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IDENTIFICATION AND MAPPING OF RISK FACTORS ASSOCIATED WITH CHOLERA IN SELECTED COMMUNITIES IN THE GREATER ACCRA REGION, GHANA

BY

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DECLARATION

I, **ANTHONY DONGDEM** hereby declare that apart from the references cited to other people's work which is duly acknowledged, this research work is the result of my own research work conducted under the supervision and has never on any previous occasion been submitted in part or whole to any Institution or Board for the award of any degree.

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DEDICATION

This work is dedicated to my wife Rita, children Maximilian, Krispin, Elsa-Keziah, Jaiden, mother Philomena, and late father Mr. Luke Dongdem.

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ABSTRACT

Background

Cholera is an acute diarrhoeal disease caused by the toxigenic *Vibrio cholerae* O1 or O139 strains. Globally, it is estimated to infect over 1.3 million people with over 21, 000 deaths annually with the most affected countries being in Africa and Asia. In Ghana, cholera has become endemic in some communities in the Greater Accra Region (GAR) with reported focal epidemics. The factors contributing to its persistence and spread in these communities are not well understood. To prevent future epidemics, it is important to identify the specific risks in these communities that may account for its persistence. This study, therefore, identified and mapped risk factors associated with cholera at the household and community levels in the GAR.

Methods

A community-based cross-sectional comparative study was conducted in 24 (12 each) from cholera endemic and non-endemic communities from March 2019 to March 2020. The multistage cluster sampling procedure was used in selecting households and a structured questionnaire was used to collect data from the head of households. Drinking water from selected households was sampled for water quality and the community water bodies were assessed for the presence of toxigenic strains of *V. cholerae* during the wet and dry seasons. A sanitation inspection guide was used to assess the sanitation conditions of the communities and GPS coordinates of the identified risk taken. Data were analysed using STATA version 14 software. Associations between the history of cholera and the independent variables were determined using Chi-square/Fisher exact test and multivariable penalized logistic regression. The differences in bacterial counts were determined using the Kruskal Wallis rank test. A p-value less than 0.05 was considered significant for the associations. Principal Component Analysis was used to categorize the

household wealth index and the environmental sanitation conditions. Significantly identified risk factors were mapped with the historical cholera cases using Arc GIS.

Results

Results of the multivariable penalized logistic regression showed that the presence of waste dumpsites (AOR=2.96, 95% CI: 1.11-7.88, p=0.030) and big open drainage (AOR=5.78, 95% CI: 1.89-17.72, p=0.002) were predictors of cholera occurrence. Whereas cooking in a detached kitchen (AOR=0.22, 95% CI: 0.06-0.82. P=0.024), or in yard (AOR=0.42, 95% CI: 0.21-0.86, p=0.017), and the availability of public toilets in the neighbourhood (AOR=0.28, 95% CI: 0.08-0.97, p=0.046) were significantly protective against cholera. The household drinking water was mostly contaminated with faecal coliforms with counts exceeding the zero cfu/100ml standard. *Klebsiella penumoniae* and *Escherichia coli* were the dominant organisms isolated. The household stored water was more contaminated than that obtained from the direct source and the differences were significant. The contamination was generally higher in the wet season than in the dry season. No toxigenic *Vibrio cholerae* O1 or O139 was isolated from the household drinking water and the environmental water bodies. However, *Vibrio fluvialis* and *Vibrio algionolyticus* were isolated from the environmental water bodies. Environmental sanitation revealed 66.2% (300/453) of the endemic communities to have poor sanitation compared to the 67.5% (306/453) of the non-endemic communities with good sanitation conditions.

Conclusion/Recommendation

The presence of public toilets, waste dumpsites, and open drainage systems close to each other and their combined effect may account for the cholera endemicity in the endemic communities. Household drinking water contaminated with faecal coliforms is an indicator of high risk for cholera transmission. The absence of toxigenic strains of *Vibrio cholerae*

O1 or O139 in the drinking and environmental water bodies could predict the absence of cholera occurrence. *Vibrio fluvialis* and *Vibrio algionolyticus* emerging cholera-like diarrhoea pathogens were isolated from the environmental water bodies. Poor environmental sanitation is still prevalent in the endemic communities. It is recommended that sanitation laws and the treatment of household drinking water at the point-of-used as well as siting of public toilets, waste dumpsites, and open drainages beyond 500 m from households are enforced.

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µl	microlitres
µm	micron
ABC	Ablekuma Central
ABW	Ablekuma West
ACC	Accra Metropolitan
ACP	Accuracy Check Point
ALN	Ablekuma North
AMA	Accra Metropolitan Assembly
ANOVA	Analysis of Variance
AOR	Adjusted Odd Ratio
APHA	American Public Health Association
API 20E	Antigenic Profile Index for 20 Enterobacteriaceae
ASH	Ashiaman
AYW	Ayawaso West
cAMP	Cyclic adenosine 5'-monophosphate
CFR	Case Fatality Rate
cfu	Colony-Forming Unit
CHPs	Community-based Health Planning Services
CLTS	Community-Led Total Sanitation
ctx	Cholera toxin complex
DHS	Demographic and Health Survey
EDTA	Ethylenediaminetetraacetic acid

EMB	Eosin Methylene Blue Agar
FC	Faecal Coliform Bacteria
FCC	Faecal Coliform Count
GAR	Greater Accra Region
GHS	Ghana Health Service
GHS-ERC	Ghana Health Service Ethical Review Committee
GIS	Geographic Information System
GPS	Geographic Positioning System
GSS	Ghana Statistical Service
GTFCC	Global Task Force on Cholera Control
HIV	Human Immunodeficiency Virus
HlyA	Haemolysin
IHR	International Health Regulations
IND	Indole
KKT	Korle Klottey
KVIP	Kumasi Ventilated Improved Pit latrine
LDK	La Dade Kotopon
LEKMA	Ledzokuku Krowor Municipal
m	metres
MAC	MacConkey Agar
ml	millilitre
^{o}C	Degree Celsius
OCV	Oral Cholera Vaccines
OKN	Okaikoi North

