues et al. 1997		Family washes hands before eating	L	0.60	0.4-0.9		
43	Shultz A et al. 2009		Washes hands before eating	M	1.70	0.2-16.7		
44	Shultz A et al. 2009		Washes hands after eating	M	2.20	0.8-6.5		
45	Kirk MD et al. 2005		Clorox present in the house	L			0.17	0.04–0.81
46	Mugoya et al. 2008	Not washing hands with soap before eating‡		L	3.70	1.3–10.3		
47	Kur L et al. 2009	soap not present in house		L	1.00	0.3-2.9		
48	Cummings et al. 2012	Does not wash hand following defecatio		L	11.00	4.4-27.4		
49	Mugoya et al. 2008	Not washing hands after visiting toilet		L	4.30	1.2–15		
50	Fatiregun AA et al. 2013	Lack of hand washing practice after latrine use		L	3.75	0.78-17.9		
51	Kur L et al. 2009	Not washing hands before eating with soap		L	1.30	0.6-2.9		
52	Hutin et al. 2003	No hand washing with soap before meals		L			1.90	1.1-3.3
53	Izadi et al. 2006	No hand washing after with soap after toilet use		L			22.06	2.91-167.1
54	Kur L et al. 2009	Not washing hands post defecation with soap		L	1.70	0.7-3.9		
55	Izadi et al. 2006	No hand washing before meals		L			3.64	1.03-12.9
56	Hutin et al. 2003	No hand washing before meals		L	1.00	0.14-8.6		

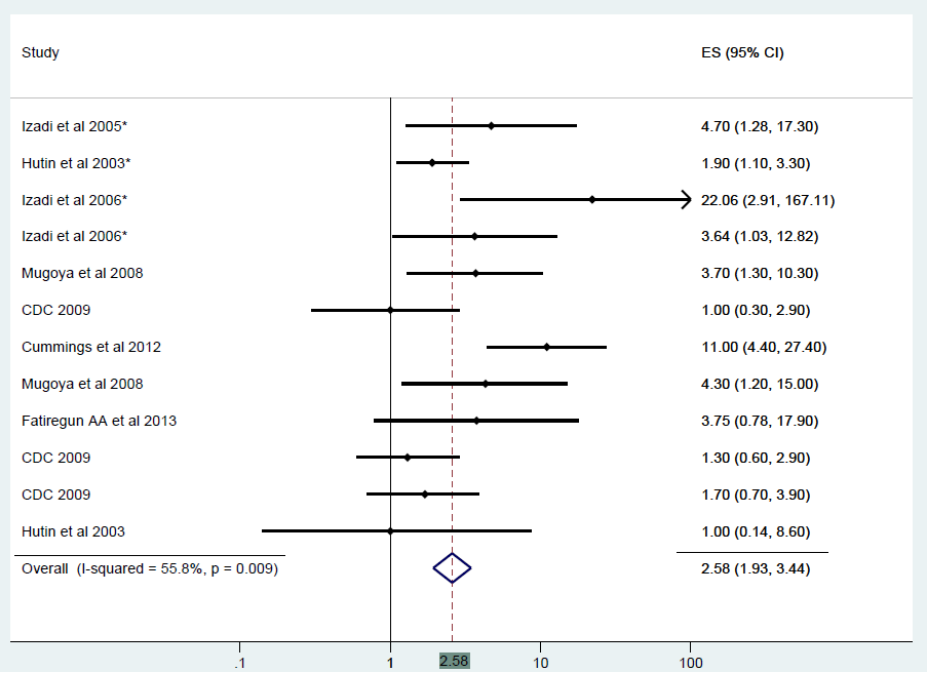
[+]Please note the exposure is highlighted in pink when it is not as expected, in orange when there is no association, and results in red when they had errors/were removed [88, 85, 86, 87, 89, 92, 94, 95, 97, 98, 100, 101, 108, 111, 113, 114, 116 124, 125, 126, 128, 129, 131, 132, 134, 136, 142, 144]

Based on the forest plot analysis of 44 studies, categorized as predicted protective factors in Table 5.2, the cumulative effect of safe hygiene practices and cholera is 0.43 (0.38– 0.50). As expected the overall association, odds ratio and 95% CI, is significant, which suggests that safe hygiene practices are protective against cholera. The χ^2 test has a low p-value <0.001 and an I^2 test value of 54.9%, which suggests that 54.9% of variation in studies is due to heterogeneity.



Graph 5.0.0. Forest plot analysis of the effect of hygiene (predicted protective factors) on outcome of cholera [Note: Studies with * are multivariate analysis]

Based on the forest plot analysis of 12 studies, characterized as predicted risk factors in Table 5.2, the cumulative effect of unsafe hygiene practices and cholera is 2.58 (1.93 – 3.44). As expected the overall association, odds ratio and 95% CI, is significant, which suggests that unsafe hygiene practices are risk factors for cholera. The χ^2 test has a low p-value < 0.05 (p-value = 0.009) and I^2 test value of 55.8%, which suggests that none of variation in studies is due to heterogeneity.



Graph 5.0.1. Forest plot analysis of the effect of lack of hygiene (predicted risk factors) on outcome of cholera [Note: Studies with * are multivariate analysis]

6) Association between knowledge of cholera prevention and cholera

In 25 studies association between knowledge of cholera transmission and prevention and cholera was determined. Knowledge of cholera treatment methods such as ORS are expected to be protective against cholera as it can decrease exposure to contaminated food and water. Protective factors include going to a cholera treatment center or hospital when sick (5), having knowledge of disease transmission or receiving information on disease transmission and treatment from a church, TV, radio, pamphlet, health workers, town meeting (14), vaccinated (2), and receiving home treatment such as ORS (4). Predicted risk factors include no knowledge of the disease, transmission, and treatment methods. Using antibiotics to treat cholera and/or using incorrect recipe for home based ORS are also expected to be risk factors for the disease. As can be seen from the studies below having knowledge and going to a cholera treatment center are protective against cholera and not attending a health center, receiving antibiotics or incorrect composition of ORS are risk factors for cholera. However, in one study deceased were less likely to receive ORS, which was not a risk factor for cholera.

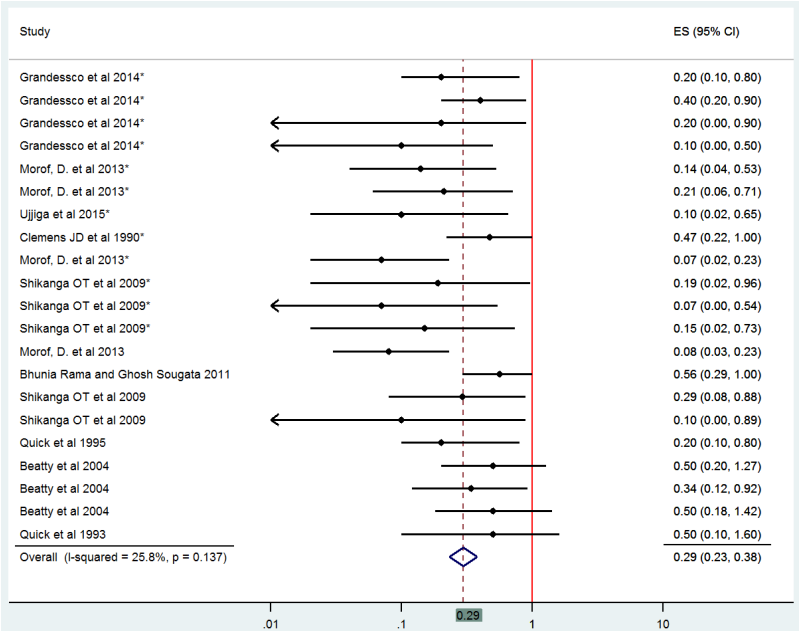
Table 5.3. Association between knowledge of cholera prevention and cholera outcome[+]

Study	Papers	Predicted Risk Factors	Predicted Protective Factors	Bias	OR univariate	95% CI	OR multivariate	95% CI
1	Morof, D. et al. 2013		Went to CTC	L	0.08	0.03-0.23		
2	Morof, D. et al. 2013		Went to CTC	L			0.07	0.02-0.23
3	Shikanga OT et al. 2009		Treated in a government facility	H			0.19	0.018-0.96
4	Shikanga OT et al. 2009		Admitted overnight (hospitalization)	H			0.07	0.001-0.54
5	Gunnlaugsson et al. 2000	Not attending a health center		H			5.40	0.7-43.2
6	Bhunia Rama and Ghosh 2011		Knowledge on diarrhea transmission	L	0.56	0.29–1.0		
7	Shikanga OT et al. 2009		Educated on cholera by a health worker	H			0.15	0.015-0.73
8	Shikanga OT et al. 2009		Educated on cholera before illness (any source)	H	0.29	0.08–0.88		
9	Shikanga OT et al. 2009		Heard about cholera before illness	H	0.10	0.002–0.89		
10	Quick et al. 1995		Claimed to know how to prevent cholera	L	0.20	0.1-0.8		
11	Beatty et al. 2004		Exposure by pamphlet	L	0.50	0.20-1.27		
12	Grandesso et al. 2014		Receiving information cholera prevention via television	L			0.20	0.1-0.8
13	Grandesso et al. 2014		Receiving information cholera prevention via television	L			0.40	0·2–0·9
14	Beatty et al. 2004		Exposure through radio	L	0.34	0.12-0.92		
15	Grandesso et al. 2014		Receiving information cholera prevention in training session	L			0.20	0.0 - 0.9
16	Beatty et al. 2004		Exposure through town meeting	L	0.50	0.18-1.42		
17	Grandesso et al. 2014		Receiving information cholera prevention at church	L			0.10	0.0-0.5
18	Quick et al. 1993	Home prepared oral rehydration solution SSS -not protective against death		H	1.20	0.3-4.4		
19	Bhunia R et al. 2009	Ignorance regarding cholera transmission		M	4.00	2.1–7.7		
20	Shikanga OT et al. 2009	Home treatment with antibiotics		H			0.04	<0.001-0.4
21	Quick et al. 1993		Among patients treate only at home deceased were less likely than survivors to have received ORS	H	0.50	0.1-1.6		
22	Morof, D. et al. 2013		Received home based ORS/SSS/water	L			0.14	0.04-0.53
23	Morof, D. et al. 2013		Received home based ORS/SSS/water	L			0.21	0.06-0.71
24	Ujjiga et al. 2015		Had 2 OCV doses	M			0.10	0.02-0.65

25	Clemens JD et al. 1990		Maternal recipient of vaccination and risk o Severe cholera in non vaccinated infant	L			0.47	0.22-1.00

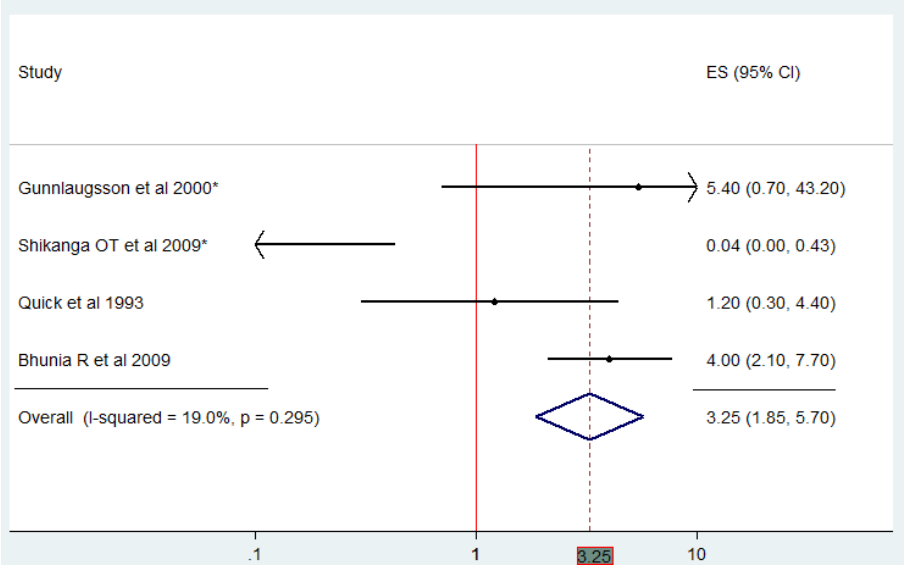
[+]Please note the exposure is highlighted in pink when it is not as expected [81, 84, 92, 95, 103, 113, 118, 122, 128, 133, 137]

Based on the forest plot analysis of 21 studies, categorized as predicted protective factors in Table 5.3, the cumulative effect of knowledge of cholera prevention and cholera is 0.29 (0.23 – 0.38). As expected, the overall association, odds ratio and 95% CI, is significant, which suggests that knowledge of cholera prevention is protective against cholera. The χ^2 test has a high p-value of 0.120 and an I^2 test value of 25.8% that is 25.8% of variation in studies is due to heterogeneity.



Graph 6.0.0. Forest plot analysis of the effect of knowledge of cholera prevention (predicted protective factors) on outcome of cholera [Note: Studies with * are multivariate analysis]

Based on the forest plot analysis of four studies, characterized as predicted risk factors in Table 5.3, the cumulative effect of no knowledge of cholera prevention and cholera is 3.25(1.85 – 5.70). The overall association, odds ratio and 95% CI, is significant, which suggests that no knowledge of cholera prevention is a risk factor for cholera. The χ^2 test has a high p-value = 0.295 and an I^2 test value of 19% that is 19% of variation in studies is due to heterogeneity. It can be concluded from the two tests (χ^2 and I^2 test) that the studies evaluating the effect have low heterogeneity.



Graph 6.0.1. Forest plot analysis of lack of knowledge for cholera prevention (predicted risk factors) on outcome of cholera [Note: Studies with * are multivariate analysis]

7) Association between food and cholera

Food items can either be risk or protective factors for cholera depending on the food, whether the produce is washed, how the food is consumed that is (cooked or uncooked) [62]. To determine the association of food on cholera, Food is divided into the follow sub-categories: 1) Eating cooked food, 2) Eating uncooked food, 3) Cold food/ left over food, 4) Eating out, and 5) Other, which are described below.

7.1. Eating cooked food and cholera outcome

In 63 studies association between eating cooked food and cholera was determined. Cooked food is expected to be a protective factor however reheating food may not be protective [68]. Eating cooked, dried seafood and canned meat/fish are protective factors. However, fresh fish and seafood may not be fully cooked and maybe risk factors. If cooking food takes more than six hours, it is expected to be a risk factor as contamination might occur on cooked food lying outside. Cooked food served hot is a protective factor. Four studies determining the association between reheated food and cholera however, in two studies reheating food or eating remaining food hot are not protective. In 11 studies eating a cooked dish, reheating food, cooked beef, fish or shellfish is not protective against cholera. This is perhaps because cooking and heating food kills bacteria and lack of competing bacteria provide the right conditions for cholera

to grow [69, 70]. Fresh fish and shellfish is expected to be a risk factor since cholera can reside in shellfish however in six out of 29 studies, consumption of fish and shellfish is not a risk factor for cholera.

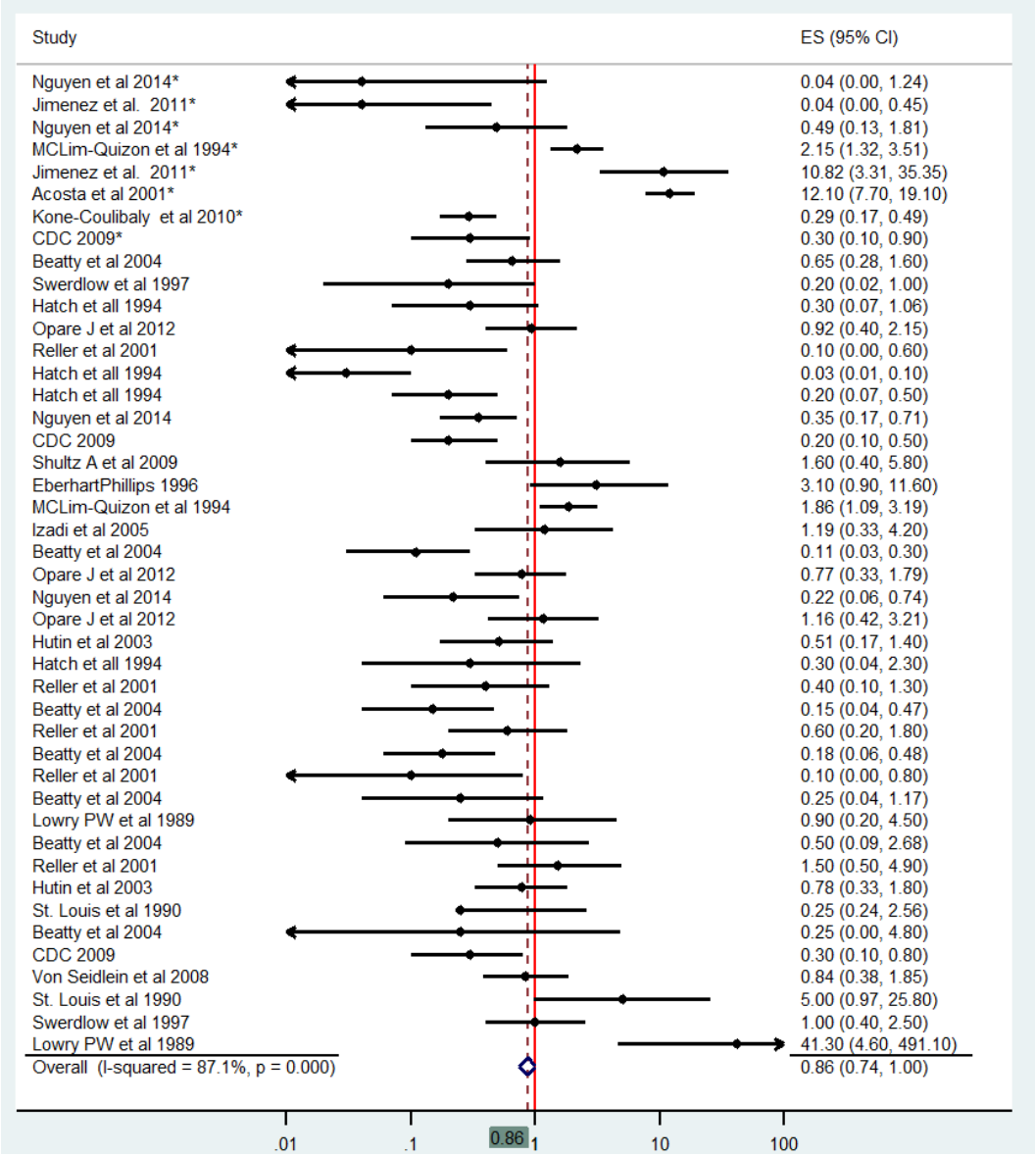
Table 5.4. Association between eating cooked food and cholera outcome[+]

Study	Papers	Predicted Risk Factors	Predicted Protective Factors	Bias	OR univariate	95% CI	OR multivariate	95% CI
1	Nguyen et al. 2014		Hot rice	L			0.04	0.002-1.24
2	Jimenez et al. 2011		Mixed rice and vegetables	M			0.04	0.003-0.45
3	Beatty et al. 2004		Rice at room temp	L	0.65	0.28-1.60		
4	Swerdlow et al. 1997		Reheated leftover peas	L	0.20	0.02-1.0		
5	Kone-Coulibaly et al. 2010		Ate hot food	M			0.29	0.17-0.49
6	Hatch et al.l 1994		Cabbage leaves	M	0.30	0.07-1.06		
7	Opare J et al. 2012		Fufu (Cassava flour)	M	0.92	0.4-2.15		
8	Reller et al. 2001		Heated rice water	L	0.10	0.0-0.6		
9	Hatch et al.l 1994		Tanapas leaves	M	0.03	0.01-0.1		
10	Hatch et al.l 1994		Pumpkin leaves	M	0.20	0.07-0.5		
11	Nguyen et al. 2014		Reheated rice	L	0.35	0.17– 0.71		
12	Kur L et al. 2009		Consumed meal with hot meat vs. without hot meat	L	0.20	0.1-0.5		
13	Kur L et al. 2009		Only consuming at least one hot meal containing meat in the 3 days before becoming ill	L			0.30	0.1-0.9
14	Shultz A et al. 2009		Reheats food cooked previous day	M	1.60	0.4-5.8		
15	EberhartPhillips 1996		Cooked ham	L	3.10	0.9-11.6		
16	MCLim-Quizon et al. 1994		Spaghetti	M	1.86	1.09-3.19		
17	Izadi et al. 2005		Use of remaining food – hot	**H**	1.19	0.33-4.20		
18	Nguyen et al. 2014		Okra	L			0.49	0.13-1.81
19	Beatty et al. 2004		Vegetables	L	0.11	0.03-0.30		
20	Opare J et al. 2012		Banku (cooked fermente corn and cassava dough)	M	0.77	0.33-1.79		
21	Nguyen et al. 2014		Potato leaf	L	0.22	0.06– 0.74		
22	MCLim-Quizon et al. 1994		Pansit	M			2.15	1.32-3.51
23	Opare J et al. 2012		Kenkey	M	1.16	0.42-3.21		
24	Hutin et al. 2003		Eating Alefu	L	0.51	0.17-1.4		
25	Hatch et al. 1994		Beans	M	0.30	0.04-2.3		
26	Reller et al. 2001		Vegetables	L	0.40	0.1-1.3		
27	Beatty et al. 2004		Eggs fresh	L	0.15	0.04-0.47		
28	Reller et al. 2001		All meat	L	0.60	0.2-1.8		
29	Beatty et al. 2004		Beef fresh	L	0.18	0.06-0.48		
30	Reller et al. 2001		Chicken	L	0.10	0.0-0.8		
31	Beatty et al. 2004		Pork fresh	L	0.25	0.04-1.17		
32	Lowry PW et al. 1989		Chicken	L	0.90	0.2-4.5		
33	Beatty et al. 2004		Chicken fresh/frozen	L	0.50	0.09-2.68		
34	Reller et al. 2001		Beef	L	1.50	0.5-4.9		
35	Hutin et al. 2003		Eating fried fish	L	0.78	0.33-1.8		
36	St. Louis et al. 1990		Smoked fish	L	0.25	0.24-2.56		