OR	Odds Ratio
ORS	Oral Rehydration Salt
PBS	Phosphate Buffered Saline
PC	Plate Count Agar
PCA	Principal Components Analysis
PCR	Polymerase Chain Reaction
PI	Principal Investigator
RDTs	Rapid Diagnosis Tests
RDTs	Rapid Diagnostic Tests
RTA	Repeat in toxin
SDG	Sustainable Development Goal
SES	Socioeconomic Status
SSA	Sub Saharan Africa
stn	Heat-stable enterotoxin
TC	Total Coliform Bacteria
TCBS	Thiosulfate-Citrate-Bile-Sucrose Agar
TCC	Total Coliform Count
tcp	Toxin co-regulator
TDA	Tryptophan deaminase
TSA	Tryptone Soy Agar
TSI	Triple Sugar Iron Agar
TTC	Thermotolerant Coliform
TTSS	Type 3 Secretion System
UNICEF	United Nations Children Fund

USA	United States of America
USD	United States Dollars
VC	Vibrio Count
VIFs	Variance inflation factors
VP	Acetoin
VRB	Violet Red Bile Agar
WASH	Water, Sanitation and Hygiene
WC	Water Closet
WGB	Weija Gbawe
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background

1.1.1 Epidemiology

Cholera is an acute diarrheal infection caused by the bacterium *Vibrio cholerae*, which causes disease exclusively in humans. The bacterium naturally inhabits aquatic water sources such as brackish riverine, estuarine, and coastal waters (Almagro-Moreno & Taylor, 2014). The survival and multiplication of the bacterium in the aquatic environment are influenced by both biotic and abiotic factors (de Magny et al., 2011; Lutz, Erken, Noorian, Sun, & McDougald, 2013). The abiotic factors include; pH, temperature, salinity, and organic nutrients in the water that helps the zooplankton and phytoplankton to bloom with increasing concentration of *V. cholerae* O1 (Hug, Small, West, & Colwell, 1984). This makes it possible to make predictions of a likely cholera outbreak by monitoring these abiotic factors in the aquatic environment (Jutla et al., 2013). Nonetheless, cholera is difficult to eradicate because of the presence of the *V. cholerae* within and outside of the aquatic water environment but can be prevented by controlling exposure to the bacterium from the environment.

There are over 200 serogroups of *V. cholerae*, with only the serogroups O1 and O139 being responsible for cholera epidemics (Islam et al., 2013). The *V. cholerae* O1 is responsible for most of the cholera cases globally with O139 restricted to India and Bangladesh (Mukhopadhyay, Takeda, & Nair, 2014). The *V. cholerae* O1 strains are classified based on biochemical characteristics into classical and El Tor. The classical biotype was responsible for the sixth cholera pandemic, while the El Tor biotype is the causative agent of the current seventh pandemic (Hu et al., 2016).

Cholera is an ancient disease that has plagued every continent of the world in the past resulting in the deaths of hundreds and thousands of people, and remains a major public health problem in developing countries especially in parts of Asia, Africa, and Latin America where the basic amenities such as safe water and sanitation are still lacking or have been disrupted by war, civil conflicts or natural disasters such as earthquakes and floods (WHO, 2010, 2017b).

Globally it is estimated that 1.3 to 4.0 million cholera cases and 21, 000 – 143, 000 deaths occur annually with the majority of these deaths occurring in Africa (Ali, Nelson, Lopez, & Sack, 2015). In the African continent, cholera cases are not equally distributed in all the regions. The incidences are higher in Sub-Saharan Africa (SSA) and in the Great Lake regions where frequent outbreaks continue to occur (Mengel, Delrieu, Heyerdahl, & Gessner, 2014). In the West African Coast (i.e stretching from Benin to Mauritania) from 2009 to 2015, at least 54% of the reported cases were among populations living in three urban cities (i.e Accra, Freetown, and Conakry) (Moore et al., 2018). Of the reported cases, Ghana alone contributed 52.4% (51,333 out of 97,887 of all the suspected cases) within the same period as reported by Moore et al. (2018) in the WHO, Health Observatory Data Repository. The annual cholera cases in the country have also been estimated to be 41,732 with an average case fatality of 3.8% (Ali et al., 2015). The coastal regions (Greater Accra, Volta, Central, and Western) and Eastern region are mostly affected (Ghana Health Service, 2015).

1.1.2 Clinical Presentation, diagnosis, and management

Cholera is contracted by ingesting food or water contaminated with the toxigenic *V. cholerae* O1 or O139. On ingestion, the bacterium colonizes the small intestine and releases the cholera toxin which binds the epithelial cells and induces excessive secretion of water and electrolytes into the lumen of the small intestines, resulting in diarrhoea.

Although the infection is often mild or asymptomatic in most people, those infected may shed the bacteria in their faeces for 1 to 10 days (WHO, 2019). In severe cases, patients present with profuse watery diarrhoea and vomiting. If left untreated, severe cases result in massive loss of bodily fluids and lead to dehydration, hypovolemic shock, and death. The incubation period is short from few hours to 5 days after ingestion of food or water contaminated with the bacterium and thus can kill rapidly (Azman, Rudolph, Cummings, & Lessler, 2013). Cholera can spread rapidly among children and adults (WHO, 2019).

To diagnose quickly to prevent spread, a rapid diagnostic test (RDTs) is used. However, confirmation of the diagnosis is done by the isolation of the bacterium from the stool of infected persons (GTFCC, 2017). Treatment of cholera is usually by the replacement of the electrolytes loss, through rehydration fluid such as oral rehydration salt, and in severe cases by intravenous rehydration with the appropriate antibiotics to reduce the volume of diarrhoea and bacterial shedding in their stool (WHO, 2018a). Without prompt and appropriate management case-fatality rates can rise to 50% (GTFCC, 2017).