37	Beatty et al. 2004		Canned meat/fish	L	0.25	0.00-4.8		
38	Kur L et al. 2009		Consumed meal with hot fish	L	0.30	0.1-0.8		
39	St. Louis et al. 1990	Fresh fish		L	0.64	0.23-1.75		
40	DuBois et al. 2006	Kapenta		L			0.30	0.1–0.7
41	Lowry PW et al. 1989	Antacids taken with crabs		M	0.00	0-9.9		
42	Jimenez et al. 2011		Cooked shrimp on ice	M			10.82	3.31-35.35
43	Beatty et al. 2004	Lagoon fish		L	4.21	0.50-197.(		
44	Acosta et al. 2001	Prawns		L	3.10	1.6-5.9		
45	Rodrigues et al. 2000	Shellfishh		L			1.00	0.59-1.70
46	Killewo et al. 1989	Eating fresh fish		L	3.86	1.54-9.65		
47	Beatty et al. 2004	Pelagic fish		L	0.61	0.25-1.50		
48	Beatty et al. 2004	Salt fish		L	0.23	0.04-0.94		
49	Beatty et al. 1994	Crab		L	5.10	1.4-19.2		
50	MCLim-Quizon et al. 1994	Vegetables with salty brine shrimp		M	2.01	1.24-3.27		
51	Von Seidlein et al. 2008		Ate dried fish 5 days before	M	0.84	0.38–1.85		
52	Beatty et al. 2004	Ocean fish		L	0.46	0.07-2.41		
53	St. Louis et al. 1990		Shellfish (clams) – well cooked	L	5.00	0.97-25.8		
54	Lowry PW et al. 1989		Cooked crab	L	41.30	4.6-491.1		
55	Reller et al. 2001	Shellfish		L	1.40	0.6-3.3		
56	Jimenez et al. 2011	Langostinos		M			2.23	0.56-8.81
57	Acosta et al. 2001		Eating dried fish	L			12.10	7.7-19.1
58	Swerdlow et al. 1997		Ate dried fish	L	1.00	0.4-2.5		
59	Weber et al. 1994	Shellfishh		L	1.60	0.8-3.0		
60	Lowry PW et al. 1989	Shrimp/crab		L	45.00	4.4-2021.2		
61	Weber et al. 1994	Concha/ mussels		L	1.60	0.5-4.7		
62	Lowry PW et al. 1989	Time taken to cook crabs >=6h		M	22.80	2.6-211.3		
63	Lowry PW et al. 1989	Time taken to cook crabs >= 12h		M	35.00	3.4-447		

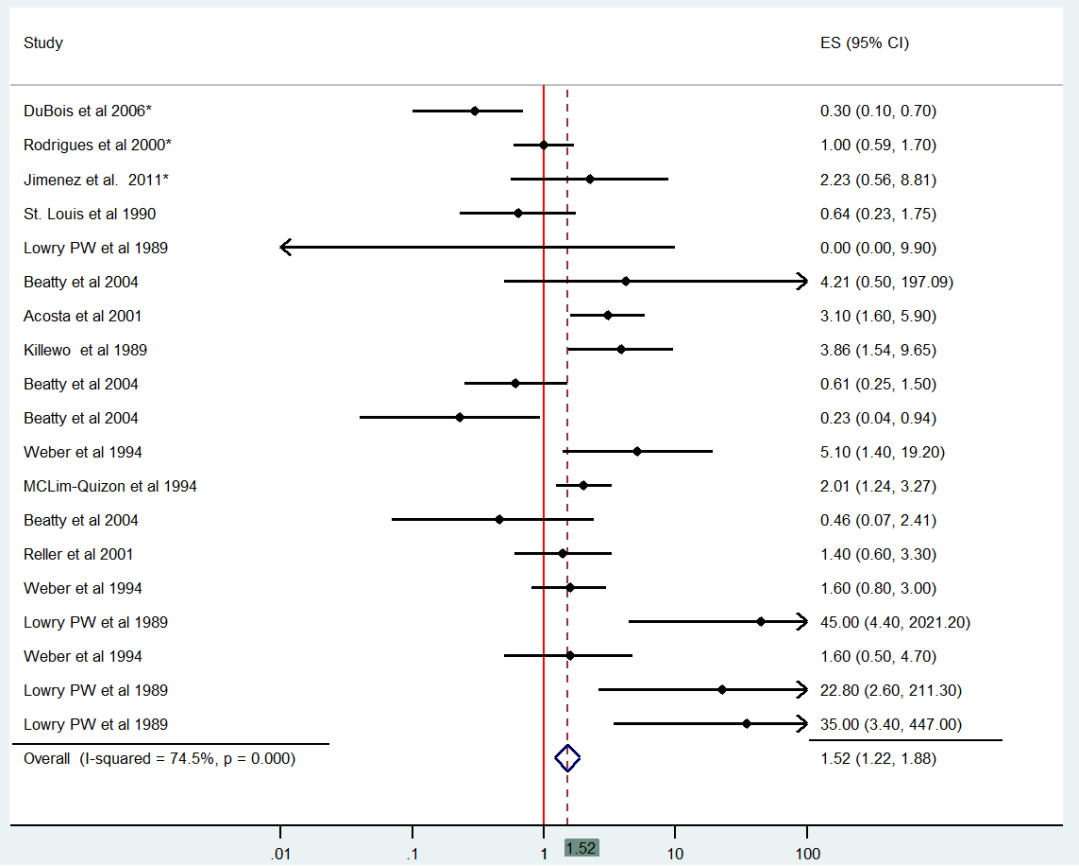
[+] Please note the exposure is highlighted in pink when it is not as expected, in orange when there is no association, and results in red when they had errors/were removed [83, 90, 97, 99, 101, 103, 104, 108, 112, 114, 116, 117, 125, 126, 129, 132, 136, 138, 140, 142, 143]

Based on the forest plot analysis of 44 studies, categorized as predicted protective factors in table 5.4, the cumulative effect of eating cooked food and cholera is 0.86 (0.74 – 1.00). The overall association is protective but the 95% CI is not significant. The χ^2 test has a low p-value of <0.001 and an I^2 test value of 87.1%, which suggests that 87.1% of variation in studies is due to heterogeneity. Cooked food is expected to be protective however given the varying food items being tested the results as expected are not significant.



Graph 7.1.0. Forest plots analysis of the effect of eating cooked food (predicted protective factors) on the outcome of cholera [Note: Studies with * are adjusted]

Based on the forest plot analysis of 19 studies, categorized as predicted risk factors in Table 5.4, the cumulative effect of eating cooked food and cholera is 1.53 (1.22 – 1.88). As expected the overall association of eating cooked food, is significant, which suggests that some cooked food items are risk factors for cholera. The χ^2 test has a low p-value < 0.001 and an I^2 test value of 74.5%, which suggests that 74.5 % of variation in studies is due to heterogeneity.



Graph 7.1.1. Forest plots analysis of the effect of eating cooked food (predicted risk factor) on the outcome of cholera [Note: Studies with * are multivariate analysis]

7.2. Consuming uncooked food items and cholera outcome

In 58 studies the association between uncooked/raw food and cholera was determined. Uncooked food items such as raw seafood, fruits, juice are expected to be risk factor however if the food is washed before consumption it may not be a risk factor, fruits with skin such as bananas and oranges are expected to be protective, and acidic food items such as lime and citrus are also expected to be protective [68]. In 28 studies eating uncooked, raw food is a risk factor for cholera. In 17 studies however, uncooked, unwashed produce is not a risk factor for cholera. In five studies lime and citrus is not protective however, in three studies lime and orange juice are protective against cholera. In four studies on fruits with skin, eating bananas, oranges, and breadfruit are protective against cholera but consuming melon is not protective.

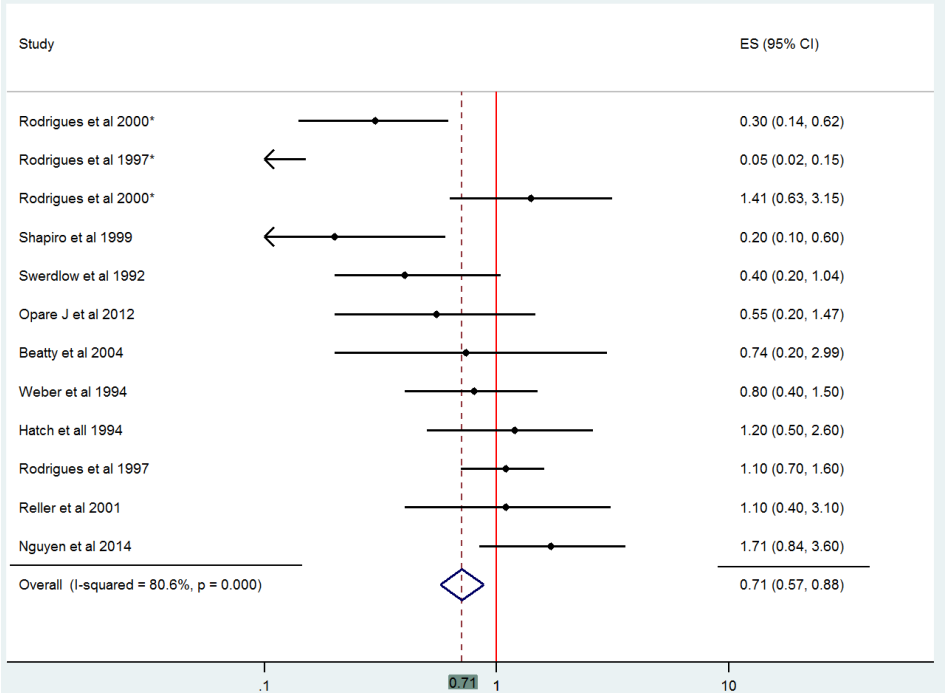
Table 5.5. Association between consuming uncooked/raw food items and cholera outcome[+]

Study	Papers	Predicted Risk Factors	Predicted Protective Factors	Bias	OR univariate	95% CI	OR multivariate	95% CI
1	Von Seidlein et al. 2008	Eating uncooked food		M	0.36	0.16–0.84		
2	Dunkle et al. 2011	Raw food		L	1.20	0.2-6.2		
3	DuBois et al. 2006	Consumption of raw vegetables		L			4.70	1.7–13
4	Acosta et al. 2001	Uncooked vegetables		L	1.30	0.9-2.0		
5	Hoge et al. 1996	Raw/partially cooked seafood		L			3.19	1.37-7.44
6	Hoge et al. 1996	Raw/partially cooked seafood		L	1.92	0.87-4.25		
7	EberhartPhillips 1996	Raw ham		L	3.30	0.9-12.6		
8	Quick et al. 1995	Ate seafood prepared home served raw / col		L	7.00	1.4-35		
9	Weber et al. 1994	Raw shellfish		L	3.20	0.9-11		
10	Swerdlow et al. 1992	Ate cabbage		L	2.70	0.97-7.6		
11	Hutin et al. 2003	Eating salad		L	1.50	0.6-3.9		
12	Lowry PW et al. 1989	Cross contamination of crabs (Placing cooked crabs in the same container used for raw crabs)		M	25.30	0.9-1530.5		
13	Weber et al. 1994	raw seafood		L	4.00	1.4-11.5		
14	Beatty et al. 2004	Fruit (excluding breadfruit)		L	0.41	0.16-1.00		
15	Rodrigues et al. 2000	Tomato		L			0.43	0.26–0.70
16	Mujica et al. 1994	Toronja (grapefruit) drink		L	0.30	0.1-0.7		
17	Shapiro et al. 1999		Eating washed fruits or vegetables	L	0.20	0.1-0.6		
18	EberhartPhillips 1996	Fresh fruit		L	0.90	0.2-3.1		
19	Hatch et al.l 1994	Tomato		M	0.20	0.09-0.4		
20	Swerdlow et al. 1992		Ate bananas	L	0.40	0.2-1.04		
21	Opare J et al. 2012		Orange	M	0.55	0.2-1.47		
22	Reller et al. 2001	Fruit		L	0.30	0.1-1.4		
23	Acosta et al. 2001	Fruits		L	1.10	0.7-1.6		
24	Beatty et al. 2004		Fermented breadfruit	L	0.74	0.20-2.99		
25	Shapiro et al. 1999	Eating raw fruits or vegetables		L	2.30	1.1-4.6		
26	EberhartPhillips 1996	Melon		L	2.00	0.6-7.2		
27	Mujica et al. 1994	Unwashed fruits/ vegetables		L	5.30	1.4-19.5		
28	St. Louis et al. 1990	Fresh vegetables		L	2.16	0.65-7.19		
29	St. Louis et al. 1990	Raw fish		L	0.86	0.23-3.25		
30	Lowry PW et al. 1989	Ate crab fat		M	0.70	0.1-3.9		
31	Lowry PW et al. 1989	Ate crab eggs		M	0	0.0-1.4		
32	MCLim-Quizon et al. 1994	Blood meat (dinuguan		M	1.96	1.08-3.57		
33	Killewo et al. 1989	Handling and eating fish		L	7.00	2.38-20.57		
34	Weber et al. 1994	Raw fish		L	10.00	1.2-85.6		
35	MCLim-Quizon et al. 1994	Fish balls		M	1.76	1.04-2.9		
36	Beatty et al. 2004	Shellfish (lobster, crab shrimp, bivalves)		L	0.50	0.04-4.51		
37	Lowry PW et al. 1989	Placed crab shells in mouth		M	0.80	0.1-5.5		

38	Beatty et al. 2004	Raw fish		L	0.71	0.21-2.41		
39	Dunkle et al. 2011	Seafood		L	0.50	0.2-1.2		
40	Weber et al. 1994	Seafoods		L	1.20	0.6-2.4		
41	MCLim-Quizon et al. 1994	Mussel Soup		M			2.29	1.06-4.95
42	Lowry PW et al. 1989	Raw/cooked shrimp		L	19.30	2.8-149.4		
43	Nguyen et al. 2014	Crab		L			3.29	1.03-10.56
44	Swerdlow et al. 1992	Ate raw seafood		L	1.00	0.06-16		
45	Weber et al. 1994	Juice		L	0.60	0.2-1.8		
46	Weber et al. 1994		Orange juice	L	0.80	0.4-1.5		
47	Rodrigues et al. 2000		lime juice	L			0.30	0.14-0.62
48	Rodrigues et al. 1997		Lime	L			0.05	0.02-0.15
49	O'Connor et al. 2011	Sugar cane juice		L			9.1	1.0 – ∞
50	Nguyen et al. 2014	Sugar cane		L	0.93	0.26–2.9		
51	Reller et al. 2001	unwashed produce		L	0.50	0.6-3.4		
52	Nguyen et al. 2014	Omolay (fermented sugar drink) (fermented drink)		L	8.76	1.8–83		
53	Rodrigues et al. 2000		Vinegar lime	L			1.41	0.63–3.15
54	Hatch et al. 1994		Citrus	M	1.20	0.5-2.6		
55	Rodrigues et al. 1997		lime juice	L	1.10	0.7-1.6		
56	Reller et al. 2001		Lemonade	L	1.10	0.4-3.1		
57	Weber et al. 1994	Raw concha		L	2.50	0.7-9.3		
58	Nguyen et al. 2014		lime	L	1.71	0.84–3.6		

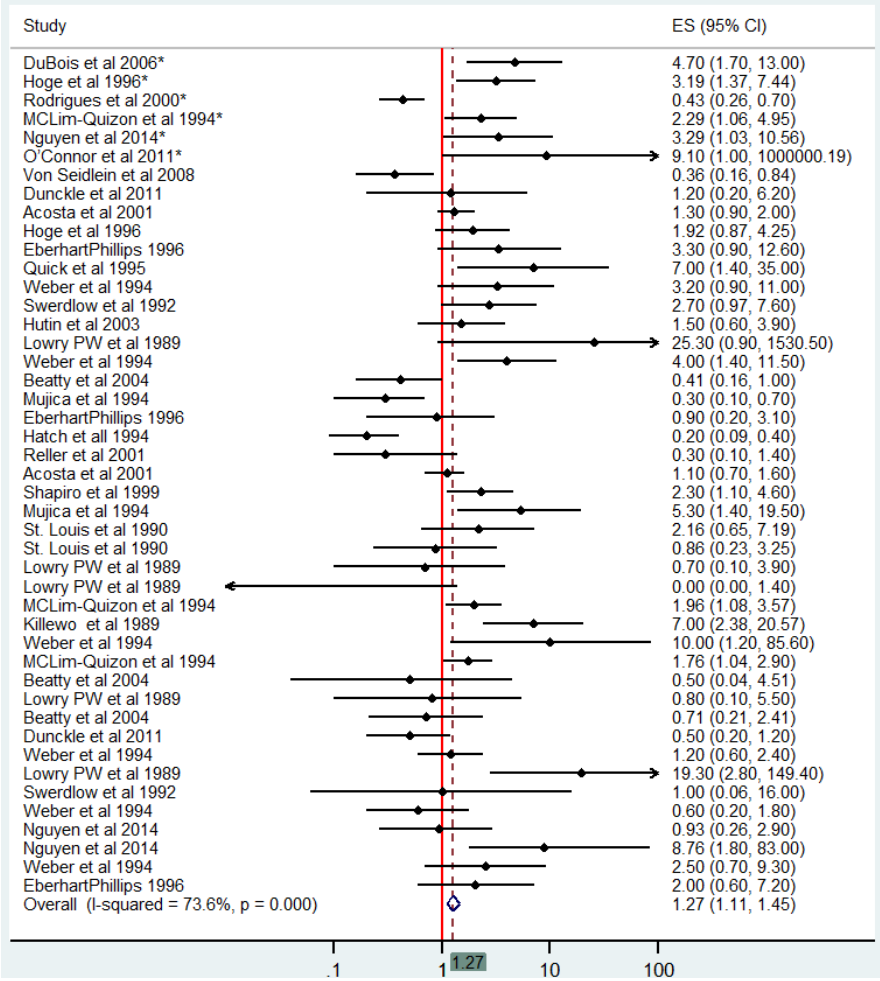
[+] Please note the exposure is highlighted in pink when it is not as expected and results in red when they are reported with errors [∞ values are replaced by $1*10^8$ is STATA] [88, 89, 99, 101, 103, 104, 106, 116, 119, 111, 112, 113, 114, 125, 132, 134, 136, 138, 140, 142]

Based on the forest plot analysis of 12 studies, categorized as predicted protective factors in Table 5.5, the cumulative effect of eating uncooked food and cholera is 0.71 (0.57 – 0.88). As expected, the overall association (OR and 95% CI) is protective but only marginally significant. The χ^2 test has a low p-value of <0.001 and an I^2 test value of 80.6%, which suggests that 80.6% of variation in studies is due to heterogeneity. As expected acidic foods and washed food items characterized as uncooked predicted protective factors are protective against cholera.



Graph 7.2.0. Forest plot analysis of the effect of eating uncooked food (predicted protective factors) on outcome of cholera [Note: Studies with * are multivariate analysis]

Based on the forest plot analysis of 43 studies, categorized as predicted risk factors in table 5.5, the cumulative effect of eating uncooked food and cholera is 1.27 (1.11 – 1.45). The overall association (OR and 95%CI) is significant, which suggests that some uncooked food items are a risk factor for cholera. The χ^2 test has a low p-value <0.001 and an I^2 test value of 73.6%, which suggests that 73.6% of variation in studies is due to heterogeneity. As expected uncooked food characterized by raw vegetables, meat, and uncooked seafood are significant risk factors for cholera.



Graph 7.2.1. Forest plot analysis of the effect of eating uncooked food (predicted risk factors) on the outcome of cholera [Note: Studies with * are multivariate analysis]

7.3. Cold food and cold/left over food and cholera outcome

Cold left over food is expected to be a risk factor for cholera as bacteria is allowed to grow on food [68]. Thirty-seven studies determine the association between consuming cold, let over food and cholera. Eating cold let over food and cold food items such as sandwiches, beverages with ice, are risk factors for cholera. In twenty-six studies consumption of cold food or leftover food is a risk factor for cholera. In nine studies however, cold left over food is not a risk factor for transmission. Out of six studies on beverages with ice, in five studies ice is a risk factor however in one study ice is not a risk factor for cholera. In one study discarding remaining food is protective.

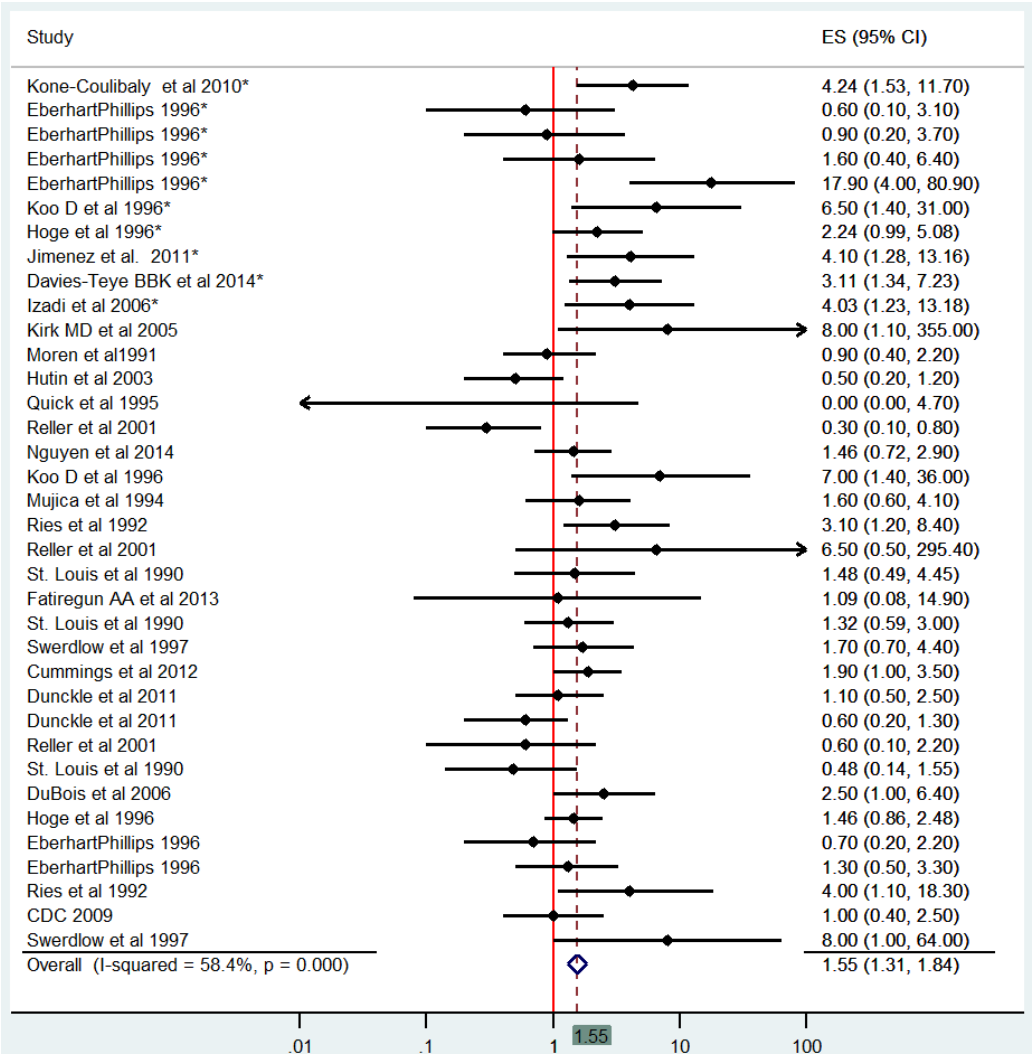
Table 5.6. Association between consuming cold food and cold/left over food and cholera outcome [+, **]

Study	Papers	Predicted Risk Factors	Predicted Protective Factor	Bias	OR univariate	95% CI	OR multivariate	95% CI
1	Kirk MD et al. 2005	Store cooked food outside uncovered > ½ day		L	8.00	1.10-355		
2	Moren et al.1991	Food left-over		L	0.90	0.4-2.2		
3	Hutin et al. 2003	Eating cold leftovers (rice or two)		L	0.50	0.2-1.2		
4	Quick et al. 1995	Ate left over rice without reheating		L	0.00	0.0-4.7		
5	Kone-Coulibaly et al. 2010	Ate cold food		M			4.24	1.53-11.70
6	Reller et al. 2001	Leftover rice		L	0.30	0.1-0.8		
7	Nguyen et al. 2014	Cold rice		L	1.46	0.72–2.9		
8	Koo D et al. 1996	Left over rice		L	7.00	1.4-36		
9	Mujica et al. 1994	Unreheated cooked rice		L	1.60	0.6-4.1		
10	Ries et al. 1992	Eating rice >3 h old without reheating		L	3.10	1.2-8.4		
11	Reller et al. 2001	Unheated leftover rice		L	6.50	0.5-295.4		
12	St. Louis et al. 1990	Habitual consumption: leftov Rice		L	1.48	0.49-4.45		
13	Izadi et al. 2005		Remaining food discarded	**H**	0.74	0.13-3.99		
14	Fatiregun AA et al. 20	Ate food cold before onset of illness		L	1.09	0.08-14.9		
15	Izadi et al. 2006	Eating remaining food from previous meals without reheating		L			4.03	1.23-13.18
16	St. Louis et al. 1990	Tomato sauce eaten un-reheated as a leftover		L	1.32	0.59-3.00		
17	Swerdlow et al. 1997	Left peas out overnight		L	1.70	0.7-4.4		
18	EberhartPhillips 1996	Ham and cheese sandwhich		L			0.60	0.1-3.1
19	EberhartPhillips 1996	Chicken Sandwhich		L			0.90	0.2-3.7
20	EberhartPhillips 1996	Turkey sandwhich		L			1.60	0.4-6.4
21	EberhartPhillips 1996	Seafood salad		L			17.90	4.0-80.9
22	Davies-Teye BBK et al.2014	Cold/warm food		**H**			3.11	1.34-7.23
23	Cummings et al. 2012	Eats mostly cold meal		L	1.90	1-3.5		
24	Dunkle et al. 2011	Cold rice		L	1.10	0.5-2.5		
25	Dunkle et al. 2011	Cold left over food		L	0.60	0.2-1.3		
26	Reller et al. 2001	Cold rice water		L	0.60	0.1-2.2		
27	St. Louis et al. 1990	Habitual consumption leftover sauces		L	0.48	0.14-1.55		
28	DuBois et al. 2006	Ate leftover nshima (maize flour and water)		L	2.50	1.0–6.4		
29	Jimenez et al. 2011	Ice cubes in beverages		M			4.10	1.28-13.16
30	Hoge et al. 1996	Ice		L	1.46	0.86-2.48		
31	Hoge et al. 1996	Ice		L			2.24	0.99-5.08
32	EberhartPhillips 1996	Ice drink		L	0.70	0.2-2.2		
33	EberhartPhillips 1996	Ice drink		L	1.30	0.5-3.3		
34	Ries et al. 1992	Beverage with ice		L	4.00	1.1-18.3		
35	Kur L et al. 2009	Eat or stored leftover food		L	1.00	0.4-2.5		
36	Koo D et al. 1996	Rice and helados (ice cream)		L			6.50	1.4-31
37	Swerdlow et al. 1997	For those who ran out of wood- Left peas out overnigh		L	8.00	1.0-64.0		

[+] Please note the exposure is highlighted in pink when it is not as expected and results in red when they had errors/were removed

** No forest plot analysis for <4 studies (protective factor for cold/ left over food has only 1 study) [82, 83, 86, 89, 90, 101, 108, 111, 112, 113, 120, 121, 124, 126, 131, 132, 136, 142, 143, 144]

Based on the forest plot analysis of 36 studies, categorized as predicted risk factors in Table 5.6, the cumulative effect of eating cold/left over food and cholera is 1.55 (1.31 – 1.84). The overall association, OR and 95% CI, suggests that cold or left over food is a significant risk factor for cholera. The χ^2 test has a high p-value <0.001 and an I^2 test value of 58.4%, which suggests that 58.4% of variation in studies is due to heterogeneity.



Graph 7.3.0. Forest plot analysis of the effect of eating cold food and left over food (predicted risk factors) on outcome of cholera [Note: Studies with * are multivariate analysis]

7.4. Eating out and cholera outcome

The association between eating out and cholera is determined in 41 studies and five studies determine the association between eating at home and cholera. Eating out from street vendors and in restaurants is expected to be a risk factor in cholera endemic areas [68]. Eating out is categorized as eating or consuming beverages outside home. In eight studies

out of 41, eating out is not a risk factor and in one study there is no association between drinking beverages sold by street vendors and cholera, the remaining 32 studies show that eating out is a risk factor for cholera. Eating at home in all five studies is protective. One study from eating at home reported results with errors therefore association between eating at home and cholera is determined in four studies.

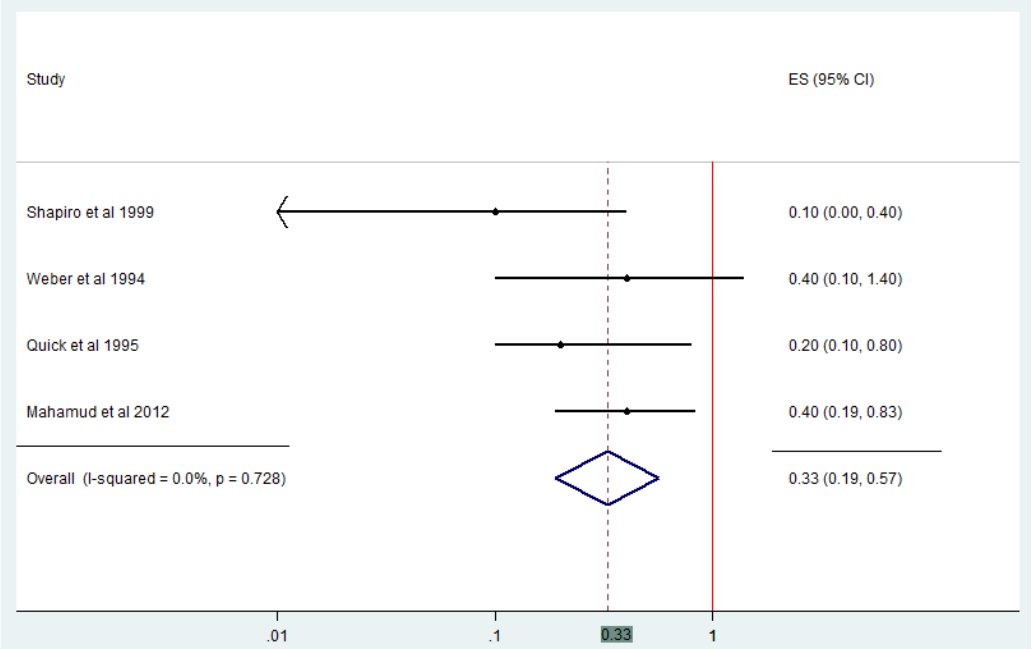
Table 5.7. Association between eating outside and cholera outcome [+, **]

Study	Papers	Predicted Risk Factors	Predicted Protective Factors	Bias	OR univariate	95% CI	OR multivariate	95% CI
1	Dunkle et al. 2011	Food/drink from street vendor		L	0.70	0.3-1.7		
2	Das A et al. 2009	Consuming food in hotel		M	0.85	0.34-2.1		
3	Das A et al. 2009	Consuming street vended food		M	0.75	0.2-2.5		
4	Von Seidlein et al. 2008	Ate Food away from home in previous 5 days		M	0.64	0.21–1.9		
5	Quick et al. 1995	Drank street vended beverages		L	0.70	0.2-2.7		
6	Hutin et al. 2003	Eating food sold by street vendors		L	0.60	0.2-1.3		
7	Reller et al. 2001	Food or beverage from street vendor		L	0.90	0.3-2.5		
8	Grandesso et al. 2014	Ate a meal away from home at least once in week before illness		L			35.90	7.9-163.4
9	Grandesso et al. 2014	Ate a meal away from home at least once in week before illness		L			1.80	0·9–3·7
10	Nguyen et al. 2014	Vended food/drink		L	5.11	2.3–13.0		
11	Cummings et al. 2012	Eats roadside food often		L			2.91	1. 24–6. 81
12	Mahamud et al. 2012	Eat or drink anything outside the home		L	1.57	0.61-4.0		
13	Bhunia Rama and Ghosh 2011	Eating outside		L	1.50	0.72–3.1		
14	Mridha P et al. 2011	Consumption of food From local vendor		L			2.60	0.5-14.7
15	Von Seidlein et al. 2008	Ate street food in previous 5 days		M	1.16	0.11–12.2		
16	Mugoya et al. 2008	Eating ugali outside the home		L			6.80	1.3–35
17	DuBois et al. 2006	Ate food away from home		L	1.50	0.8–2.9		
18	Rodrigues et al. 2000	Consumption of food at ceremonies		L	6.21	2.1–18.7		
19	Koo D et al. 1996	Foods purchased on streets		L	11.00	2.3-54		
20	Hoge et al. 1996	Food sold by street vendor		L			2.19	1.04-4.62
21	Hoge et al. 1996	Food sold by street vendor		L	1.43	0.76-2.71		
22	Quick et al. 1995	Ate street-vended food		L	2.10	0.5-8.4		
23	Weber et al. 1994	Street vendor orange juice		L	4.30	1.3-14.0		
24	Ries et al. 1992	Eating from street vendor		L	24.00	3-191		
25	Ujjiga et al. 2015	Ate outside home before illness		M			9.17	1.89-44.41

26	Swaddiwudhipong et al. 2008	History of frequently having food purchased from one food handler in their community		M	3.40	1-11.7		
27	St. Louis et al. 1990	Any prepared foods in the market place		L	1.31	0.54-3.19		
28	Reller et al. 2001	Food or beverage in market		L	1.10	0.4-2.6		
29	Reller et al. 2001	Food or beverage during trip outside Fort-Dauphin		L	2.80	0.6-13.2		
30	Izadi et al. 2006	Drinks beverages from street vendors		L			10.16	2.55-40.50
31	Rodrigues et al. 2000	Consumption of drinks at ceremonies		L	4.04	1.33–12.2		
32	Koo D et al. 1996	Drinks purchased on streets		L	3.80	1.2-12		
33	Eberhart Phillips 199	Drink without ice		L	2.00	0.5-8.7		
34	Eberhart Phillips 199	Drink without ice		L	2.00	0.7-5.7		
35	Weber et al. 1994	Street vendor beverage		L	2.80	1.3-5.9		
36	Ries et al. 1992	Drinking beverage from street vendor		L	14.60	4.2-51		
37	Hutin et al. 2003	Drinking local drinks sold by street vendors		L	1.00	0.5-2		
38	Reller et al. 2001	Beverage outside of home		L			0.70	0.2-1.9
39	Mugoya et al. 2008	Eating beans outside the ho		L	2.90	1.1–7.5		
40	Koo D et al. 1996	Any items from the street		L	6.30	1.4-29		
41	Izadi et al. 2005	Eating food at parties		H			34.48	4.76-250
42	Davies-Teye BBK et al. 2014		Home food	H			0.08	0.39-0.18
43	Shapiro et al. 1999		Eating food at home compound	L	0.10	0.0, 0.4		
44	Weber et al. 1994		Always boiling water for refreshments at home	L	0.40	0.1-1.4		
45	Quick et al. 1995		Ate rice prepared at home	L	0.20	0.1-0.8		
46	Mahamud et al. 2012		Ate cooked vegetables at home	L	0.40	0.19-0.83		

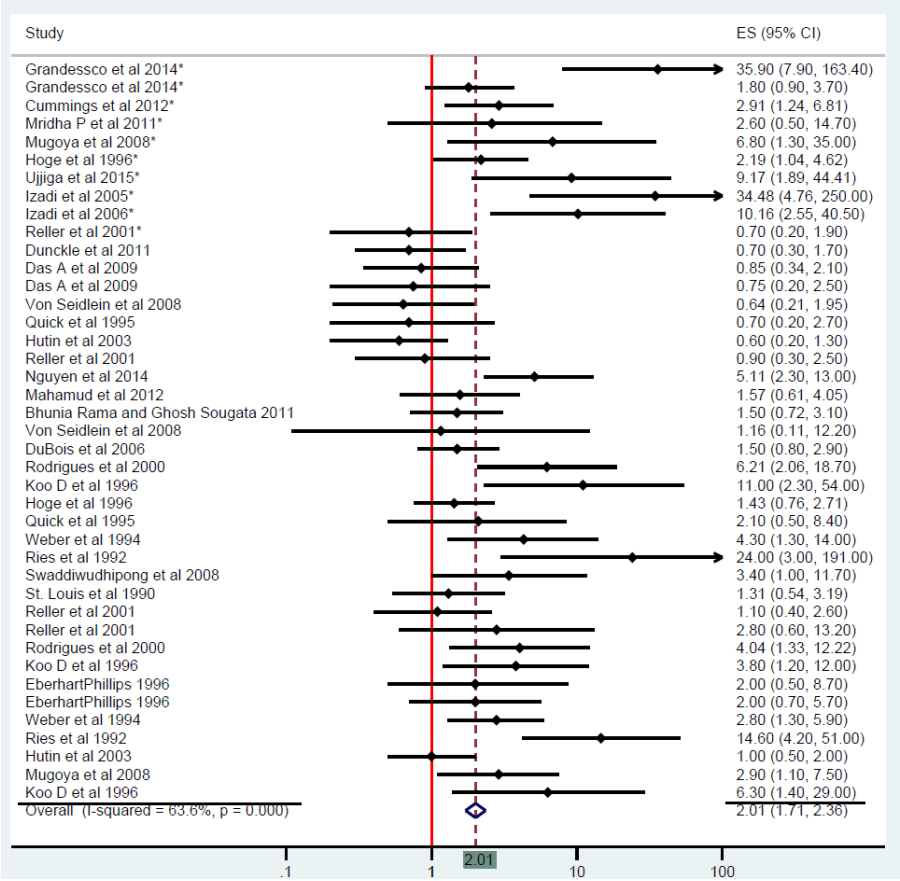
[+] Please note the exposure is highlighted in pink when it is not as expected, in orange when there is no association, and results in red when they are reported with errors, ** No forest plot analysis for <4 studies (eating at home) [81, 82, 83, 86, 87, 89, 92, 93, 99, 101, 106, 111, 112, 113, 116, 121, 130, 132, 136, 142, 143]

Based on the forest plot analysis of four studies, characterized as predicted protective factors in Table 5.7, the cumulative effect of eating out and cholera is 0.33 (0.19 - 0.57). The overall association (OR and 95% CI) is significant, which suggests that eating out is a risk factor for cholera. The χ^2 test has a high p-value 0.728 and an I^2 test value of 0.0%, which suggests that none of the variation in studies is due to heterogeneity. As expected, eating at home is a significant protective factor against cholera outcome.



Graph 7.4.0. Forest plot analysis of the effect of eating at home (predicted protective factor) on outcome of cholera [Note: Studies with * are multivariate analysis]

Based on the forest plot analysis of 41 studies, characterized as predicted risk factors in Table 5.7, the cumulative effect of eating out and cholera is 2.01(1.71 – 2.36). The overall association (OR and 95% CI) is significant, which suggests that eating out is a risk factor for cholera. The χ^2 test has a high p-value <0.001 and an I^2 test value of 63.6%, which suggests that 63.6% variation in studies is due to heterogeneity. As expected, eating out is a significant risk factor for cholera outcome.



Graph 7.4.1. Forest plot analysis of the effect of eating out (predicted risk factor) on outcome of cholera [Note: Studies with * are multivariate analysis]

7.5. Other food items and cholera outcome

In 26 studies association between other food items and food consumption behavior and outcome of cholera was determined. Other food items include consuming different sauces, milk and milk products, alcohol, nuts, sugarcane, food diversity, powdered drink mix. Seven studies determine association between sauces and developing cholera. Rodrigues et al. 2000 and St. Louis et al. 1990 determined that peanut sauce has a pH of 6.0-7.0 and provides a suitable environment for cholera to grow rapidly, therefore peanut sauce is expected to be a risk factor for cholera [71, 72]. Tomato sauce however is acidic and is therefore expected to not be a risk factor [68]. Two out of four studies on peanut sauce determined that consuming peanut sauce is not a risk factor for cholera. One study on tomato sauce determined that tomato sauce is not a risk factor for cholera. Milk and dairy products are expected to be risk factors for cholera [68]. In ten studies association between milk, dairy products and cholera is determined however, five out of 10 studies show that milk and dairy products are not risk factors. Alcohol is expected to be protective however three out of four

studies show that alcohol is not protective. In two studies nuts are risk factors for cholera. Large food diversity is protective against cholera and consuming a powdered drink mix (citric and ascorbic acid, excess sugar, not ORS) is also protective against cholera.

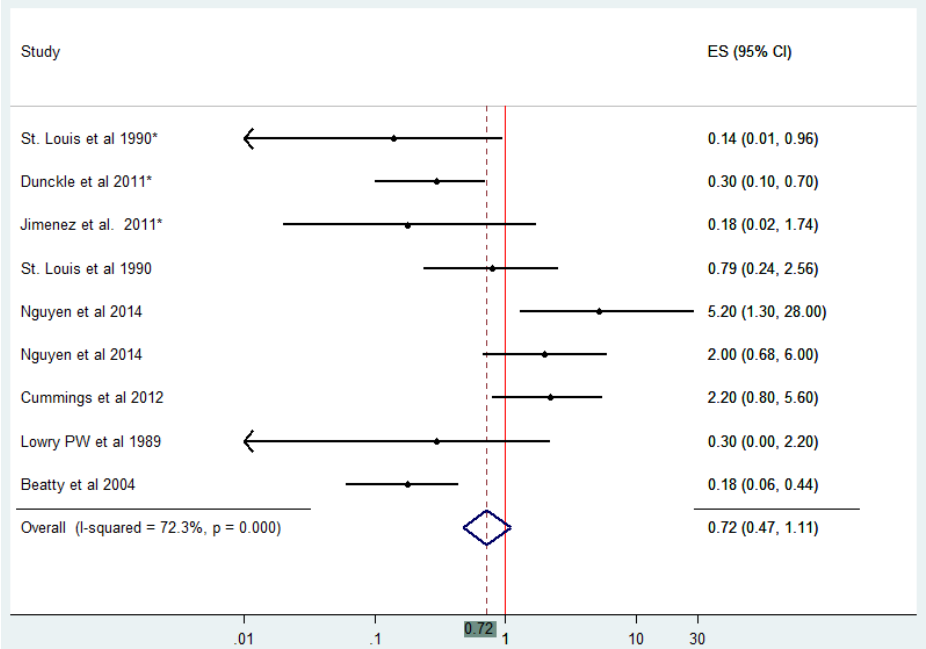
Table 5.8. Association between consumption of other food items and cholera outcome[+]

Study	Papers	Predicted Risk Factors	Predicted Protective Factors	Bias	OR univariate	95% CI	OR multivariate	95% CI
1	Rodrigues et al. 2000	Peanut sauce		L			0.75	0.41-1.36
2	Rodrigues et al. 1997	Peanut sauce		L	0.40	0.2-0.6		
3	St. Louis et al. 1990	Peanut sauce eaten at the noon meal		L	1.17	0.3-4.51		
4	St. Louis et al. 1990		Tomato sauce eaten at noon meals	L			0.14	0.01-0.96
5	St. Louis et al. 1990	Peanut sauce leftover, cold		L			3.10	1.17-8.24
6	St. Louis et al. 1990		Meat sauce	L	0.79	0.24-2.56		
7	St. Louis et al. 1990	Other sauce		L	2.50	0.57-10.9		
8	Kone-Coulibaly et al. 2010	Drank cold milk		M	5.48	1.54-19.38		
10	EberhartPhillips 1996	Cheese		L	1.50	0.6-3.9		
11	Mahamud et al. 2012	Drank milk at home		L	0.52	0.27-0.99		
12	Rodrigues et al. 2000	Curdled milk		L			0.51	0.23-1.10
13	Reller et al. 2001	Milk		L	0.30	0.1-0.9		
14	Nguyen et al. 2014	Milk		L	0.48	0.23-0.98		
15	Rodrigues et al. 1997	Curdled milk		L	0.60	0.3-1.1		
16	Das A et al. 2009	Consuming milk products		M	5.70	1.7-30		
17	Von Seidlein et al. 2008	Dairy products 5 day before		M			1.03	0.48–2.2
18	Nguyen et al. 2014		Poyo (palm wine)	L	5.20	1.3–28		
19	Nguyen et al. 2014		Alcoholic Beverage	L	2.00	0.68– 6.0		
20	Cummings et al. 2012		Drinks local alcoholic beverage (kwete) often	L	2.20	0. 8–5. 6		
21	Lowry PW et al. 1989		Alcohol consumed with crabs	M	0.30	0-2.2		
22	Rosewell, A. et al. 2012	Chews betel nut		M	1.74	0.68-4.67		
23	Dunkle et al. 2011		food diversity >23 items	L			0.30	0.1-0.7
24	Beatty et al. 2004		Use of powdered drink mix (citric and ascorbic acid, excess sugar, not ORS)	L	0.18	0.06-0.44		
25	Jimenez et al. 2011		Other food	M			0.18	0.02-1.74
26	Hatch et al. 1994	Nuts		M	1.20	0.5-3.2		

[+] Please note the exposure is highlighted in pink when it is not as expected and results in red when they had errors/were removed [83, 85, 86, 87, 89, 90, 93, 99, 103, 112, 114, 126, 134, 136, 138, 142]

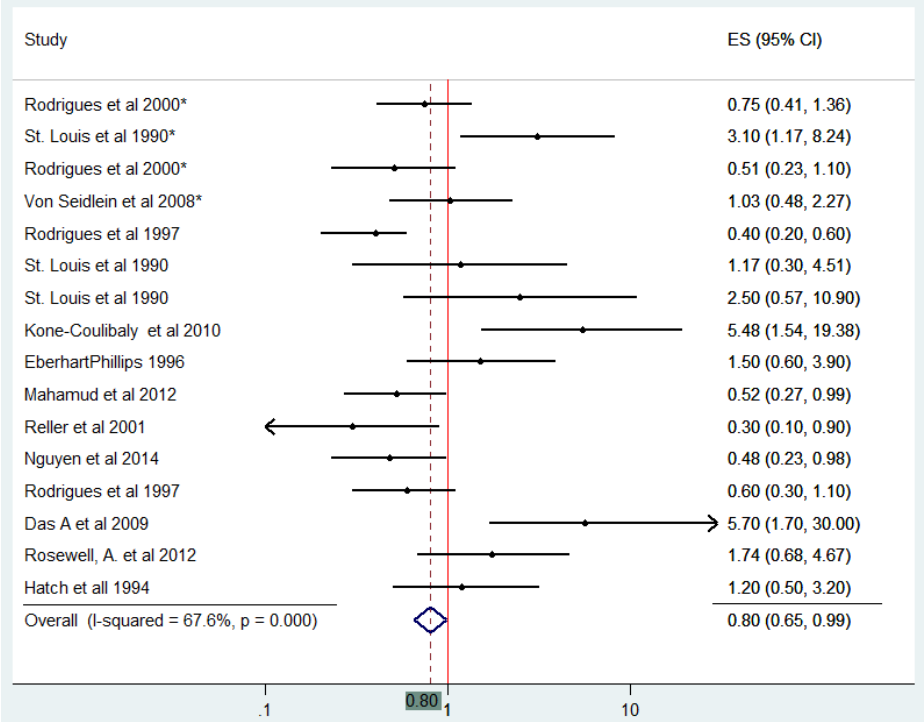
Based on the forest plot analysis of nine studies, categorized as predicted protective factors in Table 5.8, the cumulative effect of eating other protective food and cholera is 0.72(0.47 – 1.11). The overall association (OR and 95%CI) is

protective but not significant. The χ^2 test has a high p-value < 0.001 and an I^2 test value of 72.3%, which suggests that 72.3% variation in studies is due to heterogeneity.