1.1.3 Cholera Risk Factors

Although cholera is transmitted through ingestion of food or water contaminated with toxigenic *Vibrio cholerae* O1 or O139, several demographics, socioeconomic, environmental, and climatic risk factors also promote the spread of the disease (Bwire et al., 2017; Leckebusch & Abdussalam, 2015; Stoltzfus et al., 2014). However, the specific mechanism and factors involved in its persistence and spread may vary from one area to another.

Accra Metropolitan Assembly, Ledzokuku, and Krowo Municipal Assemblies in the Greater Accra Region are highly vulnerable to cholera because they are the most urbanized and highly commercialized assemblies in the region with traders and civil workers across the country visiting on daily basis (Ghana Statistical Service, 2013). The capital city of the country, Accra which is located in the Accra Metropolis is also an important city as it links major cities within West Africa. There is therefore an influx of people every day to the city putting pressure on the existing sanitation facilities and thus increasing the risk of cholera. Accra is also a coastal town with fisher folks fishing along the West African Coast. The long days they spend and the unhygienic conditions they face while at sea also pose a risk to cholera (Khonje et al., 2012; Ohene-Adjei et al., 2017). There are also rivers passing through the city along with some crowded communities. These rivers are contaminated with human excreta and sewage due to a lack of access to toilet facilities in the households (Ghana Statistical Service, 2013). However, during times of water shortages, such polluted water bodies are used for household activities (Ghana Statistical Service, 2013).

There are slums and squatter settlements within the city. The inhabitants in these settlements are less privileged and live in advert poverty including having poor access to portable water and sanitation (Accra Metropolitan Assembly and UN Habitat, 2011). Most of these slum communities are mostly located in low-lying areas that are prone to flooding. There is indiscriminate dumping of waste especially the non-biodegradable waste in the urban communities (Yoada, Chirawurah, & Adongo, 2014). This waste is washed by rain waters to choke the drains or gutters. Heavy rains may aggravate the situation with flood waters that may contaminate broken pipelines conveying water to the households and increases the risk of cholera. To control cholera in the Greater Accra Region, it is important to identify the cholera 'hotspots' and to investigate the specific risks to prevent recurrence of cholera.

1.1.4 Spatial Distribution

The potential risk factors of cholera have been well demonstrated by spatial epidemiology in many studies (Olanrewaju & Adepoju, 2017; Osei, Duker, & Stein, 2012; Osei & Stein, 2018). Spatial epidemiology allows data to be collected to show the spatial distribution of disease incidences and their relationship with the risk factors. This was first demonstrated by John Snow during the London cholera epidemic in 1855. Snow was able to map the location of cholera cases which he traced to the water source (Tulchinsky, 2018). With the current advancement in technology, Geographical Information System (GIS) is used not only to ensure accurate mapping of locations but also to provide the application in studying the correlation between the identified risk (Ngwa et al., 2016; Olanrewaju & Adepoju, 2017; Osei & Stein, 2018). By mapping disease in geographical space, local authorities will be able to easily identify risk factors, their distribution, and spread in the area to plan interventions.

1.2 Problem Statement

In Ghana, cholera has become endemic since the first case was reported in 1970 with focal epidemics occurring every 4 to 6 years (Dzotsi, Odoom, Opare, & Davies-Teye, 2016; Ofori-Adjei & Koram, 2014) (Figure 1.1). From 1970 to 2011, Ghana cumulatively reported 128, 525 cholera cases and 6,400 deaths with a case fatality rate (CFR) of 4.98 (Mengel et al., 2014). Despite the decline in the trend of cases from 1991 to 2010, large outbreaks occurred in 2011, 2012, and 2014 (Figure 1.1) (Ghana Disease Surveillance Department, 2016). In 2014, the country recorded the worst outbreak after 30 years with over 28,000 cases and 240 deaths (Ghana Health Service, 2015).

In recent years the epidemics have become more frequent and unpredictable with outbreaks occurring from 2010 to 2012 and 2014 to 2016. Also, complete lull periods in 2013, 2017 to 2020 were experienced with no confirmed case reported despite the ongoing surveillance. Most cholera epidemics in Ghana occur along the four coastal regions (i.e Western, Central, Volta, and Greater Accra) (Ghana Health Service, 2015). However, it is more prevalent in communities in the Greater Accra Region where there are crowded living conditions, poor sanitation, erratic clean water supply, inadequate food safety, and hygiene practices (Mireku-Gyimah, Apanga, & Awoonor-Williams, 2018). Outbreaks often start in such communities and quickly spread with heavy rains and flooding to the rest of the metropolis, eventually reaching the other parts of the country by population movement (Ghana Disease Surveillance Department, 2016; UNICEF, 2013). Twelve (12) of these cholera endemic communities described as 'cholera hotspots' are located in the Accra Metropolis (Adabraka, Agbogbloshie, Agege, Chorkor, Dansoman, James Town, Kaneshie, Maamobi, Mamprobi, Nima), Ledzokuku Municipal Assembly (Teshie) and Krowor Municipal Assembly (Nungua).

These communities have persistently reported the highest numbers of cases with high attack rates for the past four years (2011 to 2014) (Disease Surveillance Department, 2015). Despite the persistence of cholera in these communities, the underlying factors behind its endemicity and spread remain unclear. Although cholera transmission is through the consumption of food or water contaminated with toxigenic *Vibrio cholerae* O1 or O139, several demographics, socioeconomic, environmental, and climatic risk factors are known to promote the spread of the disease (Bwire et al., 2017; Leckebusch & Abdussalam, 2015; Stoltzfus et al., 2014).

This study assessed the behavioural, socioeconomic, bacteriological water quality, and environmental factors in the cholera endemic communities (Agbogbloshie , James Town, Adabraka, Kaneshie, Maamobi, Nima, Chorkor, Mamprobi, Agege, Dansoman, Teshie, Nungua) and non-endemic communities (Dawhenya North, Kofikope, Awhiam, Dawa, Addokope, Dogobom, Anyaman East, Adjumanikope, Adedetsekope, Angomya-Ada, Kophemm Kasseh, Asigbekope) to identify amendable risk factors to give up to date and relevant information for policy makers to appropriately plan and allocate resources to control the disease.

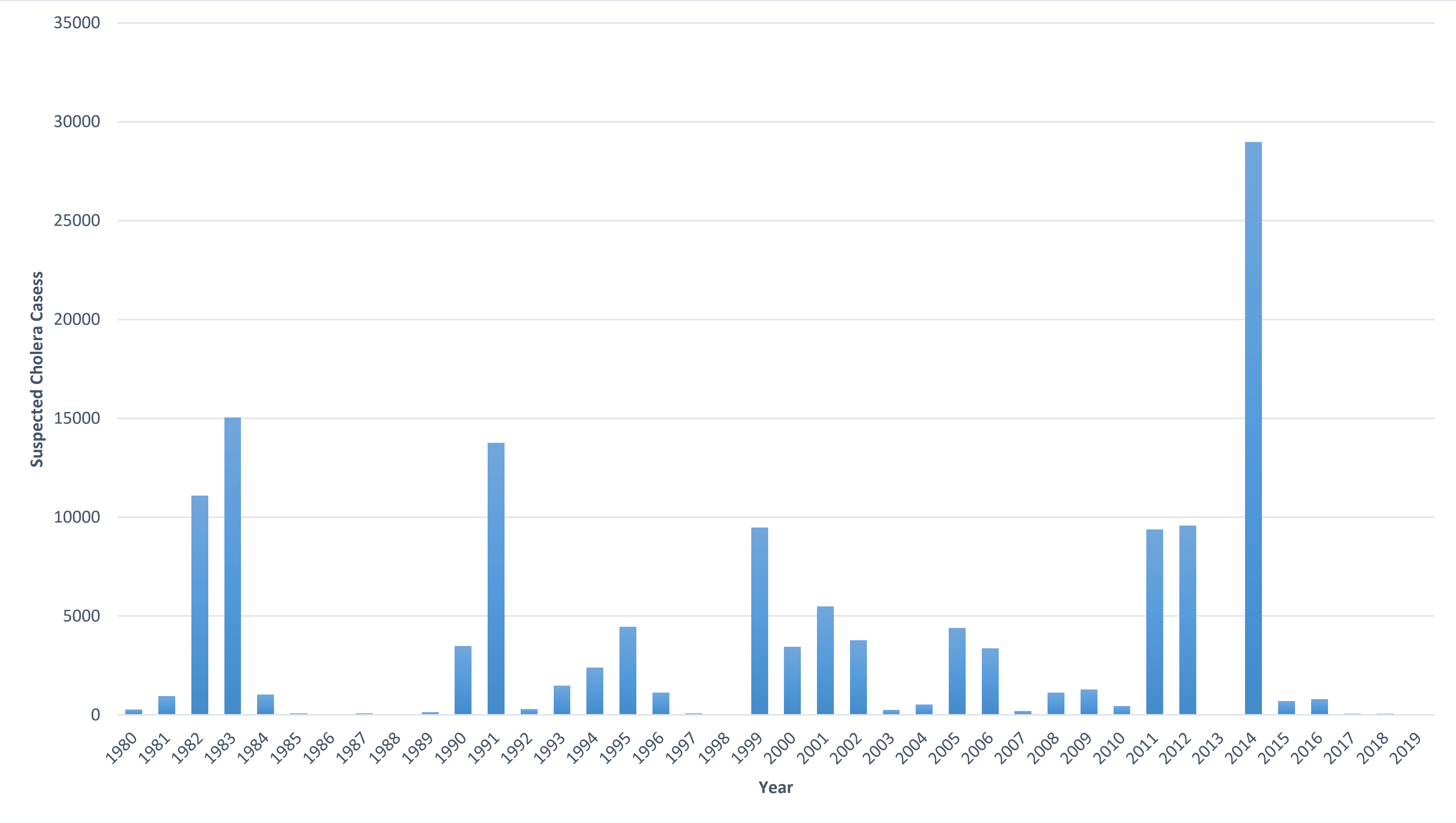

Figure 1.1: Annual cholera cases reported in Ghana according to the Disease Surveillance department records (1980 to 2019)