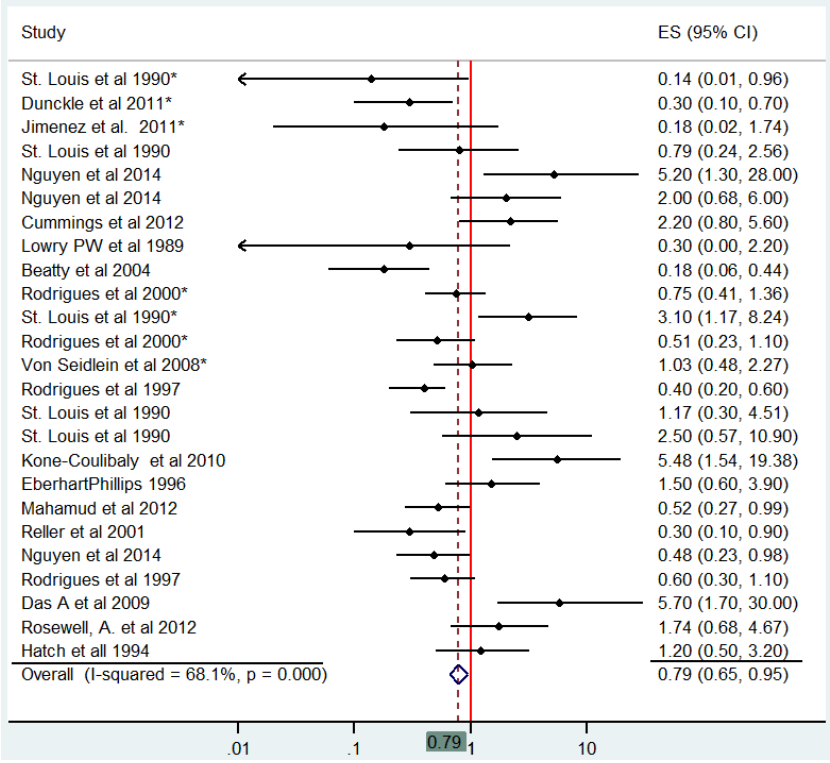
Graph 7.5.0 Forest plot analysis of the effect of other food items (predicted protective factors) on outcome of cholera [Note: Studies with * are adjusted]

Based on the forest plot analysis of 16 studies, initially characterized as predicted risk factors in Table 5.8, the cumulative effect of eating other food and cholera is 0.80 (0.65 – 0.99). The overall association (OR and 95% CI) is protective but not significant. The χ^2 test has a low p-value <0.001 and an I^2 test value of 67.6%, which suggests that 67.6% variation in studies is due to heterogeneity.



Graph 7.5.1. Forest plot analysis of the effect of other food items (predicted risk factors) on outcome of cholera [Note: Studies with * are adjusted]

Eating other food items is protective for exposures that were initially categorized as risk factors. Based on a forest plot analysis of all 25 studies on other food items, the cumulative effect of eating other food items is 0.79 (0.65-0.95). The overall association, OR and 95% CI, of consuming other protective food items and cholera is protective. The χ^2 test has a low p-value <0.001 and an I^2 test value of 68.1%, which suggests that 68.1% of variation in studies is due to heterogeneity.



Graph 7.5.2. Forest plot analysis of the effect of other food items (all factors) on outcome of cholera [Note: Studies with * are adjusted]

8) Socioeconomic Status (SES) and cholera

Association between SES and cholera is identified in 69 studies. Higher SES is expected to decrease the outcome of cholera whereas as lower SES is expected to increase the outcome of cholera [73]. High SES includes formal residence, high to middle SES score, income, small family size (4-5 people), amenities and appliance, ownership of land. Low SES includes informal residence and people in IDP-camps, low SES score, low income, large family size no appliances and amenities and ownership of pigs.

In 10 studies the association between residence type and cholera was determine. Informal residents and settlements are high risk areas associated with cholera. Residents in formal settlements of cholera affected area are also at risk of cholera. In one study however, IDP camp settlement (informal residence) is not a risk factor.

In five studies, that determine the association between socioeconomic score and cholera, high and middle SES score is protective however, in one of the five studies, low SES score is not a risk factor for cholera.

In 13 studies the association between education and cholera was determined. In one study no primary education was not a risk factor and in two other studies education below tertiary and secondary level respectively were not protective factors for cholera.

In eight studies the association between being employed, having income and cholera was determined. Employed and having income are protective factors whereas being unemployed and having low to no income are risk factors however, in one study being unemployed is not a risk factor.

The association between family size and cholera was determined in 17 studies. Small household of 4-5 people and 0-1 children is expected to be protective however, in three studies a large household of over five people is not a risk factor.

The association between owning appliances and amenities and developing cholera was determined in 15 studies. Owning appliances and amenities indicates higher SES and is expected to be a protective factor however, in two studies owning a cooking pot is not protective and running out of firewood is not a risk factor.

Table 5.9. Association between socioeconomic status and cholera outcome[+]

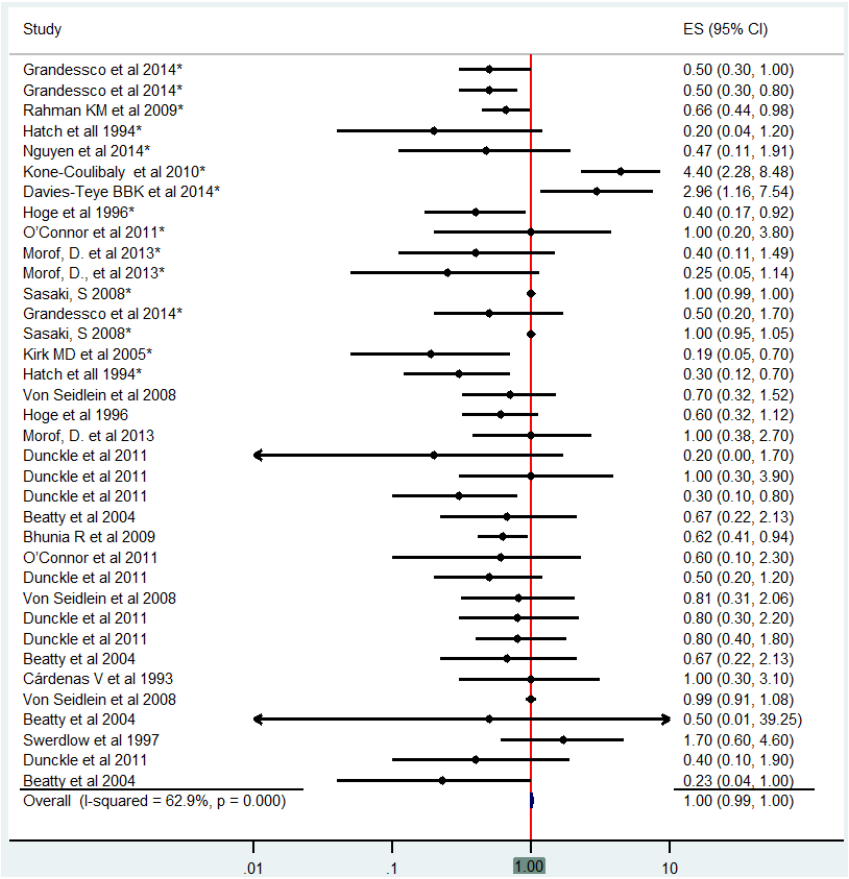
Study	Papers	Predicted Risk Factors	Predicted Protective Factors	Bias	OR univariate	95% CI	OR multivariate	95% CI
1	Rosewell, A.et al. 2012	Residence informal settlement		M	3.20	1.4-7.9		
2	Dunkle et al. 2011	Tarp roof		L	1.60	0.3-8.8		
3	Acosta et al. 2001	Mud housing		L	1.70	1.2-2.5		
4	Hatch et al.l 1994	Reside in transit center (informal)		M			17.60	3.6-85.9
5	Acosta et al. 2001	Non iron sheet roof		L	2.00	1.4-2.8		
6	Gunnlaugsson 1998	Cholera in their compound		M	4.20	1.3–14.1		
7	Hatch et al.l 1994	Resident in Malawi <3 months		M			3.60	0.6-22.9
8	Dunkle et al. 2011	IDP CAMp- self reported		L	2.10	0.5-8.7		
9	Dunkle et al. 2011	IDP camp - observed		L	0.70	0.1-7.3		
10	Von Seidlein et al. 2008		Household members o a machamba (land for farming)	M	0.70	0.32–1.52		
11	Grandesso et al. 2014	Household owns pigs		L			10.30	2·3–46·6
12	Grandesso et al. 2014		Socioeconomic score	L			0.50	0·3–1·0
13	Grandesso et al. 2014		Socioeconomic score	L			0.50	0.3-0.8
14	Rahman KM et al. 2009		SES (quintile)	M			0.66	0.44-0.98
15	Hatch et al.l 1994		Middle SES	M			0.20	0.04-1.2
16	Hatch et al.l 1994	lowest SES		M			0.70	0.1-3.5
17	Nguyen et al. 2014		Secondary education	L			0.47	0.113-1.9
18	Kone-Coulibaly et al 2010		Did not have secondary education level	M			4.40	2.28-8.48

19	Davies-Teye BBK et 2014		Education below tertiary	H			2.96	1.16- 7.54
20	Hoge et al. 1996		Education, Completed primary school	L	0.60	0.32-1.12		
21	Hoge et al. 1996		Education, completed primary school	L			0.40	0.17-0.92
22	Bhunia R et al. 2009	No primary education		M	0.72	0.45–1.1		
23	Bhunia Rama and Ghosh 2011	Education ≤ primary		L	1.40	0.83–2.5		
24	Morof, D. et al. 2013		Any education	L	1.00	0.38-2.7		
25	O'Connor et al. 2011		Completed primary	L			1.0	0.2–3.8
26	Dunkle et al. 2011		Completed primary	L	0.20	0.0-1.7		
27	Dunkle et al. 2011		Literate	L	1.00	0.3-3.9		
28	Izadi et al. 2006	Illiteracy		L			5.76	2.63-30.0
29	Dunkle et al. 2011		Speaks French	L	0.30	0.1-0.8		
30	Morof, D. et al. 2013		Any Income	L			0.40	0.11- 1.49
31	Morof, D., et al. 2013		Any Income	L			0.25	0.05-1.14
32	Sasaki, S 2008		Average monthly inco (USD$)	L			1.00	0.99-1.00
33	Bhunia R et al. 2009	Monthy income <US $3		M	1.60	0.96–2.7		
34	Dunkle et al. 2011	Unemployed		L	0.70	0.2-2.4		
35	Beatty et al. 2004		Employyed	L	0.67	0.22-2.13		
36	Shikanga OT et al. 2009	Not working at time of illness onset		H	9.10	1.00–434		
37	Cárdenas V et al. 1993	Head of household engaged in fishing-related trades		L	2.70	0.2-80.7		
38	Morof, D. et al. 2013	Average number of people sleeping in the house at night		L			1.21	0.99-1.48
39	Morof, D., et al. 2013	Average number of people sleeping in the house at night		L			1.21	1-1.46
40	Von Seidlein et al. 2008	Mean number of household members (SI		M	1.14	1.01–1.30		
41	Izadi et al. 2005	Household size (person)		H			1.30	1.07-1.57
42	Grandesso et al. 2014		Household with 4 to 5	L			0.50	0.2-1.7
43	Bhunia Rama and Ghosh 2011	Household ≥5 members		L	0.85	0.47–1.5		
44	Sasaki, S 2008		Number of family members with case =4.89 and with no cases =5.03	L			1.00	0.949–1.0
45	Bhunia R et al. 2009		Household members <5	M	0.62	0.41–0.94		
46	Izadi et al. 2005	Household with 5-7		H	2.50	0.27-23.23		
47	Grandesso et al. 2014	Household with 6-8		L			0.70	0.2-2.6
48	Morof, D. et al. 2013	≥ 6 persons sleeping in the house		L	2.23	1.00 – 4.83		
49	Izadi et al. 2005	Household with 8-10		H	5.33	0.63-44.98		
50	Grandesso et al. 2014	≥9		L			0.10	0.0-0.5
51	Izadi et al. 2005	Household with 11-12		H	6.40	0.47-86.34		
52	Izadi et al. 2005	Household with ≥13		H	5.33	0.25-110.7		
53	Hatch et al.l 1994	Household with 2 childern		M			9.70	2.1-44.9
54	Hatch et al.l 1994	Household with 3+ children		M			38.20	7.2-202
55	O'Connor et al. 2011		Has electricity	L			0.60	0.1–2.3

56	Dunkle et al. 2011		Has electricity	L	0.50	0.2-1.2		
57	Von Seidlein et al. 2008		With electricity in the household	M	0.81	0.31–2.06		
58	Dunkle et al. 2011		Owns radio	L	0.80	0.3-2.2		
59	Dunkle et al. 2011		Owns TV	L	0.80	0.4-1.8		
60	Beatty et al. 2004		Owned a freezer	L	0.67	0.22-2.13		
61	Kirk MD et al. 2005		Working refrigerator/ice box	L			0.19	0.05–0.70
62	Cárdenas V et al. 1993		Refrigerator in household	L	1.00	0.3-3.1		
63	Von Seidlein et al. 2008		Mean number of kitchen implements, pots, pans, etc. (SD)	M	0.99	0.91–1.08		
64	Beatty et al. 2004		Owned a stove	L	0.50	0.01-39.25		
65	Hatch et al. 1994		Any cooking pots	M			0.30	0.12-0.7
66	Swerdlow et al. 1997		Owned a cooking pot	L	1.70	0.6-4.6		
67	Dunkle et al. 2011		Owns car/motorcycle	L	0.40	0.1-1.9		
68	Beatty et al. 2004		Owned a car	L	0.23	0.04-1.00		
69	Swerdlow et al. 1997	Ran out of firewood in previous week		L	0.70	0.3-2.7		

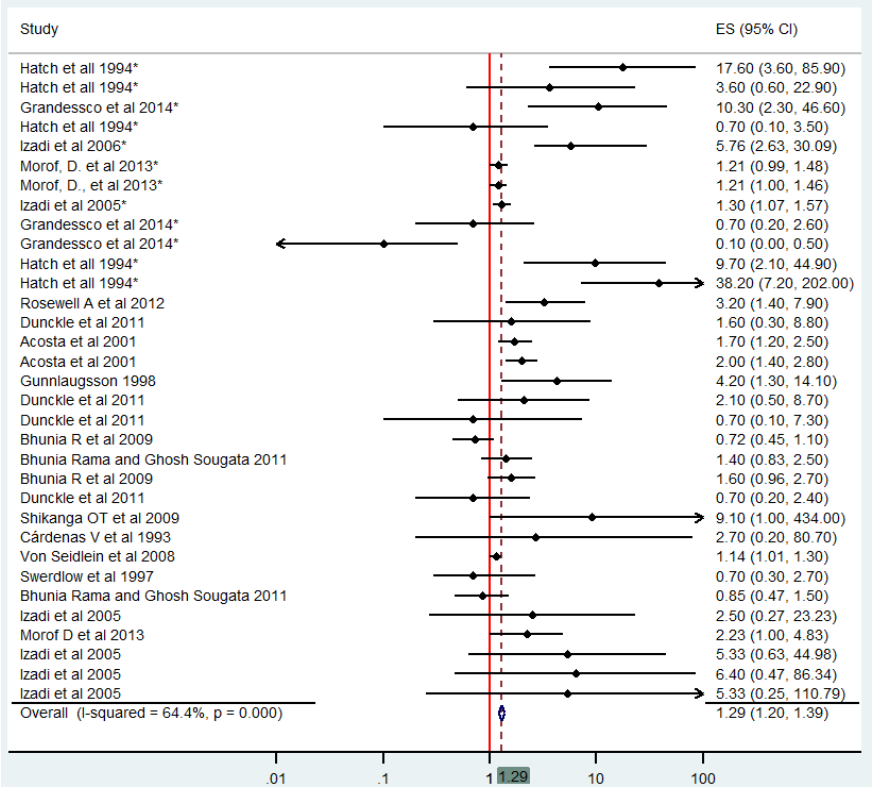
[+] Please note the exposure is highlighted in pink when it is not as expected, and in orange when there is no association [75, 81, 82, 83, 84, 85, 88, 89, 92, 95, 96, 98, 99, 103, 104, 108, 111, 114, 131, 135, 126, 128, 143]

Based on the forest plot analysis of 36 studies, categorized as predicted protective factors in Table 5.9, the cumulative effect of high to middle SES and cholera is 1.00 (0.99 – 1.00). There is no overall association and the 95% CI is not significant. The χ^2 test has a low p-value of <0.001 and an I^2 test value of 62.9%, which suggests that 62.9% of variation in studies is due to heterogeneity.



Graph 8.0.0. Forest plot analysis of the effect of higher SES (predicted protective factor) on outcome of cholera
[Note: Studies with * are multivariate analysis]

Based on the forest plot analysis of 33 studies, categorized as predicted risk factors in Table 5.9, the cumulative effect of low SES and cholera is 1.29 (1.20 – 1.39). There overall association (OR and the 95% CI) is significant, which suggests that lower SES is a risk factor for cholera. The χ^2 test has a low p-value of <0.001 and an I^2 test value of 64.4%, which suggests that 64.4% of variation in studies is due to heterogeneity.



Graph 8.0.1. Forest plot analysis of the effect lower SES (predicted risk factors) on outcome of cholera [Note: Studies with * are multivariate analysis]

9) Association between other exposures and cholera

Association between other exposures and cholera is given in 110 studies. Exposures characterized under the other category include: 9.1. know a case of cholera or have contact with a cholera patient, 9.2. gender, 9.3. age, 9.4. marital status, 9.5. religion, 9.6. attending a function, 9.7. eating at a funeral, 9.8. infection, 9.9 nation, 9.10. breastfeeding, 9.11. travel, and 9.12. using utensils. The exposures in the sub-categories are described below.

9.1. Knowing a cholera patient or contact with a cholera patient and cholera outcome

In 28 studies the association between knowing a cholera case and developing cholera was determined. Nineteen out of 27 studies determine association between contact with a cholera patient and developing cholera. The study by Weil et al. 2009, determined higher incidence of *V. cholerae* infection and associated clinical symptoms in household contacts of patients with cholera [74]. Contact with a patient is therefore expected to be a risk factor. In 17 studies contact with a cholera patient is associated with the disease however, in one study visiting a person with diarrhea is not a risk factor.

Nine cases determine association between knowing a case of cholera and developing the disease. In all nine studies knowing a case of cholera is a risk factor for developing the disease.