1.3 Conceptual Framework

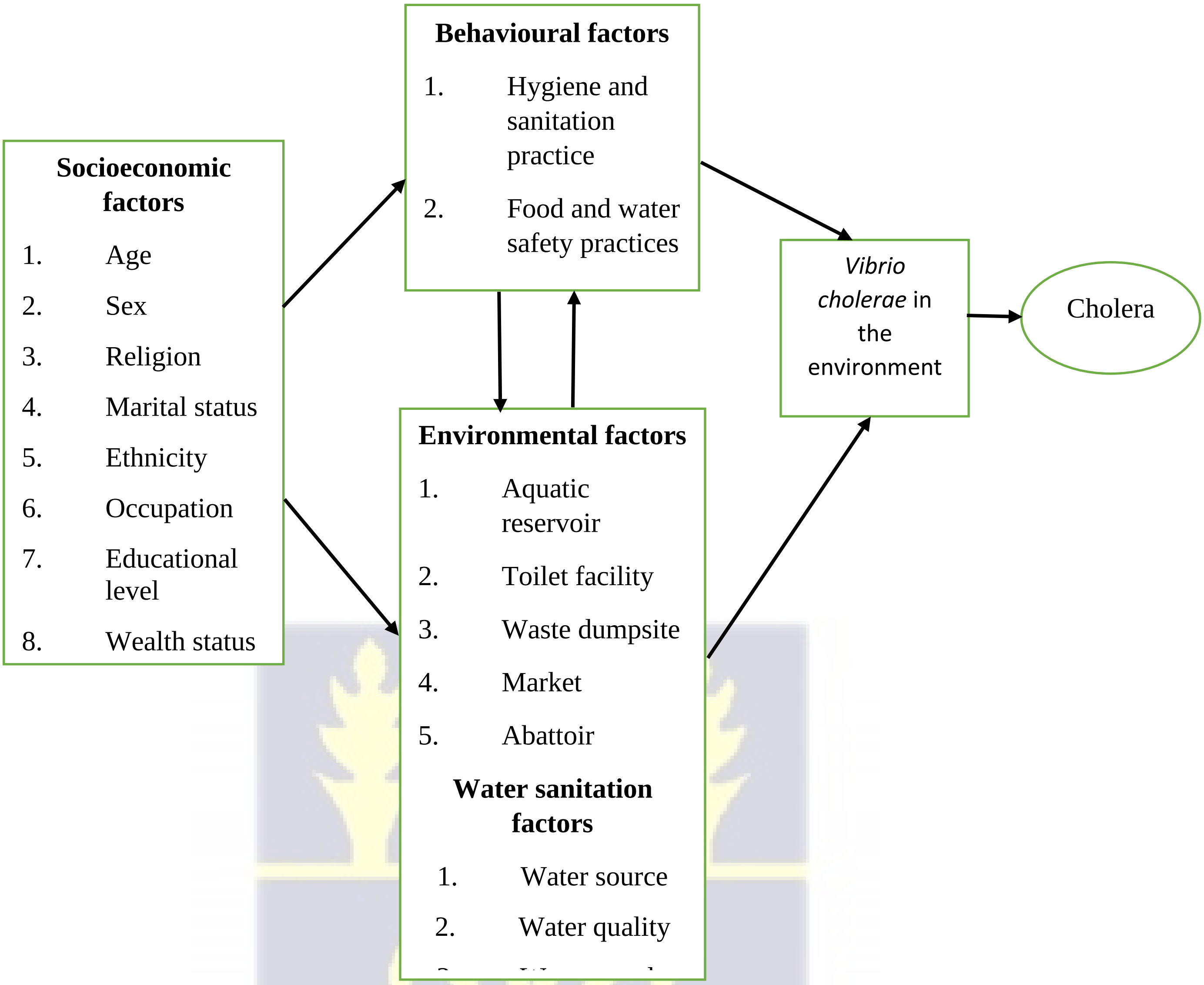

Figure 1.2: Conceptual framework of the factors influencing cholera recurrence

Narrative: The framework explains the influence of environmental, water sanitation, socioeconomic and behavioural factors on cholera. The socioeconomic factors such as sex, marital status, ethnicity (beliefs), occupation (e.g traders, fishermen), educational level, income level or wealth status, and housing (crowdedness) may influence the individual hygiene practices including their food eating and water drinking habits which could lead to cholera. The behavioural factors also affect the web of environmental factors that influence how sanitation is maintained and solid or liquid waste is disposed of which could lead to outbreaks once the cholera bacterium is exposed to the environment. The poor sanitary conditions from the environment may lead to the contamination of the water distribution network system and affect the quality of the treated water. This situation may be aggravated by heavy rains and flooding due to the poor drainage system, resulting in cholera outbreaks. The hygiene practices of households to ensuring safe drinking water may be compromised in the household through the type of storage containers, cups dipped into the storage water, or length of storage of the water (Agensi, Tibyangye, Tamale, Agwu, & Amongi, 2019; Gizachew, Admasie, Wegi, & Assefa, 2020). Behavioural factors involving food safety practices could also expose households to cholera. The proximity of environmental factors including an aquatic water reservoir which may be inhabited with crustaceans and other natural hosts of *Vibrio cholerae* may pose a risk once these water bodies contaminate the household drinking water sources (Almagro-Moreno & Taylor, 2014). The closeness of waste dump sites, abattoirs, and markets to the households may also expose households to flies and other rodents that could contaminate foods and influence cholera recurrence (Osei & Duker, 2008b).

1.4 General Objectives

To identify the risk factors associated with cholera outbreaks and map the environmental factors in the Greater Accra Region.

1.4.1 Specific Objectives

1. To determine the behavioural risk factors associated with cholera in the Greater Accra Region
2. To assess the bacteriological quality of household drinking water and cholera risk in the Greater Accra Region

3. To determine the risk of toxigenic strains of *V. cholerae* O1 and O139 in the environmental water bodies.
4. To assess the environmental sanitation risk factors for cholera in the Greater Accra Region
5. To identify and map the significant environmental risk factors using Geographic Information System maps

1.5 Justification

Greater Accra Region has been the epicenter of most of the cholera outbreaks from where it spreads to other parts of the country (UNICEF, 2013). Therefore, preventing cholera in Greater Accra Region is likely to help control cholera in the country. Few studies have attempted to identify the risk factors that contributed to cholera persistence in the endemic communities in GAR. Most of the publications have been limited to determining risk factors during localized outbreaks with a focus on case management (Dzotsi et al., 2016; Ohene-Adjei et al., 2017). To control cholera in GAR, it is important to identify the specific risk factors in the cholera endemic communities that aid its persistence, transmission, and spread any time cholera occurs. Also, many of the proposed solutions to cholera epidemics are generic and poorly adapted to the local needs and specificities. Understanding these community risk factors will help target effective interventions to control and prevent future epidemics.

The use of GIS technology to map the risk of cholera will help provide information on endemicity and guide the prevention and control of the disease in some communities. It will also help to provide information on the location where the intervention is most needed to ensure efficient use of resources. The spatial distribution of the cases could provide clues to the transmission and spread of the disease in the communities (Guo et al., 2013; Lessler, Salje, Grabowski, & Cummings, 2016).

This work will support the effort of the Global Task Force on Cholera Control (GTFCC) strategy in stopping cholera transmission in cholera 'hotspots' communities by 2030 (Legros, 2018). This study is important as it will provide current knowledge on the potential risks factors aiding cholera persistence and the results will help influence policy makers to identify measures to prevent cholera epidemics in GAR.

CHAPTER TWO

LITERATURE REVIEW

2.1 History of Cholera

Cholera has existed since the ancient times of Hippocrates and Galen when the disease was described as sporadic diarrhoea. Many other hints indicated that cholera had occurred since antiquity in the plains of the Ganges River. However, it was not until the 19th Century that John Snow demonstrated an association between cholera transmission and contaminated water in the Soho district of London cholera outbreak (Tulchinsky, 2018). This event disabused the highly upheld 'miasma theory' in the early 1880s that suggested the disease to be caused by 'bad air' or 'bad smell'. About the same time, an Italian anatomist Filippo Pacini isolated the bacterium from the intestinal contents of the corpse of cholera patients in Florence, Italy but his work was unnoticed. It was not until 1884, that Robert Kock identified the pathogen as *Vibrio cholerae* O1 from a pure culture during an outbreak in Egypt.

So far, seven pandemics have occurred globally. The first pandemic started in 1817, followed by six subsequent pandemics in 1829, 1852, 1863, 1881, 1889, and 1961 (Azizi & Azizi, 2010). The first six pandemics originated from Ganges in Bangladesh, thus was nicknamed 'homeland of cholera' and the seventh pandemic is suggested to have begun in Sulawesi, Indonesia in 1961 (WHO, 2019). It then spread to other continents including Africa, Europe and finally reached Latin America in 1991 which has been free from cholera for more than a century, resulting in a large number of cases and deaths.

The seventh cholera pandemic has continued to ravage the world especially in countries with poor access to safe water and sanitation or disrupted by war or civil conflicts or natural disasters such as earthquakes, tsunamis, volcanic eruptions, landslides, and floods (WHO, 2019). Currently, the disease affects about 3 to 5 million people globally with an annual estimated death of 100,000 to 120,000 with recent large outbreaks in Haiti, Yemen, and Sub-Saharan Africa (Ali et al., 2015; Tulchinsky, 2018).

2.2 Cholera in Africa

Five cholera pandemics have so far affected Africa in the 19th century. These occurred particularly in North Africa where the epidemic killed several thousands of people, especially Egypt. The epidemics that occurred at the time were associated with pilgrims who returned from Hajj in Mecca. However, it was not until during the seventh pandemic that Africa bore the major burden of cholera. The seventh pandemic started in 1970 in West Africa, with Guinean students travelling to Conakry where there was an ongoing epidemic. The epidemic then spread rapidly from Guinea to most parts of the continent where it has become endemic (Echenberg, 2011).

Since then, cholera incidence has continued to increase with high Case Fatality Rates (CFRs) (Mengel et al., 2014). The majority of the reported cholera cases occur in the Central African Great Lakes region and Sub-Saharan African countries (Gaffga, Tauxe, & Mintz, 2007; Nkoko et al., 2011). From 1970 to 2011, the African countries alone contributed 3,221,000 cases which constituted 46% of the global cases (Mengel et al., 2014). In 2017, large outbreaks occurred in the Democratic Republic of the Congo (DRC), Nigeria, Ethiopia, South Sudan, Sudan, Somalia, and Zambia (Legros, 2018). The true burden, however, could be more than reported by WHO because of the inadequate laboratory and surveillance reporting systems notwithstanding the countries' fear of restriction on travel and trade if known with cholera (Ali et al., 2012; Zuckerman, Rombo, & Fisch, 2007). The cholera epidemic in Africa has largely been associated with migration, socioeconomic, climatic, environmental factors, and political instability (Ajayi & Smith, 2019).

2.3 Cholera in Ghana

The cholera epidemic started in Ghana during the seventh pandemic. The first case was a Togolese native who died on arrival at the airport in Accra on the first of September, 1970, while in transit to Conakry, Guinea (Pobee, 1970). The case however did not start an outbreak. Ghana's first outbreak was imported by Ghanaian fishermen who have been fishing along the Coast of Togo, Liberia, and Guinea (Ofori-Adjei & Koram, 2014). One of the fishermen died and the corpse was 'smuggled' into the country by the relatives for the burial rituals despite the sanitary cordon at the border (Ofori-Adjei & Koram, 2014). The disease subsequently spread to other coastal fishing communities. The two worst-hit fishing communities were Akplabanya (in the then Ada District) and Nyanyano near Kasoa (Ofori-Adjei & Koram, 2014). The disease further spread to start outbreaks in the Ashanti region (Ashitey, 1994).

Since then cholera has become endemic with focal outbreaks occurring every 4 to 6 years (Dzotsi et al., 2016; Ofori-Adjei & Koram, 2014). Between 1970 and 2011, Ghana reported cumulatively 128, 525 cases and 6,400 deaths with a CFR of 4.98 to WHO (Mengel et al., 2014). Although there was a decline in the trend of cases from 1990 to 2010, large outbreaks occurred in 2011, 2012, and 2014 (Ghana Disease Surveillance Department, 2016). In 2014, Ghana recorded over 28,000 cases with 240 deaths, the worst after 30 years, and affected all the then ten regions, with 72% of the cases occurring in the GAR (Ghana Health Service, 2015). The GAR has thus become cholera-endemic but the underlying mechanism behind its endemicity and spread in some communities remains unclear.