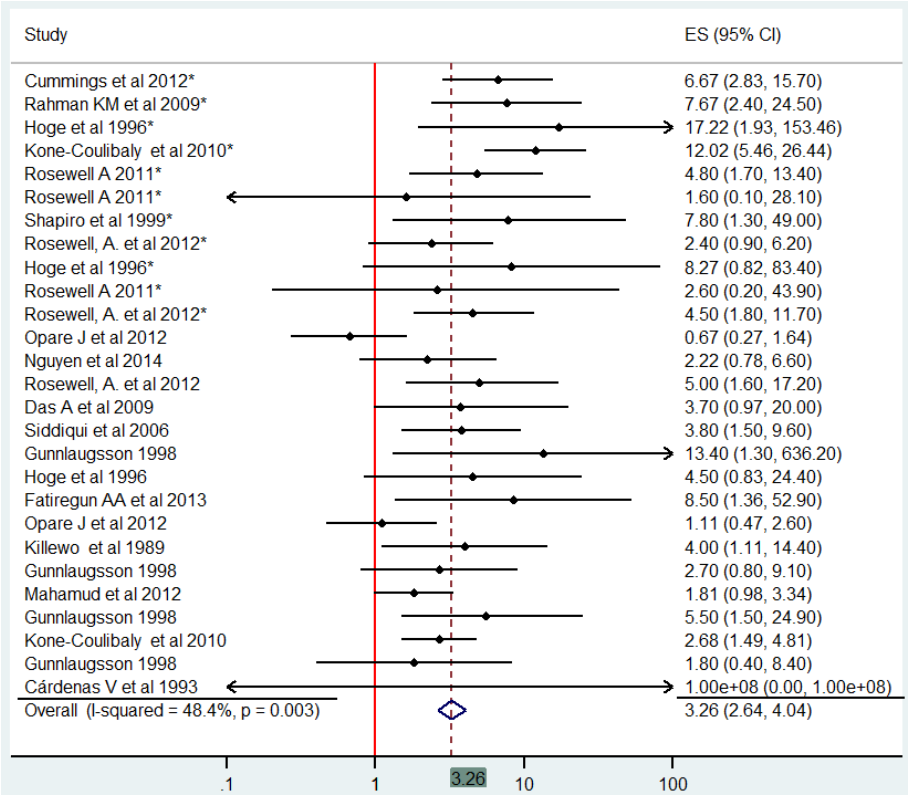
Table 6.0. Association between knowledge of or contact with a cholera patient and cholera outcome[+]

Study	Papers	Predicted Risk Factors	Predicted Protective Factors	Bias	OR univariate	95% CI	OR multivariate	95% CI
1	Opare J et al. 2012	Visited any person with diarrhoea		M	0.67	0.27-1.64		
2	Nguyen et al. 2014	Contact with cholera patient		L	2.22	0.78– 6.6		
3	Rosewell, A. et al. 2012	Shares housing with diarrhoea case		M	5.00	1.6-17.2		
4	Cummings et al. 2012	Resides in same household as another cholera case		L			6. 67	2.83–15.70
5	Das A et al. 2009	Exposure to case-patient		M	3.70	0.97-20		
6	Rahman KM et al. 2009	Sibling of case		M			7.67	2.4-24.5
7	Siddiqui et al. 2006	Household member with cholera		L	1.30	5.2-29.2		
8	Siddiqui et al. 2006	Household member with cholera		L	3.80	1.5-9.6		
9	Gunnlaugsson 1998	Touched the corpse		M	13.40	1.3–636.2		
10	Hoge et al. 1996	History of contact with person who had diarrhea within household		L	4.50	0.83-24.40		
11	Hoge et al. 1996	History of contact with person who had diarrhea within household		L			17.22	1.93-153.46
12	Fatiregun AA et al. 201	Diarrhoea contact at home/neighborhood 7 days prior to illness		L	8.50	1.36-52.9		
13	Opare J et al. 2012	Contact with person with diarrhoea		M	1.11	0.47-2.6		
14	Kone-Coulibaly et al. 2010	Had diarrheal contact at home		M			12.02	5.46-26.44
15	Killewo et al. 1989	Previous case in family		L	4.00	1.11-14.40		
16	Rosewell A 2011	Had close contact with diarrhea patient		L			4.80	1.7–13.4
17	Rosewell A 2011	Washed the body/clothes deceased		L			1.60	0.1–28.1
18	Gunnlaugsson 1998	Visitors to their compound		M	2.70	0.8–9.1		
19	Shapiro et al. 1999	Sharing food with a person with watery diarrhea		L			7.80	1.3-49.0
20	Rosewell, A. et al. 2012	Knows case of cholera		M			2.40	0.9-6.2
21	Mahamud et al. 2012	Neighbor/family member had diarrhoea		L	1.81	0.98-3.34		
22	Gunnlaugsson 1998	Related to index case		M	5.50	1.5–24.9		
23	Hoge et al. 1996	History of contact with person who had diarrhea outside household		L			8.27	0.82-83.40
24	Kone-Coulibaly et al. 2010	Had diarrhoea contact outside home		M	2.68	1.49 - 4.81		
25	Cárdenas V et al. 1993	Index case in house		L			∞	0 - ∞
26	Rosewell A 2011	Had death in the family		L			2.60	0.2–43.9
27	Gunnlaugsson 1998	Entered funeral house		M	1.80	0.4–8.4		

28	Rosewell, A. et al. 2012	Knows someone who travelled to cholera area		M			4.50	1.8- 11.7

[+] Please note the exposure is highlighted in pink when it is not as expected and in results are in red when they are reported with errors [Note: ∞ values are given a value of $1*10^8$ in STATA] [83, 85, 86, 87, 93, 96, 102, 106, 75, 111, 124, 125, 126, 135, 140, 141]

Based on the 28 studies, categorized as risk factors in Table 6.0, the cumulative effect of knowing a cholera patient/ contact with a cholera patient on cholera outcome is 3.26 (2.64-4.04). As expected, the overall association (OR and 95% CI) is significant, which suggests that knowing or contact with a cholera patient is a risk factor for cholera. The χ^2 test has a p-value of 0.003 and an I^2 test value of 48.4%, which suggests that 48.4% variation in data is because of heterogeneity.



Graph 9.1.0. Forest plot analysis of the effect of knowing/contact with a cholera patient (predicted risk factor) on the outcome of cholera [Note: Studies with * are multivariate analysis; studies with ∞ values are given a value of $1*10^8$ in STATA]

9.2. Gender and cholera outcome

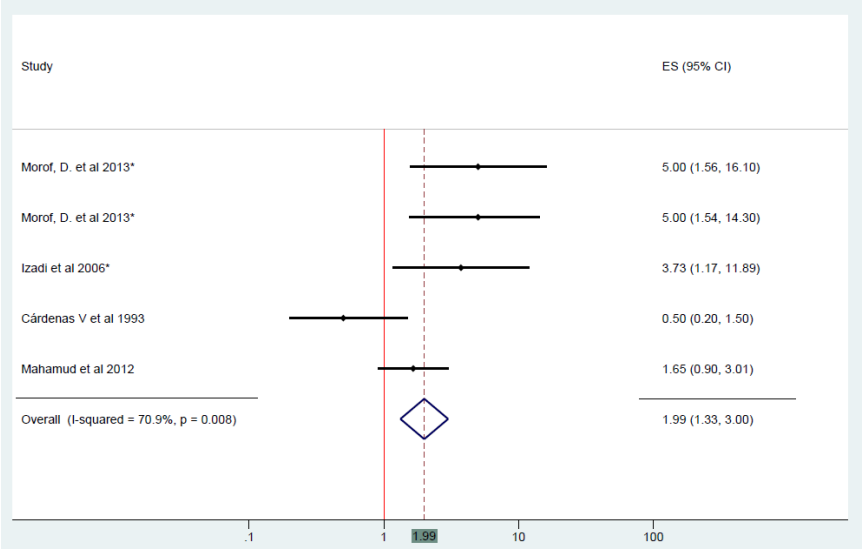
Ten studies determine the association between gender and developing cholera. Five studies determine the association between being male and cholera, in four studies being male is a risk factor however, in one study being male is not a risk factor. Five other studies determine that being female is a risk factor for cholera.

Table 6.1. Association between gender and cholera outcome[+]

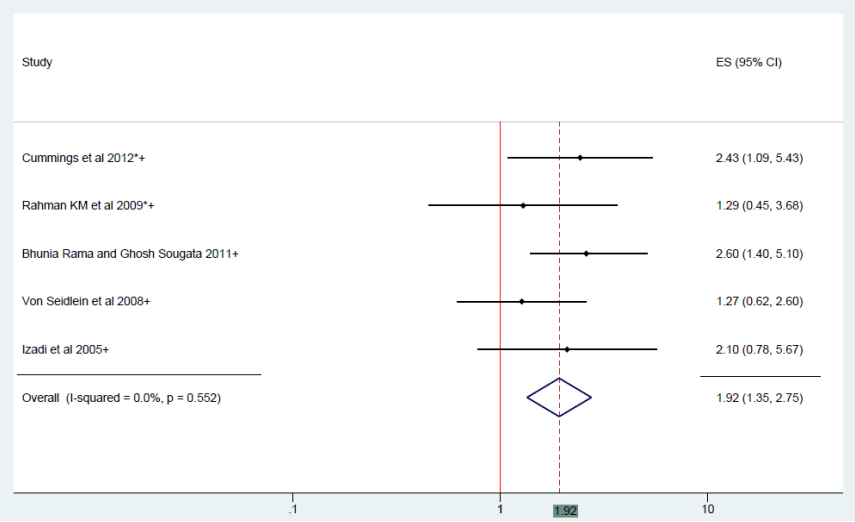
Study	Papers	Predicted Risk Factors	Predicted Protective Factors	Bias	OR univariate	95% CI	OR multivariate	95% CI
1	Cárdenas V et al. 1993		Male	L	0.50	0.2-1 .5		
2	Morof, D. et al. 2013	Male sex from those who did not go to CTC		L			5.00	1.56-16.10
3	Morof, D. et al. 2013	Male sex - all including those who went to CTC		L			5.00	1.54 –14.30
4	Cummings et al. 2012	Female		L			2. 43	1.09–5.43
5	Mahamud et al. 2012	Males		L	1.65	0.90-3.01		
6	Bhunia Rama and Ghosh 2011	Female		L	2.60	1.4-5.1		
7	Rahman KM et al. 2009	Female		M			1.29	0.45-3.68
8	Von Seidlein et al. 2008	Sex (female/total)		M	1.27	0.62–2.60		
9	Izadi et al. 2006	Male		L			3.73	1.17-11.89
10	Izadi et al. 2005	Female		**H**	2.10	0.78-5.67		

[84, 86, 87, 92, 96, 99, 135, 143, 144]

Based on the forest plot analysis, given in the graphs below, the cumulative effect of being male on cholera is 1.99 (1.33-3.00) and of being female on cholera is 1.92 (1.35-2.75). As expected, the overall association suggests that being male or female, are both, risk factors for cholera. For the analysis of being male and outcome of cholera the χ^2 test has a high p-value < 0.05 (p-value = 0.008) and I^2 test value of 70.9%, which suggests that 70.9% of variation in studies is due to heterogeneity. However, for the analysis of being female on cholera the χ^2 test has a high p-value > 0.05 (p-value = 0.552) and I^2 test value of 0.0%, which suggests that none of variation in studies is due to heterogeneity.



Graph 9.2.0 Forest plot analysis of the effect of being male on the outcome cholera [Note: Studies with * are multivariate analysis]



Graph 9.2.1 Forest plot analysis of the effect of being a female on the outcome of cholera [Note: Studies with * are multivariate analysis]

9.3. Age and cholera outcome

In eighteen studies the association between age and cholera was determined. Three studies showed no association between age and cholera. Two studies showed that age is protective against cholera. In one study being over 45 years was protective, four other studies concluded that being 12-17months, 18-23months, 24-29 months, 30-35 months is protective. 8 studies showed that age is a risk factor for ages 5-14 years, 6-10 years, 11-15 years, <18 years, 16-20 years, 10-17 years, >20years, and 35-44 years.

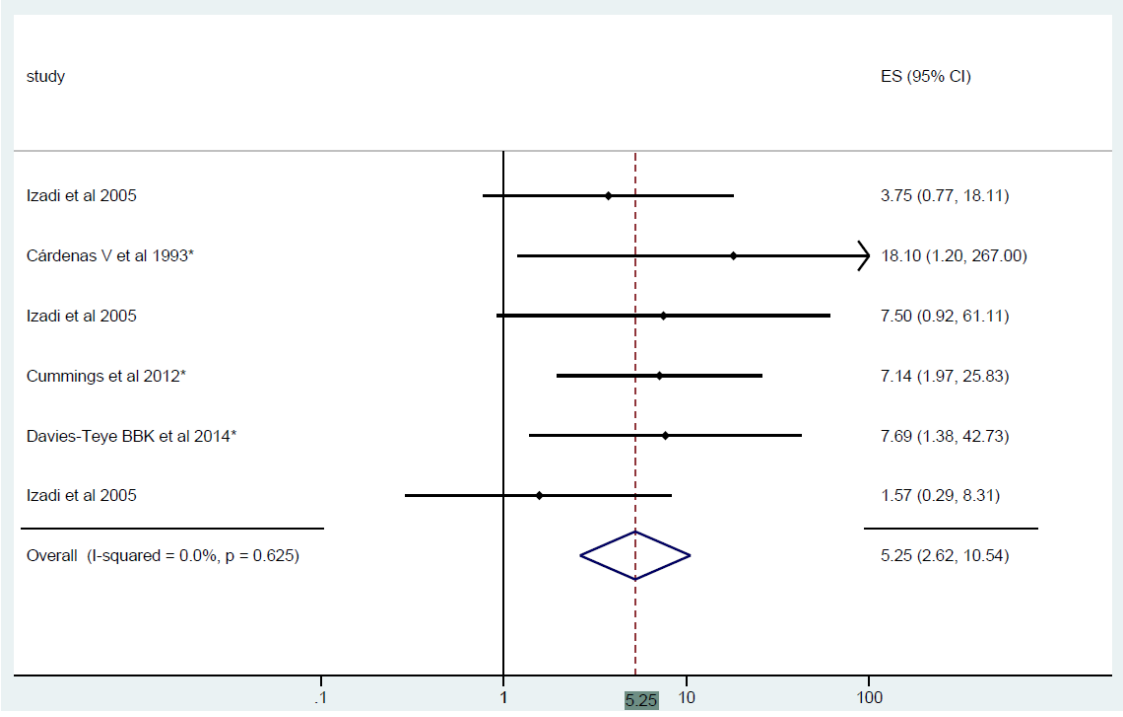
Table 6.2. Association between age and cholera outcome[+]

Study	Papers	Predicted Risk Factors	Predicted Protective Factors	Bias	OR univariate	95% CI	OR multivariate	95% CI
1	Cárdenas V et al. 1993		>=45	L			0.70	0.1-7.0
2	Clemens JD et al. 1990		12-17 months decline in reduced risk of cholera with breast feeding with age	L			0.06	0.01-0.31
3	Izadi et al. 2005		age	H			0.84	0.72-0.97
4	Izadi et al. 2005	6-10 years		H	3.75	0.77-18.11		
5	Clemens JD et al. 1990		18-23months decline in reduced risk of cholera with breast feeding with age	L			0.21	0.07-0.6
6	Cárdenas V et al. 1993	5-14 years		L			18.10	1.2-267.0
7	Izadi et al. 2005	11-15 years		H	7.50	0.92-61.11		
8	Clemens JD et al. 1990		24-29months decline in reduced risk of cholera with breast feeding with age	L			0.29	0.13-0.6
9	Cárdenas V et al. 1993		15-24 years	L			1.00	referent
10	Izadi et al. 2005	16-20 years		H	1.57	0.29-8.31		
11	Cárdenas V et al. 1993		25-34 years	L			1.00	0.1-9.8
12	Clemens JD et al. 1990		30-35months decline in	L			0.60	0.27-1.23

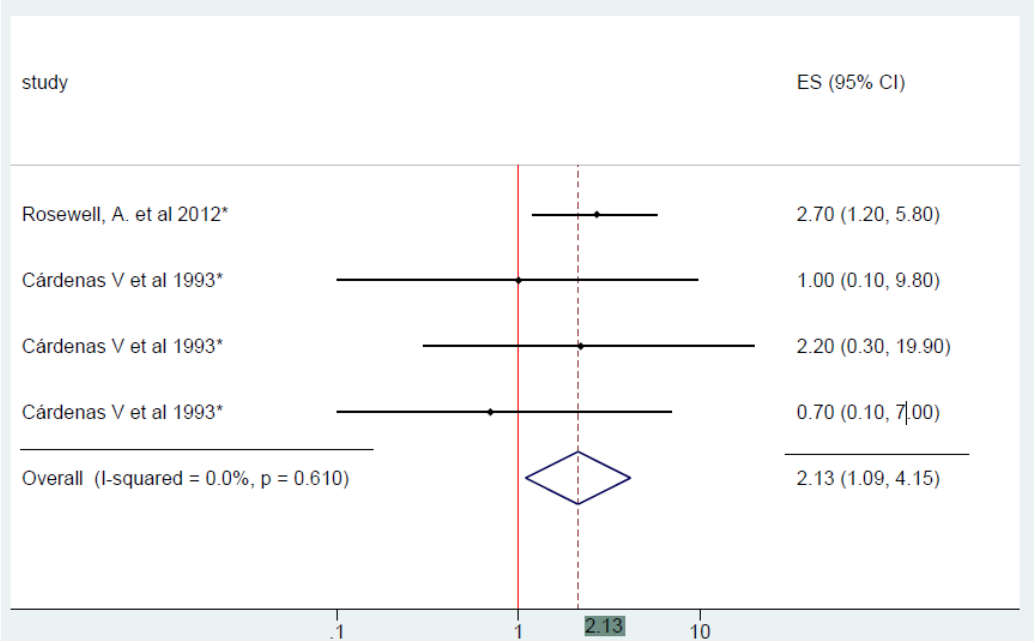
			reduced risk of cholera with breast feeding with age					
13	Cárdenas V et al. 1993	35-44 years		L			2.20	0.3-19.9
14	Rahman KM et al. 2009		Age years	M			0.95	0.83-1.09
15	Davies-Teye BBK et al. 2014	age < 18 years		H			7.69	1.38-42.73
16	Rosewell, A. et al. 2012	>20 years		M			2.70	1.2-5.8
17	Cummings et al. 2012	10 to 17 years		L			7.14	1.97–25.83
18	Von Seidlein et al. 2008		Mean age in years (SD)	M	1.00	0.97–1.02		

[+] Please note the exposure is highlighted in orange when there is no association and results in red when they are reported with errors [82, 85, 86, 96, 99, 135, 137, 143]

For a homogeneous comparison, the age data was grouped into three categories including: 1-3 years, 5-18 years, and over 20 years old. The association between 1-3 years of age and cholera is determined in only one study, which is not sufficient for a forest plot analysis. In the age groups from 5-18years and >20 years, cholera outcome was a significant risk factor and the overall estimate was (5.25; 2.62-10.54) and (2.13; 1.09-4.15) respectively. In both these age groups there was no heterogeneity between studies. The results suggest that the 5-18years age group are at a higher odds of having cholera outcome than the over 20 year age group.



Graph 9.3.0 Forest plot analysis of the effect of being 5-18 years of age on the outcome of cholera [Note: Studies with * are multivariate analysis]



Graph 9.3.1 Forest plot analysis of the effect of being over 20 years of age on the outcome of cholera [Note: Studies with * are multivariate analysis]

9.4. Marital Status and cholera outcome

Two studies determine that association between marital status and developing cholera. In one study being married is a protective factor however, in another study a homemaker at home is a risk factor.

Table 6.3. Association between marital status and cholera outcome**

Study	Papers	Predicted Risk Factors	Predicted Protective Factors	Bias	OR univariate	95% CI	OR multivariate	95% CI
1	Morof, D. et al. 2013		Married	L			0.26	0.08-0.83
2	Bhunia R et al. 2009	Homemaker		M	1.80	0.79–4.0		

** Forest plot analysis not conducted on exposures with <4 studies [84, 128]

9.5. Religion and cholera outcome

Two studies determine the association between religion and cholera. Being an Apostolic in one study is not a risk factor for developing cholera however, in another study being a Muslim compared with other religions, including Catholics, Protestants, and others is a risk factor.

Table 6.4. Association between religion and cholera outcome **

Study	Papers	Predicted Risk Factor	Predicted Protective Factor	Bias	OR univariate	95% CI	OR multivariate	95% CI
1	Morof, D. et al. 2013		Apostolic	L	0.67	0.28–1.63		
2	Acosta et al. 2001	Muslims compared with		L	2.30	1.1-3.4		

		other religions, including Catholics, Protestants, and others						

** Forest plot analysis not conducted on exposures with <4 studies [84, 104]

9.6. Attending a function and cholera outcome

Twenty-one studies determine the association between attending a function and cholera. Attending a funeral, gathering, ceremony, or going to the market place is expected to be a risk factor for cholera because individuals may be exposed to unsanitary and unhygienic conditions present in crowded areas. In four studies however, attending a funeral, wedding, party, and participating in a ceremony are not risk factors for cholera.

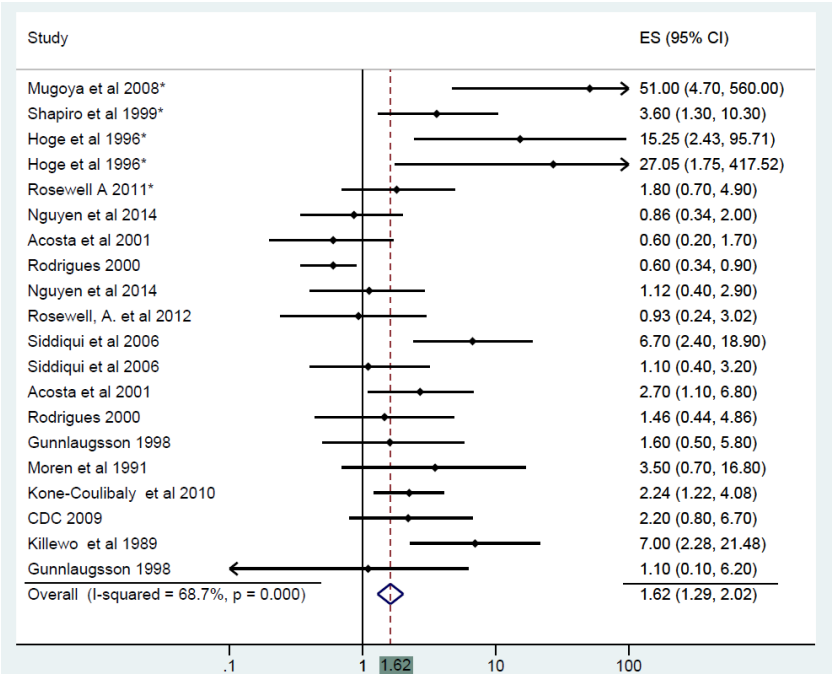
Table 6.5. Association between attending a function and cholera outcome [+]

Study	Papers	Predicted Risk Factor	Predicted Protective Factor	Bias	OR univariate	95% CI	OR multivariate	95% CI
1	Nguyen et al. 2014	Attended wedding		L	0.86	0.34-2		
2	Acosta et al. 2001	Attended party recently		L	0.60	0.2-1.7		
3	Rodrigues 2000	Participating in a ceremony		L	0.60	0.34–0.9		
4	Nguyen et al. 2014	Attended funeral		L	1.12	0.40–2.9		
5	Rosewell, A. et al. 2012	Attended funeral		M	0.93	0.24-3.0		
6	Mugoya et al. 2008	Attended funeral		L			51.00	4.7–560.0
7	Siddiqui et al. 2006	Attending any gathering		L	6.70	2.4-18.9		
8	Siddiqui et al. 2006	Attending any gathering		L	1.10	0.4-3.2		
9	Acosta et al. 2001	Attended funeral recently		L	2.70	1.1-6.8		
10	Rodrigues 2000	Attending a funeral		L	1.46	0.44–4.8		
11	Shapiro et al. 1999	Attending a funeral feast		L			3.60	1.3- 10.3
12	Gunnlaugsson 1998	Attended funeral of index case		M	1.60	0.5–5.8		
13	Hoge et al. 1996	Attendance at gathering (party, festival)		L			15.25	2.43-95.71
14	Hoge et al. 1996	Attendance at gathering (party, festival)		L			27.05	1.75-417.5
15	Swerdlow et al. 1992	Went to a fiesta		L	3.60	1.1-1.11		
16	Moren et al. 1991	market contact		L	3.50	0.7-16.8		
17	Kone-Coulibaly et al. 2010	Attended any gathering		M	2.24	1.22 - 4.0		
18	Kur L et al. 2009	Attended a large gathering		L	2.20	0.8-6.7		
19	Killewo et al. 1989	Attendance to gatherings		L	7.00	2.28-21.4		
20	Rosewell A 2011	Attended a funeral		L			1.80	0.7–4.9
21	Gunnlaugsson 1998	Attended other cholera funerals		M	1.10	0.1–6.2		

[+] Please note the exposure is highlighted in pink when it is not as expected and results in red when they are reported with errors [71, 83, 85, 102, 104, 106, 75, 111, 119, 120, 126, 140, 141]

Based on 20 studies (1 study deleted), categorized as predicted risk factors in Table 6.5, the cumulative effect of attending a function is 1.62 (1.29-2.02). The overall association, OR and 95% CI, is significant which suggests that

attending a function is a risk factor for cholera. The χ^2 test has a low p-value < 0.001 and I^2 test value of 68.7%, which suggests that 68.7% variation in studies is due to heterogeneity.



Graph. 9.6.0. Forest plot analysis of the effect of attending a function (predicted risk factor) on the outcome of cholera
[Note: Studies with * are multivariate analysis]

9.7. Eating at a funeral and cholera outcome

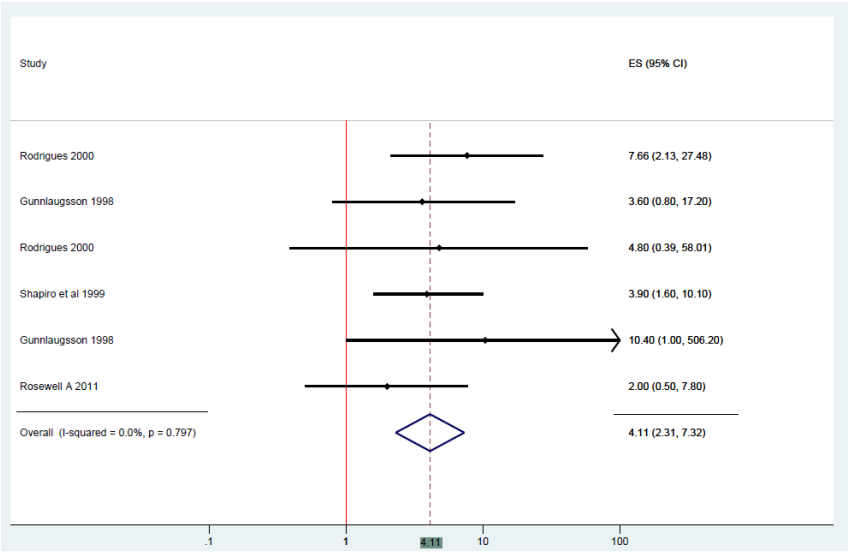
Six studies determine the association between eating at a funeral and outcome of cholera. Eating or drinking at a funeral is expected to be a risk factor for cholera especially when communities engage in high risk funeral practices such as not disinfecting the corpse [75]. In all six studies eating or drinking at a funeral is associated with cholera outcome.

Table 6.6. Association between eating at a funeral and cholera outcome

Study	Papers	Predicted Risk Factor	Predicted Protective Factor	Bias	OR univariate	95% CI	OR multivariate	95% CI
1	Rodrigues 2000	Eating at ceremonies where there is a corpse		L	7.66	2.13–27.48		
2	Gunnlaugsson 1998	Drank alcohol at the funeral		M	3.60	0.8–17.2		
3	Rodrigues 2000	Eating at memorial funeral feasts		L	4.80	0.39–58.01		
4	Shapiro et al. 1999	Eating food at a funeral/gathering		L	3.90	1.6, 10.1		
5	Gunnlaugsson 1998	Ate at the funeral		M	10.40	1.0–506.2		
6	Rosewell A 2011	Consumed food during funera		L	2.00	0.5–7.8		

[71, 106, 75, 141]

Based on the six studies, categorized as predicted risk factors in Table 6.6, the cumulative effect of eating at a funeral on cholera is 4.11 (2.31-7.32). The overall association, OR and 95% CI, is significant, which suggests that eating at a funeral is a risk factor for cholera. The χ^2 test has a high p-value > 0.05 (p-value = 0.797) and I^2 test value is 0.0%, which suggests that none of the variation is due to heterogeneity.



Graph 9.7.0. Forest plot analysis of the effect of eating at a funeral (predicted risk factor) on the outcome of cholera
[Note: Studies with * are multivariate analysis]

9.8. Prior Infection and cholera outcome

Three studies determine the association between prior infection and developing cholera. A previous infection may increase the risk of cholera. In all the three studies previous infection and/or poor health is associated with increased risk of developing cholera.

Table 6.7. Association between prior infection and cholera outcome**

Study	Papers	Predicted Risk Factor	Predicted Protective Factor	Bias	OR univariate	95% CI	OR multivariate	95% CI
1	Von Seidlein et al. 2008	HIV infection		M			2.59	0.90–7.49
2	Gunnlaugsson et al. 2000	Being in poor health or intoxicated at illness onset		**H**			6.40	1.1-37.7
3	Shikanga OT et al. 2009	Chronic medical condition (cancer, TB, HIV)		**H**	8.22	0.90–394		

** Forest plot analysis not conducted on exposures with <4 studies [95, 99, 75]

9.9. Nation of origin and cholera outcome

Three studies determine the association between nation and outcome of developing cholera. In two studies being from Afghanistan or Pakistan were compared to Iran. In one study being from Afghanistan was not a risk factor but being from Pakistan in the other study was a risk factor. In one study being a refugee from Somalia was not a risk factor for the disease.

Table 6.8. Association between nation and cholera outcome**

Study	Papers	Predicted Risk Factor	Predicted Protective Factor	Bias	OR univariate	95% CI	OR multivariate	95% CI
1	Mahamud et al. 2012		Somali	L	0.91	0.39 - 2.14		
2	Izadi et al. 2005		Afghan	H	0.75	0.26-2.19		
3	Izadi et al. 2005	Pakistan		H	1.32	0.26-6.76		

** Forest plot analysis not conducted on exposures with <4 studies [87, 143]

9.10. Travel to a cholera affected area and cholera outcome

Eight studies determine association between traveling and cholera. Travelling to a cholera affected is expected to be a risk factor as travelers may be exposed to contaminated food and water. All eight studies show that travel to cholera affected area, arrival at a camp during a cholera outbreak is associated with cholera. In one study however, travelling outside village has no association with cholera.

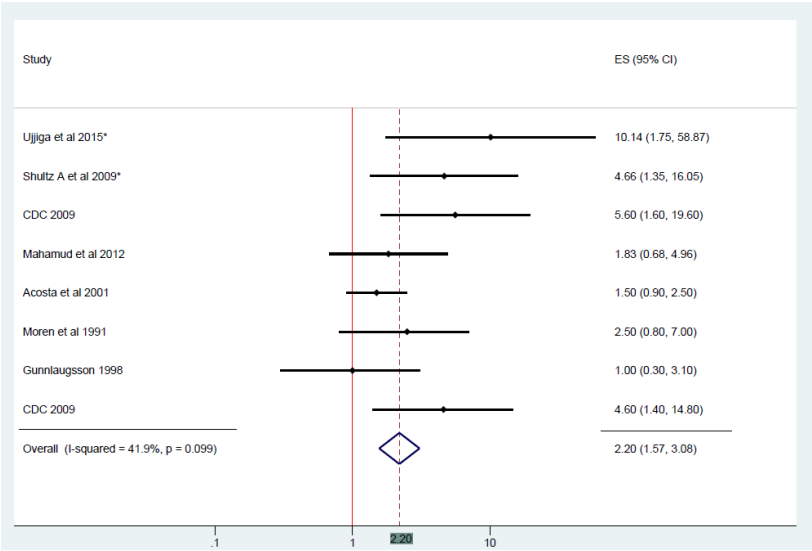
Table 6.9. Association between travelling to cholera affected areas and cholera outcome[+]

Study	Papers	Predicted Risk Factors	Predicted Protective Factors	Bias	OR univariate	95% CI	OR multivariate	95% CI
1	Ujjiga et al. 2015	Traveled outside home village before onset of illness to any area affected by cholera		M			10.14	1.75-58.87
2	Kur L et al. 2009	visitor/ nonresident of Juba, Southern Sudan		L	5.60	1.6-19.6		
3	Mahamud et al. 2012	New arrivals to camp(after 6/1/09)		L	1.83	0.68-4.96		
4	Shultz A et al. 2009	Arrived at camp on or after Nov2004.		M			4.66	1.35-16.05
5	Acosta et al. 2001	Traveled in the past 2 weeks		L	1.50	0.9-2.5		
6	Moren et al. 1991	Travel outside camp		L	2.50	0.8-7.0		
7	Gunnlaugsson 1998	Travel outside village		M	1.00	0.3-3.1		
8	Kur L et al. 2009	< 1 year in Juba, Southern Sudan		L	4.60	1.4-14.8		

[+]Please note the exposure is highlighted in orange when there is no association [75, 87, 97, 104, 75, 120, 122, 129]

Based on the eight studies, categorized as predicted risk factors in Table 7.0, the cumulative effect of traveling to cholera affected area on cholera outcome is 2.20 (1.57-3.08). The overall association, OR and 95% CI, is significant, which suggest that traveling to cholera affected area is a risk factor for cholera. The χ^2 test gives a p-value > 0.05 (p-

value = 0.09) and the I^2 test is 41.9%, which suggests that 41.9% of the variation in the studies is due to heterogeneity. As expected traveling to cholera affected area is a risk factor for cholera outcome.



Graph 9.11.0 Forest plot analysis of the effect of travelling to a cholera affected area (predicted risk factors) on the outcome cholera [Note: Studies with * are multivariate analysis]

9.11. Using utensils and cholera outcome

Four studies determine the association between use of utensils and cholera. Clean utensils are expected to be protective however using a communal bowl may be a risk factor [37]. In one study however, using a communal cup was not a risk factor. In the remaining three studies, using utensils is protective but using communal bowls is a risk factor.

Table 7.0. Association between using communal utensils and cholera outcome[+, **]

Study	Papers	Predicted Risk Factors	Predicted Protective Factors	Bias	OR univariate	95% CI	OR multivariate	95% CI
1	Rodrigues et al. 1997	use communal cup		L	0.90	0.4-1.9		
2	St. Louis et al. 1990		use utensils for noon meals	L	0.44	0.15-1.31		
3	Rodrigues et al. 1997	use communal bowl		L	1.10	0.7-1.6		
4	Rodrigues et al. 1997	someone eating from same bowl using their hands		L			2.09	1.02-4.30

[+] Please note the exposure is highlighted in pink when it is not as expected, ** Forest plot analysis not conducted on exposures with <4 studies [71, 134, 136]

Sensitivity analysis

Sensitivity analyses explores the robustness of the main results by repeating the analyses through changing the data sets included [76]. Sensitivity analysis allows assessment of data quality at the synthesis stage and gives a measure of how reliable the results are. For this thesis, sensitivity analysis was performed to allow for more homogeneous comparisons by excluding high bias studies and by grouping similar data from exposures with sufficient variable data sets.

High bias studies were dropped for all exposures. As can be seen in Table 8.0 dropping high bias studies changed the overall association for one exposure only – latrine availability (highlighted in red). The main overall association of having a latrine facility is (0.99; 0.73-1.34) which suggests that latrine facility is not a significant factor and not associated with cholera outcome. Dropping high bias studies changes the association to (1.24; 0.89-1.73) which suggests that a latrine facility is a risk factor for cholera outcome. However, both the results, with and without high bias are not significant. This is as expected since unsanitary toilets can be breeding grounds for bacteria [72].

Table 8.0. Results from dropping high bias studies to determine change in exposure-outcome relationship

Exposure	Risk Factors	Protective Factors	Overall Estimate and 95%CI	I^2 (%)	Overall Estimate and 95%CI (drop high bias)	I^2 (%)
1) Water Supply		Improved water source	0.90 (0.77–1.06)	88.9	0.63(0.52–0.77)	91.8
	Water contact		3.26 (2.84–3.74)	87.3	2.37 (1.97–2.85)	85.5
2) Water Treatment	Lack of water treatment		2.52 (2.10–3.03)	66.9	2.52 (2.10–3.03)	68.2
3) Water Storage		Safe water storage	0.65 (0.51–0.82)	55.3	0.71 (0.55–0.90)	39.4
4) Sanitation		Latrine facility present	0.99 (0.73–1.34)	83.6	1.24 (0.89–1.73)	83.6
	Inadequate/No toilet facility		4.72 (3.15–7.05)	74.7	5.26 (3.47–7.96)	73.8
5) Hygiene		Hand washing present	0.43 (0.38– 0.50)	54.9	0.43 (0.38–0.50)	54.9
	Lack of hand washing		2.58 (1.93–3.44)	55.8	2.50 (1.86–3.36)	58.4
6) Knowledge		Knowledge of cholera prevention	0.29 (0.23–0.38)	25.8	0.30 (0.23–0.39)	42.5
7) Food	Cold/left over food		1.55 (1.31–1.84)	58.4	1.55 (1.31–1.84)	58.2
	Eating out		2.01(1.71–2.36)	63.6	1.97 (1.68–2.31)	61.7
8) SES	High to middle		1.00 (0.99–1.00)	62.9	1.00 (0.99–1.00)	61.9
9) Other	Gender- females		1.92 (1.35–2.75)	0.00	1.90 (1.29–2.79)	0.00

Note: Latrine facility highlighted in red because of change in exposure-outcome relationship after dropping high bias studies

As seen in the exposure results section, summary results are given for each sub-category under every exposure. Similar data sets in summery results table were further stratified to better understand association with cholera

outcome. The additional sub-group analysis is performed on exposures that had sufficient variable data, which included: water supply and food.

Water supply: Improved drinking water

Improved drinking water included all types of improved sources. The overall association between all improved water sources and cholera outcome is (0.99; 0.77-1.06). Improved water sources were stratified into six studies on piped water, three studies on drinking water from tube well, six studies from tap water, two studies on water from borehole, three studies on drinking rainwater, and 13 on public municipal water source. Results of the sub-groups analysis conducted to determine the association between each type of improved source and cholera outcome is given below in Table 8.1. The association between these stratified studies and cholera was determine using forest plot analysis. However, as seen in Table 8.1 below, drinking rainwater and tube well were protective but rainwater has no heterogeneity and tube well has high heterogeniety. Stratification of improved water sources does not improve results or reduce heterogeneity between studies.

Table 8.1. The difference in association between types of stratified improved water sources and cholera outcome

Improved water source	Studies	Overall OR and 95%CI	χ^2 (p-value) and I^2 (%)	Heterogeneity
ALL	37	0.99; 0.77-1.06	χ^2 0.010 and I^2 test *64.4%*	Moderate
Piped	6	2.70 (1.83-4.00)	χ^2 <0.001 and I^2 test 85.0%	High
Tube well	3	0.28 (0.19-0.40)	χ^2 <0.001 and I^2 test 87.2%	High
Tap water	6	0.72 (0.51-1.02)	χ^2 <0.001 and I^2 test 96.0%	High
Borehole	2	0.43 (0.19-1.00)	χ^2 0.242 and I^2 test 26.8%	Moderate
Rainwater	3	0.39 (0.19-0.77)	χ^2 0.686 and I^2 test 0.0%	No
Well	3	0.91 (0.48-1.71)	χ^2 0.301 and I^2 test 16.6%	Low
Public/ municipal water	13	2.43 (1.68-3.52)	χ^2 <0.001 and I^2 test 80.0%	High

Food: Eating cooked protective food

Eating cooked protective food has an overall association of (0.86; 0.74-1.00). To make a more homogeneous comparison between eating cooked food and cholera outcome, cooked fish and shellfish were removed from the analysis because they are expected to be contaminated with the bacteria [22, 23]. As can be seen in Table 8.3 below, the cumulative effect of eating cooked food (without fish or shellfish included) and cholera outcome is (0.57; 0.48–0.67) and the χ^2 test has a low p-value of <0.001 and an I^2 test value of 76.4%. Compared to the association between all cooked food items and cholera and cooked food items without seafood and cholera, the

latter has a significantly protective association and lower heterogeneity. Removing seafood items allows for a more homogenous comparison between consuming cooked food and cholera outcome.

Table 8.2: The difference in association between all food and food without seafood and cholera outcome

Exposure	Protective Factors	Overall Estimate and 95%CI	χ^2 (p-value) and I^2 (%)	Heterogeneity
Food	Eating cooked food	0.86; 0.74-1.00	χ^2 <0.001 and I^2 test *87.1%*	High
	Cooked food without seafood	0.57; 0.48–0.67	χ^2 <0.001 and I^2 test 76.4%	High

Eating uncooked food (risk factors)

For a more homogeneous comparison, overall association between only uncooked fish and shellfish and cholera outcome was determined. As seen in Table 8.4 below, the overall association between uncooked seafood and cholera outcome is (1.93; 1.53–2.44). The χ^2 test has a low p-value of <0.001 and an I^2 test value of 62.6%. Compared to the overall association of eating all uncooked food items (predicted risk factors), the overall association of eating uncooked seafood (predicted risk factor) and cholera outcome is greater, and the heterogeneity is reduced.

Table 8.3 The difference in association between all uncooked food and uncooked seafood only and cholera outcome

Exposure	**Risk Factors**	**Overall Estimate and 95%CI**	**χ^2 (p-value) and I^2 (%)**	**Heterogeneity**
Food	Uncooked food	0.71; 0.57-0.88	χ^2 <0.001 and I^2 test 80.6%	High
	Uncooked seafood only	1.93;1.53–2.44	χ^2 <0.001 and I^2 test 62.6%	Moderate

Chapter 4 Discussion.

In this section, overall association between exposures and cholera outcome determined from forest plot analysis are summarized in Table 9.0 and described in detail. The effect of heterogeneity on the overall associations along with sensitivity analysis is also discussed. Limitations of the review are described in this section. Finally, the contribution of this review to the published literature is also presented here.

In Table 9 below the results from the forest plot analysis are compiled which include: list of actual risk and protective factors, as determine by the forest plot analysis, along with their overall estimates and heterogeneity. Risk factors significantly associated with the disease are highlighted in red and protective factors in dark blue if significant, and light blue if not significant. Heterogeneity is measured by the Higgin's criteria where, I^2 <25% is low (green), I^2 >50% is moderate (orange), I^2 >75% is high (dark red/brown) [57],

Table 9.0. Exposure results (overall associations) complied from forest plot analysis for each factor and cholera outcome

Exposure	Studies	Actual Protective Factor	Actual Risk Factor	Overall OR	Overall 95%CI	Heterogeneity	Description
1) Water Supply	7	1.1 Clean drinking water present		0.82	0.49-1.37	Moderate	Clean drinking water includes drinking bottled water or sachet water
	7		1.1 Lack of clean drinking water	4.41	2.85-6.80	No	Lack of clean drinking water includes drinking vended water, water in which hands had been introduced, water obtained outside the home, or at a funeral
	41	1.4 Improved Water Source		0.9	0.77-1.06	High	Drinking from piped water inside the users home, public taps, standpipes, tube wells, boreholes, protected dug wells, protected springs, aqueducts, municipal water, water from reservoir and rainwater collection
	28		1.4 Unimproved water source	3.72	3.11-4.45	Moderate	Drinking from unprotected, shallow or deep wells, water from lake, river, or stream, water from tanker
	23		1.5 Water contact	3.26	2.84-3.74	High	Contact with river, pond, lake water including bathing, cooking, washing mouth, washing utensils
2) Water Treatment	26	2.0 Water treatment present		0.46	0.38-0.56	Moderate	Treated water includes boiled water, water with chlorine present, household with chlorine present, household with other water treatment products observed. Studies that do not mention the exact method used to treat water are also included
	25		2.0 Lack of Water treatment	2.52	2.10-3.03	Moderate	Untreated water includes not boiling and not chlorinating water, untreated well, tap, piped, and rain water, and using mixed water (combination of treated and untreated water)
3) Water Storage	24	3.0 Water storage present		0.65	0.51-0.82	Moderate	Safe water storage includes plastic bottle, narrow mouth container, jerry can, bucket with lid, container that is covered/sealed, chlorine present in stored water, use of separate containers for drinking and washing, transporting and storing water via spout in vessel or pouring
	18		3.0 Unsafe/ No water storage	2.36	1.86-2.99	Low	Unsafe water storage includes bucket, open/unsealed container, presence of E.coli in stored water, container in which hands had been introduced, dirty container, shared water storage container with community, transporting and storing water via scooping water
4) Sanitation	8	4.1 Latrine facility present		0.99	0.73-1.34	High	
	8		4.1 Lack of latrine facility	4.72	3.15-7.05	Moderate	
	7		4.2 Open air defecation	2.55	1.82-3.57	Moderate	
	7		4.3 Lack of drainage	1.41	1.12-1.78	Moderate	Includes no flush system and pit toilets
5) Hygiene	44	5.0 Regular hand wash		0.43	0.38-0.50	Moderate	Safe practices include hand washing with soap and water, hand wash after visiting toilet, before eating meals, and before

							preparing food, presence of hand washing designated area, has soap, has hand washing buckets, Clorox present in the house
	12		5.0 Lack of hand wash	2.58	1.93-3.44	Moderate	Unsafe practices include no soap, no hand washing
6) Knowledge of cholera prevention	21	6.0 Have knowledge of cholera prevention		0.29	0.23-0.38	Moderate	Protective factors include going to a CTC or hospital when sick, having knowledge of disease transmission or receiving information on disease transmission and treatment from a church, TV, radio, pamphlet, health workers, town meeting, vaccinated, and receiving home treatment such as ORS
	4		6.0 No knowledge of cholera prevention	3.25	1.85-5.70	Low	Risk factors include no knowledge of the disease, transmission, and treatment methods
7) Food	44	7.1 Eating cooked food		0.86	0.74-1.00	High	Include all food items such as chicken, pork, cooked vegetables
	19		7.1 Cooked food	1.53	1.22-1.88	Moderate	Include fish and shellfish
	13	7.2 Uncooked food		0.71	0.57-0.88	High	Uncooked protective food include acidic foods such as lime, washing fruits and vegetables, eating fruits with skin such as bananas
	42		7.2 Uncooked food	1.27	1.11-1.45	Moderate	Uncooked food that may be risk factors include uncooked seafood, raw fruits and vegetables, unwashed vegetables
	36		7.3 Cold and left over food	1.55	1.31-1.84	Moderate	Cold/ left over food includes food left outside, cold sandwiches, cold rice, ice, ice-cream
	41		7.4 Eating out	2.01	1.71-2.36	Moderate	Eating out includes eating at a restaurant, street vended food and drinks, eating at parties, hotels
	9	7.5 Other food items		0.72	0.47-1.11	Moderate	Food items that may be protective include alcohol, high food diversity, meat and tomato sauce
	25	7.5 Other food items		0.79	0.65-0.95	Moderate	Food items that may be risk or protective factors include: alcohol, high food diversity, meat and tomato sauce, peanut sauce, milk and milk products, nuts
8) SES	36	8.0 High to middle		1.00	0.99-1.00		High SES includes formal residence, high to middle SES score, income, small family size (4-5 people), amenities and appliance, ownership of land
	33		8.0 Low	1.29	1.20-1.39	Moderate	Low SES includes informal residence and people in IDP-camps, low SES score, low income, large family size no appliances and amenities and ownership of pigs
9) Other	26		9.1. Knows/contact with a cholera patient	3.26	2.64-4.04	Moderate	Knowing or contact with a cholera patient is expected to be a risk factor for cholera
	19		9.2 Attending a function	1.62	1.29-2.02	Moderate	Attending a function includes funeral, gathering, fiesta, market place, ceremony, wedding
	6		9.3 Eating at a funeral	4.11	2.31-7.32	Low	

	8		9.7 Travel	2.20	1.57-3.08	Moderate	Travelling to cholera affected area is expected to be a risk factor
	5		9.8 Males	1.99	1.33-3.00	Moderate	Males and females are equally likely to have cholera
	5		9.8 Females	1.92	1.35-2.75	No	Males and females are equally likely to have cholera
	6		9.9 Age (5-18)	5.25	2.62-10.5	No	
	4		9.9 Age (>20)	2.13	1.09-4.15	No	

Color code:

Significant protective exposure
Insignificant protective exposure
Significant risk exposure
No association
No or Low heterogeneity
Moderate heterogeneity
High heterogeneity

Risk factors are exposures that are associated with developing cholera and are highlighted in red in Table 9.0 if the result of the association is significant. Risk factors for cholera: include inadequate water supply, treatment, and storage, lack of hygiene, poor sanitation, no knowledge of cholera prevention, eating food cooked and uncooked with seafood, eating cold left over food, eating out, and low SES. Other risk factors include knowing or contact with a cholera patient, travel to cholera affected area, attending a function, and eating at a funeral. As can be seen in Table 9.0, all risk factors are significantly associated with cholera. The consistency in the results suggests that these predicted exposures are risk factors for cholera outcome in all contexts that is in different populations and study locations.