2.3 The Pathogen

Cholera is caused by a bacterium belonging to the genus *Vibrio* which is described typically as small, curved rod-shaped with or without flagella which aid in motility. *Vibrio* species are facultative anaerobes with most species being oxidase-positive. There are many *Vibrio* species but the pathogenic species that are transmitted via water or seafood include; *V. cholerae, V. parahaemolyticus, V. vulnificus, V. alginolyticus, V. fluvialis, V. tubiashi* (Cabral, 2010). *Vibrio* species are primarily aquatic organisms and the distribution of their species is dependent on the sodium concentration and water temperature (Kokashvili et al., 2015).

Vibrio cholerae can survive in alkaline conditions of up to pH 10 and are inhibited by acidic conditions (pH 6 or lower). They are halophilic organisms and require sodium chloride for optimum growth thus naturally exist as free living in the estuarine and marine environment or coastal waters (Almagro-Moreno & Taylor, 2014). It has also been found to be associated with algae, shellfish, chironomid egg masses, fish, waterfowl, amoebae, and mostly copepods in its aquatic environment (Almagro-Moreno & Taylor, 2014; Senderovich, Izhaki, & Halpern, 2010). The bacterium can also survive in fresh water sources such as lakes, ponds, rivers, and water storage devices at home (Abia, Ubomba-Jaswa, & Momba, 2017; Yirenya-Tawiah, Darkwa, & Dzodzomenyo, 2018). In a non-aquatic environment *V. cholerae* can survive in refuse dumps, fruits, fresh vegetables, meat and even in cooked foods that is cold (Mrityunjoy et al., 2013).

There are over 200 serogroups of *V. cholerae* that have been classified based on the presence of the somatic O antigen. However, the most important serogroups known to cause disease and epidemics are serogroups O1 and O139 (Cho, Yi, Lee, Kim, & Chun, 2010; Islam et al., 2013). *Vibrio cholerae* O1 has further been classified based on a variety of phenotypic markers such as; susceptibility to polymycin B, Voges-Proskauer test, chicken erythrocyte agglutination, and phage IV susceptibility into classical and El Tor biotypes (Mandal, Mandal, & Pal, 2011). The El Tor biotype differs by its ability to survive better in the human host and environment than the classical biotype. However, the classical biotype causes more severe disease than the El Tor biotype (Mohammadi, Bakhshi, & Boustanshenas, 2016). At the genomic level, not much difference is observed between the classical and El Tor biotype as a complete sequence of their genome indicates that only 29 genes are missing between classical that cause the first six pandemics compared to El Tor that is responsible for the ongoing seventh pandemic (Beyhan, Tischler, Camilli, & Yildiz, 2006; Hu et al., 2016). Nonetheless, the presence of a particular biotype does not alter the management of a case but present epidemiologist with knowledge of the source and spread especially when found in areas where it has not been previously reported.

Vibrio cholerae O1 has been responsible for the epidemics globally until late 1992 when *V. cholerae* O139 was first discovered and found to also cause epidemics in Bangladesh and India (WHO, 2019). This new strain was demonstrated to be genetically different from El Tor biotype by the replacement of the biosynthetic genes with O139 biosynthetic genes (WHO, 2017d). The *V. cholerae* O139 has since been confined to causing epidemics in Asia (Mukhopadhyay et al., 2014).

The *V. cholerae* O1 strains have two serotypes, Ogawa and Inaba which differ by the antigens present in the bacteria cell wall. The Ogawa serotype has been found to possess antigens A and B whiles the Inaba contains antigens A and C. A third serotype Hikojima which is rare and unstable has antigens A, B, and C (Gustafsson & Holme, 1985). It is detected during the transition when there is a switching of Ogawa serotype to Inaba when the human population acquires immunity against the Ogawa serotype (M. T. Alam et al., 2016; Khan et al., 2010). However, immunity against Inaba is suggested to offer longer protection than Ogawa serotype infection (Longini Jr et al., 2002). Knowledge of these *V. cholerae* O1 serotypes have been used to develop the live oral vaccine candidates to protect against cholera (Karlsson et al., 2014; Sit et al., 2019).

Vibrio cholerae O1 and O139 serogroups that cause epidemics produce cholera toxin complex (ctx) and toxin co-regulated pilus (tcp), coded by ctx A and tcp A genes respectively (Danso et al., 2020). The ctx is responsible for the secretory diarrhoea whiles the tcp is involved in intestinal colonization.

The other serogroups are known collectively as *V. cholerae* non-O1/non-O139 also referred to as non-agglutinating *Vibrios*. These do not agglutinate with antisera specific to their O somatic antigen, but may cause sporadic diarrhoea and localized outbreaks. They are mostly found in aquatic environmental sources and lack the virulence gene (ctx and tcp) (Arteaga et al., 2020). However, they have other virulence factors such as; heat-stable enterotoxin (stn), haemolysin (HlyA), repeat in toxin (RTX), type 3 secretion systems (TTSS), and type 6 secretion system (T6SS) (Arteaga et al., 2020; Dutta et al., 2013).

These *V. cholerae* non-O1/non-O139 are however, thought to constitute genomic islands of potential virulence genes that may contribute to the evolution of pathogenic strains (Arteaga et al., 2020; Dutta et al., 2013; Li et al., 2014). Further surveillance and investigation are required to understand the potential pathogenicity of these environmental strains.

2.4 Infections and Symptoms

Cholera is contracted by ingesting food or water contaminated with pathogenic *V. cholerae* O1 or O139 (WHO, 2019). Once the bacterium reaches the gastric acid barrier of the stomach, the majority are destroyed, but a few make their way into the small intestines where they re-establish their population. More than 10^4 of the bacterium are required to develop symptoms of cholera in a susceptible individual (Nelson, Harris, Morris, Calderwood, & Camilli, 2009). The incubation period is dependent upon the population of the bacteria that colonize the small intestine and varies from 12 hours to 5 days, typically taking two days before causing symptoms (Spagnuolo, DiRita, & Kirschner, 2011; WHO, 2019).