Exposures associated with not developing cholera are protective factors and are highlighted in dark blue if the association is significant and in light blue if it is not significant. As listed in Table 9.0 below, factors significantly protective against the disease include presence of water treatment and storage, adequate hygiene, knowledge of cholera prevention, eating uncooked protective food, and eating at home. Factors protective against cholera but not significant include clean drinking water supply, improved water source, availability of latrine facility, eating cooked food, and other foods.

All protective factors, unlike risk factors, do not always have a significant association with the outcome of cholera. Insignificant results suggest that protective factors vary in different contexts. Clean drinking water and improved water sources do not have significant protective association with cholera. This is because although bottled water, sachet water and improved water sources such as piped, tube wells, tap water are categorized as safe, in many developing countries they do not guarantee microbial safety [65, 64]. Availability of latrine facility also has an insignificant association with cholera outcome. This is as expected because implementation of latrine facilities in some settings may be sources of microbial contamination and can aid the transmission of microbes [63, 72].

Heterogeneity

In a systematic review, variability between studies being compared is known as heterogeneity [77]. Heterogeneity can be clinical or methodological. As seen in Tale 9.0, exposures are categorized as having high (dark red/brown),

moderate (orange), or low (green) heterogeneity. Heterogeneity was determined using X^2 and I^2 test values generated by forest plots. Chi square test determines if the differences in effect estimates are due to chance alone [77]. For this review a low p-value <0.05 suggests significant difference between studies, beyond chance alone. Since there is always a degree of clinical and methodological heterogeneity between studies, I^2 test is used to quantify inconsistency between studies. As mention above percent variability between studies can be categorized as low, medium, or high however, the value of I^2 test depends on magnitude and direction of estimate effects and strength of evidence for heterogeneity given by 95% confidence intervals and X^2 test.

Clinical heterogeneity is the variability in cases and controls, exposures and outcomes studied. This review only studied one outcome, cholera, therefore clinical diversity is limited to differences in participants and exposures being study. The majority of the studies used the WHO definition (over 3 stools/ day) to define cases [36]. The majority of the cases were over five years of age and lived in the cholera affected area, controls were also from the same geographical region but did not have cholera symptoms. Although this was the standard definition used for majority of the participants, there were studies where cases and controls were not from the same populations and cases that only consisted of severe, fatal form of cholera. These discrepancies between participant selection can contribute to heterogeneity.

Exposures studied in the review vary, which also contributes to heterogeneity. For this review exposures defined in nine categories include 1) Water supply 2) Water treatment 3) Water storage 4) Sanitation 5) Hygiene 6) Knowledge of cholera prevention 7) Food 8) SES, and 9) Others. Each of the nine categories is divided into sub-categories and forest plot analysis was done on predicted protective and predicted risk factor for each sub category. However, there is degree of variability in the defined predicted protective and predicted risk exposures. For example, lack of sanitation is categorized as no latrine or inadequate latrine facility and there may be a degree of variability in how inadequate latrine facility is defined in each study.

Variability in study design and risk of bias is categorized as methodological heterogeneity [77]. Methodological heterogeneity for this review, does not pose a big problem because all the studies have the same study design; cumulative case control study design. However, variability in study design may exist because the analysis done to

determine the odds ratio, univariate, or multivariate differ. As mentioned before, 22 out of 77 studies conducted a univariate analysis and 55 out of 77 conducted a bivariate/ multivariate analysis. Using data determined from both the analysis (univariate and multivariate) may introduce heterogeneity. Risk of bias between studies being compared also varies, which can be source of heterogeneity.

To overcome the considerable level of heterogeneity and allow for more homogenous comparisons in the main results sensitivity analysis was performed. Analysis were re-run on studies after dropping high bias studies and by stratifying an exposure into further, more similar, categories. Dropping high bias studies only changed the association of one exposure, availability of latrine facility from 0.99 (0.73-1.34) to 1.24(0.89-1.73). Dropping high bias studies makes availability of latrine facility a risk factor for cholera. As mentioned above, this is not unexpected because in some settings latrine facilities may not be sanitary but instead a breeding ground for bacteria [72, 74].

Stratification of exposures was also done to better understand exposure-outcome relationship. Improved water source and cooked and uncooked food were stratified. As seen by the results in the sensitivity analysis section, only rainwater (0.39; 0.19-0.77) and tube well (0.28; 0.19-0.40) were improved sources that were protective after stratification. Eating cooked food without seafood (0.57; 0.48–0.67) was significantly protective as compared to cooked food that was protective 0.86; 0.74-1.00) but not significant. Uncooked seafood (1.93; 1.53–2.44) was a significant risk factor for cholera as compared to uncooked food that was protective (0.71; 0.57-0.88).

Limitations of the review

The limitations of this review include publication bias, which occurs when the available research is systematically different and unrepresentative of all the studies conducted in a field [78]. Out of the 77 included studies only 27 studies presented complete results. The majority of the studies did not report insignificant results. Funnel plots may be used to detect the level of publication bias. Funnel plots are scatter plots that determine publication bias, when there is no bias, studies are centered near the average and studies that have smaller effect size are equally distributed on either side [77]. When there is bias or when there is a systematic difference between studies with smaller effect size and those with larger effect estimates, the funnel plot is asymmetric.

Selection bias occurs when cases are not from the same population as the controls or when losses in one group (cases or controls) is more than the losses in the other group [47]. Selection of cases and controls may not be independent of exposure status, this may be the case since studies included are from areas with high rates of cholera, where controls may be asymptomatic cholera patients. This can lead to distortion of the true causal effect.

Systematic reviews can be conducted by more than one person, however this review was conducted by one person. Although this will result in more consistency in data selection and entry, predicted exposures may be misclassified in the summary results section resulting in inaccurate overall estimates.

For this review, 75 of the 77 studies included cases that were over five years of age even though prevalence is expected to be the highest in children under five [19]. This is because the WHO case definition for a cholera excludes children under five to maintain specificity since children under five experience diarrhea frequently and cholera like symptoms in children may be attributed to other forms of bacteria [37, 79]. In children over five and in adults other causes of diarrhea are not so common and these groups have higher specificity. Excluding children under five allows for higher specificity however, the results of this study may not extend to the under five age group

Contribution to literature

A systematic review determining the association of all exposures including water, sanitation, hygiene and additional exposures with cholera has not been performed before. This review allows policy makers to look at exposures associated with cholera in all contexts and on a global level. The review provides evidence suggesting that all risk factors must be controlled for in all contexts however, implementation of protective factors must be context specific.

Recommendation

This review allows the scientific community and policy makers to target exposures associated with cholera on a global scale. Cholera endemic countries can use the results from this paper to prevent the risks associated with

cholera in all contexts. Although in this paper all risk factors are significantly associated with cholera outcome, it is recommended that exposures with the strongest association be targeted first. These risk factors include: lack of latrine facility (4.72; 3.15-7.05), lack of clean drinking water (4.41; 2.85-6.80), and eating at a funeral (4.11; 2.31-7.32) however, simply providing clean drinking water (0.82; 0.49-1.37), and latrine facility (0.99; 0.73-1.34) is not protective as given by the insignificant association of these protective exposures with cholera. Sensitivity analysis further suggests that presence of latrine facility is also a risk factor (1.24; 0.89-1.73). Eating at a funeral may be addressed through improved funeral practices, however the exposure of eating cooked food alone (0.86; 0.74-1.00) is not significant.

Given these results, it is recommended that providing improved facilities is not always protective against the disease and much research and education is needed to change behavior such as funeral practices and to better implement and maintain the improved facilities. Educating different groups on the importance of water and sanitation will allow varying populations in different study locations to follow the same, more consistent standard of clean and improved water and sanitation facilities. As given in the results of this review, education on prevention of cholera is protective against the disease (0.29; 0.23-0.38). Even though all protective factors are not significantly associated with cholera, protective factors that are significantly associated with preventing the disease should be implemented in all cholera-affected areas. These exposures, which are protective in all contexts include: presence of water treatment (0.46; 0.38-0.56), safe water storage (0.65; 0.51-0.82), regular hand wash (0.43; 0.38-0.50), knowledge of cholera prevention (0.29; 0.23-0.38), and eating at home (0.33; 0.19-0.57).

It is recommended that for future research on this review, insignificant, unreported results be collected from the authors and included studies be weighted. Studies selected should be assigned weights, where studies that have appropriately matched the two groups or assessed the statistical difference between groups, and adjusted the results are weighted more than those that do not match groups and adjust results. Forest plot analysis on comprehensive data that has been weighted and includes all non significant results, will give a more accurate association between different exposures and cholera outcome and will allow the scientific community and policy makers to better interpret the results of the association of exposures with cholera.

References.

[1] Nasr-Azadani, F., Unnikrishnan, A., Akanda, A., Islam, S., Alam, M., Huq, A., … Colwell, R. (2015). Downscaling river discharge to assess the effects of climate change on cholera outbreaks in the Bengal Delta. Climate Research, 64(3), 257–274.

[2] Gaffga, N. H., Tauxe, R. V, & Mintz, E. D. (2007). Cholera: a new homeland in Africa? The American Journal of Tropical Medicine and Hygiene, 77(4), 705–13.

[3] Pollitzer, R., Swaroop, S., & Burrows, W. (1959). Cholera. Geneva : World Health Organization.

[4] Barua D., Burrows W., editors. Cholera. W. B. Saunders Company; Philadelphia, London, Toronto: 1974.

[5] J. N. Hays. (2005). Epidemics and Pandemics: Their Impacts on Human History. ABC-CLIO, Inc.

[6] Encyclopedia of plague and pestilence (1995). American Library Association.

[7] Cholera (pathology): Seven pandemics. Britannica Online Encyclopedia. Archived from the original on 2009-06-27. Retrieved 2014-06-29. Web. 15 December 2015.

[8] Guerrant, Richard L., and Nathan M. Thielman (1994). Vibrio Cholerae and Cholera: Molecular to Global Perspectives: Edited by I. K. Wachsmuth, Paul A. Blake and Orjan Olsvik. American Society of Microbiologists Press, Washington, D. C. 465 Pages. American Journal of Tropical Medicine and Hygiene 51.4: 512.

[9] Zhang, P., Li, F., Liang, W., Li, J., Kan, B., & Wang, D. (2014). The Seventh Pandemic Vibrio cholerae O1 El Tor Isolate in China Has Undergone Genetic Shifts. Journal of Clinical Microbiology, 52(3), 964–967.

[10] WHO | Global epidemics and impact of cholera. (2010). WHO. Retrieved from http://www.who.int/topics/cholera/impact/en/

[11] Colwell, R. R. (2004). Infectious disease and environment: cholera as a paradigm for waterborne disease. International Microbiology, 7(4), 285–289.

[12] WHO. (2015). Cholera 2014, Weekly epidemiological record (Vol. 40). Retrieved from http://www.who.int/wer

[13] Heidelberg, J. F., Eisen, J. A., Nelson, W. C., Clayton, R. A., Gwinn, M. L., Dodson, R. J., … Fraser, C. M. (2000). DNA sequence of both chromosomes of the cholera pathogen Vibrio cholerae. Nature, 406(6795), 477–83.

[14] Janda, J. M., Powers, C., Bryant, R. G., & Abbott, S. L. (1988). Current Perspectives on the Epidemiology and Pathogenesis of Clinically Significant Vibrio spp. Clinical Microbiology Reviews, 1(3), 245–267.

[15] Nair, G. B. (n.d.). Vibrio cholerae. WHO. Retrieved from http://www.who.int/water_sanitation_health/dwq/admicrob6.pdf

[16] Cash Richard A., Music Stanley I., Libonati Joseph P., Snyder Merrill J., Wenzel Richard P., & Hornick Richard B. (1974). Response of Man to Infection with Vibrio cholerae. I. Clinical, Serologic, and Bacteriologic Responses to a Known Inoculum. The Journal of Infectious Diseases, 129(1), 45–52.

[17] Levine, M. M., Kaper, J. B., Herrington, D., Losonsky, G., Morris, J. G., Clements, M. Lou, … Hall, R. (1988). Volunteer Studies of Deletion Mutants of Vibrio cholerae 01 Prepared by Recombinant Techniques. Infection and Immunity, 56(1), 161–167.

[18] 10 facts on cholera. (2014) WHO. 2014. Retrieved from http://www.who.int/features/factfiles/cholera/en/

[19] Ali, M., Lopez, A. L., Ae You, Y., Eun Kim, Y., Sah, B., Maskery, B., & Clemens, J. (2012). The global burden of cholera. Bulletin of the World Health Organization.

[20] Nygren, B. L., Blackstock, A. J., & Mintz, E. D. (2014). Cholera at the crossroads: the association between endemic cholera and national access to improved water sources and sanitation. The American Journal of Tropical Medicine and Hygiene, 91(5), 1023–8.

[21] Cholera - Vibrio Cholerae Infection. Sources of Infection & Risk Factors. (2014). CDC (2014). Web. 20 Sept. 2015.

[22] DePaola, A. (1981). Vibrio cholerae in marine foods and environmental waters: a literature review. Journal of Food Science 46: 66-70.

[23] Kaysner, C.A. and Hill, W.E. (1994). Toxigenic Vibrio cholerae O1 in food and water. In: Vibrio cholerae and Cholera: Molecular to Global Perspectives. Wachsmuth, K.I., Blake, P.A. and Olsvik, O., Eds. American Society for Microbiology. 27-39

[24] Sanitation and Hygiene: Why They Matter ? (n.d) World Bank Group. Retrieved from http://water.worldbank.org/shw-resource-guide/sanitation-and-hygiene-why-they-matter

[25] Reidl, J., & Klose, K. E. (2002). Vibrio cholerae and cholera: out of the water and into the host. FEMS Microbiology Reviews, 26(2), 125–139.
[26] Holmgren, J., & Svennerholm, A. M. (1977). Mechanisms of disease and immunity in cholera: a review. The Journal of Infectious Diseases, 136 Suppl, S105–12.
[27] Sack, D. A., Sack, R. B., Nair, G. B., & Siddique, A. K. (2004). Cholera. Lancet (London, England), 363(9404), 223–33.
[28] Huq, A., Colwell, R. R., Rahman, R., Alt, A., Chowdhury, M. A. R., Parveen, S., … Russek-Cohen3, A. E. (1990). Detection of Vibrio cholerae 01 in the Aquatic Environment by Fluorescent-Monoclonal Antibody and Culture Methods. Applied and Environmental Microbiology, 56(8), 2370–2373.
[29] Teschler, J. K., Zamorano-Sánchez, D., Utada, A. S., Warner, C. J. A., Wong, G. C. L., Linington, R. G., & Yildiz, F. H. (2015). Living in the matrix: assembly and control of Vibrio cholerae biofilms. Nature Reviews Microbiology, 13(5), 255–268.
[30] Colwell, R. R., Brayton, P. R., Grimes, D. J., Roszak, D. B., Huq, S. A., & Palmer, L. M. (1985). Viable but Non-Culturable Vibrio cholerae and Related Pathogens in the Environment: Implications for Release of Genetically Engineered Microorganisms. Bio/Technology, 3(9), 817–820.
[31] Xu Huai-Shu, Roberts N., Singleton F. L., Attwell R. W., Grimes D. J., & Colwell R. R. (1982). Survival and Viability of Nonculturable Escherichia coli and Vibrio cholerae in the Estuarine and Marine Environment. Microbial Ecology, 8(4), 313–323.
[32] Andoh, A., Fujiyama, Y., Sakumoto, H., Uchihara, H., Kimura, T., Koyama, S., & Bamba, T. (1998). Detection of complement C3 and factor B gene expression in normal colorectal mucosa, adenomas and carcinomas. Clinical and Experimental Immunology, 111(3), 477–83.
[33] Naseer, M., & Jamali, T. (2014). Epidemiology, determinants and dynamics of cholera in Pakistan: gaps and prospects for future research. Journal of the College of Physicians and Surgeons--Pakistan : JCPSP, 24(11), 855–60.
[34] Faruque, S. M., Naser, I. B., Islam, M. J., Faruque, A. S., Ghosh, A. N., Nair, G. B., . . . Mekalanos, J. J. (2005). Seasonal epidemics of cholera inversely correlate with the prevalence of environmental cholera phages. Proceedings of the National Academy of Sciences, 102(5), 1702-1707.
[35] Sanchez, J., & Holmgren, J. (2011). Cholera toxin - a foe & a friend. The Indian Journal of Medical Research, 133, 153–63.
[36] Deshpande, Abhishek. (2013). Acute Diarrhea. Disease Management. Cleveland Clinic Center for Continuing Education. Web. 8 Nov. 2015.
[37] WHO (2010). WHO | Cholera outbreak: assessing the outbreak response and improving preparedness. WHO. Global Task Force on Cholera Control.
[38] Rehydration Therapy (n.d). CDC. Retrieved from http://www.cdc.gov/cholera/treatment/rehydration-therapy.html
[39] Oral rehydration salts: Production of the new ORS. (2006). WHO/FCH/CAH/06.1. Geneva: WHO Press.
[40] WHO (2010). WHO position paper on Oral Rehydration Salts to reduce mortality from cholera. WHO Global Task Force on Cholera Control.
[41] Cholera - Vibrio cholerae infection: Zinc Treatment. (2014). CDC. Retrieved September 20, 2015, doi:http://www.cdc.gov/cholera/treatment/zinc-treatment.html
[42] Roy, S. K., Hossain, M. J., Khatun, W., Chakraborty, B., Chowdhury, S., Begum, A., … Chowdhury, R. (2008). Zinc supplementation in children with cholera in Bangladesh: randomised controlled trial. BMJ, 336(7638), 266–268.
[43] Hoque, K. M., Rajendran, V. M., & Binder, H. J. (2005). Zinc inhibits cAMP-stimulated Cl secretion via basolateral K-channel blockade in rat ileum. American Journal of Physiology. Gastrointestinal and Liver Physiology, 288(5), G956–63.
[44] Canani, R. B., Cirillo, P., Buccigrossi, V., Ruotolo, S., Passariello, A., De Luca, P., … Guarino, A. (2005). Zinc inhibits cholera toxin-induced, but not Escherichia coli heat-stable enterotoxin-induced, ion secretion in human enterocytes. The Journal of Infectious Diseases, 191(7), 1072–7.
[45] Woodin, Mark. (2014). Case Control Studies. CEE 154_Principles Epidemiology. Tufts University. Lecture.
[46] Helen Barratt, M. K. (2009). Case Control Studies: Design, Applications, Strengths & Weaknesses of Case-control Studies. Retrieved from http://www.healthknowledge.org.uk/public-health-textbook/research-methods/1a-epidemiology/cs-as-is/case-control-studies

[47] Szklo Moyses, & Nieto Javier. (2007). Epidemiology: Beyond the Basics (2nd ed.). Jones & Bartlett Learning.
[48] WHO. (2014). Cholera, Fact sheet N°107. Retrieved from http://www.who.int/mediacentre/factsheets/fs107/en/
[49] WHO. (2014). Cholera 2013, Weekly epidemiological record (Vol. 31). Retrieved from http://www.who.int/wer
[50] Laura Poppick. (2014). Deadly Cholera Outbreaks Could Increase with Climate Change. Retrieved from http://www.livescience.com/49152-cholera-outbreaks-climate-change.html
[51] White, H. (2009). International Initiative for Impact Evaluation Theory-Based Impact Evaluation: Principles and Practice. Journal of Development Effectiveness 1.3, 271–84.
[52] Travis Yates, Jelena Vujcic, Myriam Leandre Joseph, and Daniele Lantagne. (2015). 3ie Protocol: Short-term Hygiene Interventions in Emergency Response Situations: A Systematic Review and Impact Analysis
[53] Huang, X., Lin, J., & Demner-Fushman, D. (2006). Evaluation of PICO as a Knowledge Representation for Clinical Questions. AMIA Annual Symposium Proceedings, 2006, 359–363.
[54] Systematic Reviews: CRD's guidance for undertaking reviews in health care. (2009). Centre for Reviews and Dissemination
[55] Chandler, J., Churchill, R., Higgins, J., Lasserson, T., & Tovey, D. (2013). Methodological standards for the conduct of new Cochrane Intervention Reviews (Version 2.3).
[56] H. Waddington, B. Snilstveit, J. G. Hombrados, M. Vojtkova, J. Anderson, & H. White. (2012) Protocol: Farmer Field Schools for Improving Farming Practices and Farmer Outcomes in Low- and Middle-income Countries: A Systematic Review.
[57] Woodin, Mark. (2014). Tool to Assess Risk of Bias in Cohort Studies. CEE 154_Principles Epidemiology. Tufts University. Lecture.
[58] 11 Questions to Help You Make Sense of a Case Control Study. (n.d.). Retrieved September 20, 2015, from http://calder.med.miami.edu/portals/ebmfiles/UM CASP Case-Controls Assessment Tool.pdf
[59] Higgins, J. P. T., & Thompson, S. G. (2002). Quantifying heterogeneity in a meta-analysis. Statistics in Medicine, 21(11), 1539-1558.
[60] Five Basic Cholera Prevention Messages (n.d). CDC. Retrieved from http://www.cdc.gov/haiticholera/five_messages.htm
[61] Bauernfeind, A., Croisier, A., Fesselet, J., Herp, M., Saoût, E., Cluskey, J., & Tuynman, W. (2004). Cholera Guidelines (2nd ed.). Médecins Sans Frontières. doi:http://www.unicef.org/cholera/Chapter_2_basics/04_MSF_cholera_guidelines.pdf
[62] Improved and unimproved water sources and sanitation facilities. (n.d). WHO / UNICEF Joint Monitoring Program Retrieved from http://www.wssinfo.org/definitions-methods/watsan-categories/
[63] Shaheed, A., Orgill, J., Montgomery, M. A., Jeuland, M. A., & Brown, J. (2014). Why "improved" water sources are not always safe. Bulletin of the WHO, 92(4), 283–289. Retrieved from http://doi.org/10.2471/BLT.13.119594
[64] Bain, R., Wright, J., Yang, H., Pedley, S., Gundry, S. & Bartram, J. (2012) Improved but not necessarily safe: Water access and the Millennium Development Goals. GWF Discussion Paper 1225, Global Water Forum, Canberra, Australia.
[65] The Safe Water System: Safe Storage of Drinking Water.(n.d) Retrieved from http://www.cdc.gov/safewater/pdf/safestorage_2011-c.pdf
[66] WHO. (2012). Cholera 2011, Weekly epidemiological record (Vol. 31-32). Retrieved from http://www.who.int/wer
[67] The sanitation gap: Development's deadly menace. The progress of nations. (1997). UNICEF Retrieved from http://www.unicef.org/pon97/mainmenu.htm
[68] Rabbani, G. H., & Greenough, W. B. (1999). Food as a vehicle of transmission of cholera. Journal of Diarrhoeal Diseases Research, 17(1), 1–9.
[69] Teng Ph (1965) The role of foods in the transmission of cholera. IT: Proceedings of the Cholera Research Symposium, Honolulu, Hawaii, 24-29 Jan 1965. Washington, DC: U S Government Printing Office :328—32.
[70] Kolvin, J. L., & Roberts, D. (1982). Studies on the growth of vibrio cholerae biotype eltor and biotype classical in foods. Epidemiology and Infection, 89(2), 243-252.
[71] Rodrigues, A., Sandström, A., Cá, T., Steinsland, H., Jensen, H., & Aaby, P. (2000). Protection from cholera by adding lime juice to food - Results from community and laboratory studies in Guinea-Bissau, West Africa.

Tropical Medicine and International Health, 5(6), 418–422.
[72] St Louis, M. E., Porter, J. D., Helal, A., Drame, K., Hargrett-Bean, N., Wells, J. G., & Tauxe, R. V. (1990). Epidemic cholera in West Africa: The role of food handling and high-risk foods. American Journal of Epidemiology, 131(4), 719-728.
[73] Root, E. D., Rodd, J., Yunus, M., & Emch, M. (2013). The Role of Socioeconomic Status in Longitudinal Trends of Cholera in Matlab, Bangladesh, 1993-2007. PLoS Neglected Tropical Diseases, 7(1).
[74] Weil, A. A., Khan, A. I., Chowdhury, F., Larocque, R. C., Faruque, A. S. G., Ryan, E. T., … Harris, J. B. (2009). Clinical outcomes in household contacts of patients with cholera in Bangladesh. Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America, 49(10), 1473–1479.
[75] Gunnlaugsson, G., Einarsdóttir, J., Angulo, F. J., Mentambanar, S. A., Passa, A., & Tauxe, R. V. (1998). Funerals during the 1994 cholera epidemic in Guinea-Bissau, West Africa: the need for disinfection of bodies of persons dying of cholera. Epidemiology and Infection, 120(1), 7–15.
[76] Greenhouse JB, Iyengar S. (1994). Sensitivity analysis and diagnostics. In: Cooper H, Hedges LV, editors. The handbook of research synthesis. New York, NY: Russell Sage Foundation. p. 383-98.
[77] Higgins JPT, Green S (editors) (2011). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0. The Cochrane Collaboration
[78] Rothstein, H. R., Sutton, A. J., & Borenstein, M. (2006). Publication Bias in Meta-Analysis. In Publication Bias in Meta-Analysis: Prevention, Assessment and Adjustments (pp. 1–7). Retrived from http://doi.org/10.1002/0470870168.ch1
[79] Glossary of Terms Related to Cholera and Cholera Vaccine Programs. (2015). Johns Hopkins Bloomberg School of Public Health. Retrieved from https://www.stopcholera.org/sites/cholera/files/1.2_glossary_of_cholera_terms.pdf
[80] Lee, E. J., & Schwab, K. J. (2005). Deficiencies in drinking water distribution systems in developing countries. Journal of Water and Health.
[81] Grandesso, F., Allan, M., Jean-Simon, P. S. J., Boncy, J., Blake, A., Pierre, R. .& Luquero, F. J. (2014). Risk factors for cholera transmission in haiti during inter-peak periods: Insights to improve current control strategies from two case-control studies. Epidemiology and Infection, 142(8), 1625-1635.
[82] BBK Davies-Teye,Vantoo L, Yabani JB & Kwakye-Maclean C. (2014) Socio economic factors asociated with cholera outbreak in southern ghana, 2012: A case control study. The Journal of the International Society for Pharmacoeconomics and outcomes research. 17.3.
[83] Nguyen, V. D., Sreenivasan, N., Lam, E., Ayers, T., Kargbo, D., Dafae, F. . Brunkard, J. M. (2014). Cholera epidemic associated with consumption of unsafe drinking water and street-vended water--eastern freetown, sierra leone, 2012. The American Journal of Tropical Medicine and Hygiene, 90(3), 518-523.
[84] Morof, D., Cookson, S. T., Laver, S., Chirundu, D., Desai, S., Mathenge, P. . Handzel, T. (2013). Community mortality from cholera: Urban and rural districts in zimbabwe. The American Journal of Tropical Medicine and Hygiene, 88(4), 645-650.
[85] Rosewell, A., Addy, B., Komnapi, L., Makanda, F., Ropa, B., …… & MacIntyre, CR. (2012). Cholera risk factors, Papua New Guinea, 2010. BMC Infectious Diseases. 12.287.
[86] Cummings, M.J,Wamala JF., Eyura, M., Malimbo, M., Omeke, M.E., Mayer, D., & Lukwago, L. (2012;2011). A cholera outbreak among semi-nomadic pastoralists in northeastern uganda: Epidemiology and interventions. Epidemiology and Infection, 140(8), 1-10.
[87] Mahamud, A. S., Ahmed, J. A., Nyoka, R., Auko, E., Kahi, V., Ndirangu, J. . Eidex, R. B. (2012). Epidemic cholera in kakuma refugee camp, kenya, 2009: The importance of sanitation and soap. Journal of Infection in Developing Countries, 6(3), 234.
[88] O'Connor, K. A., Cartwright, E., Loharikar, A., Routh, J., Gaines, J., Fouché, M. B. . Mahon, B. E. (2011). Risk factors early in the 2010 cholera epidemic, haiti. Emerging Infectious Diseases, 17(11), 2136-2138.
[89] Dunkle, S. E., Mba-Jonas, A., Loharikar, A., Fouché, B., Peck, M., Ayers, T. . Quick, R. (2011). Epidemic cholera in a crowded urban environment, port-au-prince, haiti. Emerging Infectious Diseases, 17(11), 2143-2146.
[90] Jiménez, M. L., Apostolou, A., Suarez, A. J. P., Meyer, L., Hiciano, S., Newton, A. . Pimentel, R. (2011). Multinational cholera outbreak after wedding in the dominican republic. Emerging Infectious Diseases, 17(11), 2172-2174.
[91] Mukherjee, R., Halder, D., Saha, S., Shyamali, R., Subhranshu, C., Ramakrishnan, R. . Hutin, Y. J. (2011). Five pond-centred outbreaks of cholera in villages of west bengal, india: Evidence for focused interventions. Journal of Health, Population and Nutrition, 29(5), 421-428.

[92] Bhunia, R., & Ghosh, S. (2011). Waterborne cholera outbreak following cyclone aila in sundarban area of west bengal, india, 2009. Transactions of the Royal Society of Tropical Medicine and Hygiene, 105(4), 214-219.
[93] Das, A., Manickam, P., Hutin, Y., Pattanaik, B., Pal, B. B., Chhotray, G. P. . Gupte, M. D. (2009). Two sequential outbreaks in two villages illustrate the various modes of transmission of cholera. Epidemiology and Infection, 137(6), 906-912.
[94] Mridha, P., Biswas, A. K., Ramakrishnan, R., & Murhekar, M. V. (2011). The 2010 outbreak of cholera among workers of a jute mill in kolkata, west bengal, india. The Journal of Health, Population and Nutrition, 29(1), 9-13.
[95] Shikanga, O., Mutonga, D., Abade, M., Amwayi, S., Ope, M., Limo, H. . Feikin, D. R. (2009). High mortality in a cholera outbreak in western kenya after post-election violence in 2008. American Journal of Tropical Medicine and Hygiene, 81(6), 1085.
[96] Rahman, K. M., Duggal, P., Harris, J. B., Saha, S. K., Streatfield, P. K., Ryan, E. T. . LaRocque, R. C. (2009). Familial aggregation of vibrio cholerae-associated infection in matlab, bangladesh. Journal of Health, Population and Nutrition, 27(6), 733-738.
[97] Shultz, A., Omollo, J. O., Burke, H., Qassim, M., Ochieng, J. B., Weinberg, M. . Breiman, R. F. (2009). Cholera outbreak in kenyan refugee camp: Risk factors for illness and importance of sanitation. American Journal of Tropical Medicine and Hygiene, 80(4), 640.
[98] Sasaki, S., Suzuki, H., Igarashi, K., Tambatamba, B., & Mulenga, P. (2008). Spatial analysis of risk factor of cholera outbreak for 2003-2004 in a peri-urban area of lusaka, zambia. American Journal of Tropical Medicine and Hygiene, 79(3), 414.
[99] Von Seidlein, L., Wang, X., Macuamule, A., Mondlane, C., Puri, M., Hendriksen, I. . Lucas, M. (2008). Is HIV infection associated with an increased risk for cholera? findings from a case-control study in mozambique: HIV and cholera in mozambique. Tropical Medicine & International Health, 13(5), 683-688.
[100] Mugoya, I., Kariuki, S., Galgalo, T., Njuguna, C., Omollo, J., Njoroge, J. . Feikin, D. R. (2008). Rapid spread of vibrio cholerae O1 throughout kenya, 2005. American Journal of Tropical Medicine and Hygiene, 78(3), 527.
[101] Dubois, A. E., Sinkala, M., Kalluri, P., Makasa-Chikoya, M., & Quick, R. E. (2006). Epidemic cholera in urban zambia: Hand soap and dried fish as protective factors. Epidemiology and Infection, 134(6), 1226-1230.
[102] Siddiqui, F. J., Bhutto, N. S., von Seidlein, L., Khurram, I., Rasool, S., Ali, M. Bhutta, Z. A. (2006). Consecutive outbreaks of vibrio cholerae O139 and V. cholerae O1 cholera in a fishing village near karachi, pakistan. Transactions of the Royal Society of Tropical Medicine and Hygiene, 100(5), 476-482.
[103] Beatty, ME., Jack, t., Sivapalasingam S., Yao, SS., Paul, I., Bibb, B., Greene, KD., Kubota, K., Mintz, ED. & Brooks, JT. An outbreak of Vibrio Cholera O1 infections on Ebeye Islan, Republic of the Marshall Islands, Associated with Use of an Adequately chlorinated water source. Clinical Infectious Diseases. 38(1):1-9.
[104] Acosta, C. J., Galindo, C. M., Kimario, J., Senkoro, K., Urassa, H., Casals, C. . Alonso, P. L. (2001). Cholera outbreak in southern tanzania: Risk factors and patterns of transmission. Emerging Infectious Diseases, 7(3 Suppl), 583-587.
[105] Rodrigues, A., Sandström, A., Cá, T., Steinsland, H., Jensen, H., & Aaby, P. (2000). Protection from cholera by adding lime juice to food – results from community and laboratory studies in Guinea-Bissau, west africa. Tropical Medicine & International Health, 5(6), 418-422.
[106] Shapiro, R., Otieno, M., Adcock, P., Phillips-Howard, P., Hawley, W., Kumar, L. . Slutsker, L. (1999). Transmission of epidemic vibrio cholerae O1 in rural western kenya associated with drinking water from lake victoria: An environmental reservoir for cholera? American Journal of Tropical Medicine and Hygiene, 60(2), 271.
[107] Gunnlaugsson, G., Einarsdottir, J., Angulo, F.J., Mentambanar, S.A., Passa, A., & Tauxe, R.V. (1998). Funerals during the 1994 cholera epidemic in guinea-bissau, west africa: The need for disinfection of bodies of persons dying of cholera. Epidemiology and Infection, 120(1), 7-15.
[108] Swerdlow, DL., Malenga, G., Begkoyian, G., Nyanguliu, D., Toole, M., Waldman, RJ… & Tauxe, RV. (1997). Epidemic cholera among refugees in malawi, africa: Treatment and transmission. Epidemiology and Infection, 118(3), 207-214.
[109] Birmingham, M. E., Lee, L. A., Ndayimirije, N., Nkurikiye, S., Hersh, B. S., Wells, J. G., & Deming, M. S. (1997). Epidemic cholera in burundi: Patterns of transmission in the great rift valley lake region. The Lancet, 349(9057), 981-985.
[110] Koo, D., Aragon, A., Moscoso, V., Gudiel, M., Bietti, L., Carrillo, N. . Tauxe, R. V. (1996). Epidemic cholera in guatemala, 1993: Transmission of a newly introduced epidemic strain by street vendors. Epidemiology and Infection, 116(2), 121-126.

[111] Hoge, CW., Bodhidatta, l., Echecerria, P., Deesuwan, M., Kitporka, P. (1996). Epidemiologic Study of Vibrio cholerae 01 and 0139 in Thailand: At the Advancing Edge of the Eighth Pandemic. American Journal of Epidemiology. 143(3): 263-8.
[112] Eberhart-Phillips, J., Bessser, R. E., Tormey, M. P., Feikin, D., Araneta, M. R., Wells, J. . Mascola, L. (1996). An outbreak of cholera from food served on an international aircraft. Epidemiology and Infection, 116(1), 9-13.
[113] Quick, R. E., Thompson, B. L., Zuniga, A., Dominguez, G., De Brizuela, E. L., De Palma, O. . Blake, P. A. (1995) Epidemic cholera in rural el salvador: Risk factors in a region covered by a cholera prevention campaign. Epidemiology and Infection, 114(2), 249-255.
[114] Hatch, D. L., Waldman, R. J., Lungu, G. W., & Piri, C. (1994). Epidemic cholera during refugee resettlement in malawi. International Journal of Epidemiology, 23(6), 1292.
[115] Mujica, O. J., Quick, R. E., Palacios, A. M., Beingolea, L., Vargas, R., Moreno, D. . Tauxe, R. V. (1994). Epidemic cholera in the amazon: The role of produce in disease risk and prevention. The Journal of Infectious Diseases, 169(6), 1381-1384.
[116] Weber, JT., Mintz, ED., Canizares, R., Semiglia, A., Gomez, I., Sempertegui, R., Davila, A., Greene, KD., Puhr, ND., Cameron, Dn(1994). Epidemic cholera in Ecuador- Multidrug ressistance and Transmission by Water and Seafood. Epidemiology and Infection. 112(1): 1-11.
[117] Lim-Quizon, M. C., Benabaye, R. M., White, F. M., Dayrit, M. M., & White, M. E. (1994). Cholera in metropolitan Manila: foodborne transmission via street vendors. Bulletin of the World Health Organization, 72(5), 745–749.
[118] Quick, R. E., Vargas, R., Moreno, D., Mujica, O., Beingolea, L., Palacios, A. M. . Tauxe, R. V. (1993). Epidemic cholera in the amazon: The challenge of preventing death. American Journal of Tropical Medicine and Hygiene, 48(5), 597.
[119] Swerdlow, D. L., Greene, K. D., Tauxe, R. V., Wells, J. G., Bean, N. H., Ries, A. A. . Saldana, W. (1992). Waterborne transmission of epidemic cholera in trujillo, peru: Lessons for a continent at risk. The Lancet, 340(8810), 28-32.
[120] Moren, A., Stefanaggi, S., Antona, D., Bitar, D., Etchegorry, MG., Tchatchioka, M.,& Lungu G. (1991). Practical field epidemiology to investigate a cholera outbreak in a Mozambican refugee camp in Malawi, 1988. Journal of Tropical Medicine and Hygiene. (1):1-7.
[121] Ries, A. A., Vugia, D. J., Beingolea, L., Palacios, A. M., Vasquez, E., Wells, J. G. . Tauxe, R. V. (1992). Cholera in piura, peru: A modern urban epidemic. The Journal of Infectious Diseases, 166(6), 1429-1433.
[122] Ujjiga, T. T. A., Wamala, J. F., Mogga, J. J. H., Othwonh, T. O., Mutonga, D., Kone-Coulibaly, A. . Rumunu, J. (2015). Risk factors for sustained cholera transmission, juba county, south sudan, 2014. Emerging Infectious Diseases, 21(10), 1849-1852.
[123] Fredrick, T., Ponnaiah, M., Murhekar, M. V., Jayaraman, Y., David, J. K., Vadivoo, S., & Joshua, V. (2015). Cholera outbreak linked with lack of safe water supply following a tropical cyclone in pondicherry, india, 2012. Journal of Health, Population, and Nutrition, 33(1), 31.
[124] Fatiregun, A. A., Ajayi, I. O., & Isere, E. E. (2013). Cholera outbreak in a southwest community of nigeria: Investigation of risk factors and evaluation of a district surveillance system. West African Journal of Medicine, 32(3), 173.
[125] Opare, J., Ohuabunwo, C., Afari, E., Wurapa, F., Sackey, S., Der, J. . Odei, E. (2012). Outbreak of cholera in the east akim municipality of ghana following unhygienic practices by small-scale gold miners, november 2010. Ghana Medical Journal, 46(3), 116.
[126] Kone-Coulibaly, A., Tshimanga, M., Shambira, G., Gombe, N. T., Chadambuka, A., Chonzi, P., & Mungofa, S. (2010). Risk factors associated with cholera in harare city, zimbabwe, 2008. East African Journal of Public Health, 7(4), 311.
[127] Das, A., Manickam, P., Hutin, Y., Pal, B. B., Chhotray, G. P., Kar, S. K., & Gupte, M. D. (2009). An outbreak of cholera associated with an unprotected well in parbatia, orissa, eastern india. Journal of Health, Population and Nutrition, 27(5), 646-651.
[128] Bhunia, R., Ramakrishnan, R., Hutin, Y., & Gupte, M. D. (2009). Cholera outbreak secondary to contaminated pipe water in an urban area, west bengal, india, 2006. Indian Journal of Gastroenterology : Official Journal of the Indian Society of Gastroenterology, 28(2), 62-64.
[129] Kur, L., Mounir, C., Lagu, J., Muita, M., Rumunu, J., Ochieng, B. . Centers for Disease Control and Prevention (CDC). (2009). Cholera outbreak — southern sudan, 2007. Morbidity and Mortality Weekly Report, 58(13), 337-341.

[130] Swaddiwudhipong, W., Ngamsaithong, C., Peanumlom, P., & Hannarong, S. (2008). An outbreak of cholera among migrants living in a thai-myanmar border area. Journal of the Medical Association of Thailand = Chotmaihet Thangphaet, 91(9), 1433.
[131] Kirk, M. D., Kiedrzynski, T., Johnson, E., Elymore, A., & Wainiqolo, I. (2005). Risk factors for cholera in pohnpei during an outbreak in 2000: Lessons for pacific countries and territories. Pacific Health Dialog, 12(2), 17.
[132] Hutin, Y., Luby, S., & Paquet, C. (2003). A large cholera outbreak in kano city, nigeria: The importance of hand washing with soap and the danger of street-vended water. Journal of Water and Health, 1(1), 45.
[133] Gunnlaugsson, G., Angulo, F. J., Einarsdóttir, J., Passa, A., & Tauxe, R. V. (2000). Epidemic cholera in guinea-bissau: The challenge of preventing deaths in rural west africa. International Journal of Infectious Diseases, 4(1), 8-13.
[134] Rodrigues, A., Brun, H., & Sandstrom, A. (1997). Risk factors for cholera infection in the initial phase of an epidemic in guinea-bissau: Protection by lime juice. American Journal of Tropical Medicine and Hygiene, 57(5), 601-604.
[135] Cárdenas, V., Saad, C., Varona, M., & Linero, M. (1993). Waterborne cholera in riohacha, Colombia (1992). Bulletin of the Pan American Health Organization, 27(4), 313-330.
[136] St Louis, M. E., Porter, J. D., Helal, A., Drame, K., Hargrett-Bean, N., Wells, J. G., & Tauxe, R. V. (1990). Epidemic cholera in west africa: The role of food handling and high-risk foods. American Journal of Epidemiology, 131(4), 719-728.
[137] Clemens, J. D., Sack, D. A., Harris, J. R., Khan, M. R., Chakraborty, J., Chowdhury, S. . Yunus, M. (1990). Breast feeding and the risk of severe cholera in rural bangladeshi children. American Journal of Epidemiology, 131(3), 400.
[138] Lowry, P. W., Pavia, A. T., McFarland, L. M., Peltier, B. H., Barrett, T. J., Bradford, H. B. . Blake, P. A. (1989).
[139] Cholera in louisiana: Widening spectrum of seafood vehicles. Archives of Internal Medicine, 149(9), 2079-2084.
[140] Killewo, J. Z., Amsi, D. M., & Mhalu, F. S. (1989). An investigation of a cholera epidemic in butiama village of the mara region, tanzania. Journal of Diarrhoeal Diseases Research, 7(1-2), 13.
[141] Rosewell, A., Dagina, R., Murhekar, M., Ropa, B., Posanai, E., Dutta, S. R. . MacIntyre, C. R. (2011). Vibrio cholerae O1 in 2 coastal villages, papua new guinea. Emerging Infectious Diseases, 17(1), 154-156.
[142] Reller, M., Mong, Y., Hoekstra, R., & Quick, R. (2001). Cholera prevention with traditional and novel water treatment methods: An outbreak investigation in fort-dauphin, madagascar. American Journal of Public Health, 91(10), 1608-1610.
[143] Izadi, S., Tabatabaei, S-M., Miradi, MR. & Sheikhzadeh K. (2005). Routes of Transmission of Cholera in the Border Areas of Zahedan District, Sistan and Baluchestan Province, Summer 2003. Journal of Medical Science. 5(4): 233-238.
[144] Izadi, S., Shakeri, H., roham, P. & Sheikhzadeh K. (2006). Cholera Outbreak in Southeast of Iran: Routes of Transmission in the situation of good primary helth care Services and poor individual hygenic Practices. Japanese journal of infectious diseases. 59(3): 174-8.