In the small intestine, the bacteria adhere to the crypts of the intestine using a tcp to keep them from being flushed out (Spagnuolo et al., 2011). The bacterium releases the ctx, which is composed of an enzymatic (A) subunit and 5 binding (B) subunits. The B subunits bind to the surface of intestinal mucosal cells, allowing the A subunit to enter the cell. Once inside the cell, the A subunit stimulates cyclic adenosine 5′-monophosphate (cAMP) production. This causes secretion of water and electrolytes into the lumen of the small intestines and causes the severe diarrhoea. The amount of water losses varies between individuals, depending on the infecting *V. cholerae* strain and the amount of bacterium that colonizes the small intestine (WHO, 2017d). Within an hour a patient can lose up to a litre of fluids.

The disease is characterized by other symptoms including muscle cramps, sunken eyes, and cheeks, a scaphoid abdomen, rapid heart rate, low blood pressure, irritability, and loss of skin turgor. If early treatment is not sought, severe volume fluid depletion may result in metabolic acidosis which may lead to circulatory collapse and death (WHO, 2017d). In children, it may in addition present with drowsiness, fever, convolutions, and even a coma. The 'rice water' stool produced from a patient carries between 10^{10} and 10^{12} *Vibrio* per litre (Nelson et al., 2009).

About 75% of people infected with the *V. cholerae* are asymptomatic, although the bacterium is present in their faeces for 7 to 14 days after infection and can be shed into the environment. Of the 25% of the patients who become symptomatic, 10 to 20% may experience severe disease (WHO, 2017d). Such individuals may shed the *Vibrio* before the onset of the disease and will continue to shed the *Vibrio* for up to 14 days. A few people may habour the bacteria indefinitely with no symptoms (Adagbada, Adesida, Nwaokorie, Niemogha, & Coker, 2012). The distribution of symptomatic patients and asymptomatic carriers in a community can influence cholera transmission once the bacteria get into the environment.

2.5 Transmission of Cholera

Cholera transmission is through primary and secondary transmission routes. The primary transmission occurs through exposure to an environmental reservoir of *V. cholerae* or contaminated water source with the bacterium regardless of previously infected persons or faecal contamination (Ruiz Moreno, 2009). The primary transmission is triggered by climatic factors that make the flora and fauna in the aquatic environment bloom enabling the growth of the bacterium (Lutz et al., 2013; Mavian et al., 2020). Other studies have also suggested that the non-toxigenic strains of *V. cholerae* may evolve to become toxigenic in the aquatic ecosystem (Islam, Zaman, Islam, Ahmed, & Clemens, 2020; Mavian et al., 2020). Thus, making the aquatic environment an important source of cholera transmission.

Secondary transmission route on the other hand occurs through exposure to faecally contaminated water sources, food, or direct from person-to-person within households or at the hospital (Ruiz Moreno, 2009; Sugimoto et al., 2014; You et al., 2013). This mode of transmission plays a major role in cholera spread because it involves multiple factors. The faecal-oral transmission is increased by the level of contamination of water or food which can be influenced by other local factors such as socioeconomic, demographic as well as sanitation (WHO, 2019). To control cholera epidemics both transmission routes need to be taken into consideration for effective control of epidemics.

2.6 Diagnosis and Detection of Cholera

A review of clinical features of a suspected case of cholera may help detect cholera patients. However, it is important to distinguish between cholera cases and other acute watery diarrhoea cases. This is important for quick management of the cases to prevent the disease from rapidly spreading. Confirmation of cholera in the laboratory is by the isolation and identification of *V. cholerae* O1 or O139 from stool specimen of suspected patient or by detection with Polymerase Chain Reaction (PCR) (Dick, Guillerm, Moussy, & Chaignat, 2012; WHO, 2015).

This notwithstanding, many hospital facilities lack laboratories with the capacity to perform a culture or PCR diagnostic technique to confirm the clinical diagnosis. As such refer specimens of suspected cases to laboratory facilities where cultures or PCR identification can be done. However, there are challenges with the timely and safe referral of the specimens. This has led to the development of a rapid diagnostic test kit (RDT) to quickly test and alert public health authorities for early response to control an outbreak (Mwaba et al., 2018).

The use of RDTs, however, is limited because they cannot grow an isolate to perform antimicrobial susceptibility testing and sub-typing of strains. Other RDTs are suboptimal in terms of test kit sensitivity and specificity as published by Global Task Force on Cholera Control (WHO, 2017a). It is therefore recommended that positive RDT result are confirmed with culture or PCR technique before an outbreak is declared (WHO, 2018b). Where laboratory diagnostic testing is not available WHO recommends the use of case definitions to ascertain that all patients considered as cholera cases have the same disease (WHO, 2017a). For instance, the suspected case definition in an area where cholera is not known to be present is; 'any patient aged 2 years or more who develops severe dehydration or dies from acute watery diarrhoea'. Also, in an area where there is a cholera epidemic a case is, 'any patient aged 2 years or more who develops acute watery diarrhoea, with or without vomiting'. A confirmed case on the other hand is defined as; 'a case of cholera when *V. cholerae* O1 or O139 is confirmed by culture or PCR and, in countries where cholera is not present or has been eliminated, the *V. cholerae* O1 or O139 strain is demonstrated to be toxigenic (GTFCC, 2017). Cholera diagnosis is therefore important for ensuring an effective surveillance system and for the control of cholera outbreaks.

2.7 Treatment and Prevention of Cholera

2.7.1 Treatment

The first treatment for cholera patients is prompt rehydration through the administration of oral rehydration salt (ORS) (Munos, Walker, & Black, 2010). The WHO/UNICEF ORS standard sachets have been found to adequately treat more than 80% of patients. In severe cases, intravenous rehydration is administered, preferably Ringer lactates instead of isotonic sodium chloride. This is because the latter has the effect of correcting metabolic acidosis as well (WHO, 2008).

In addition, an appropriate antibiotic should be given in severe cases. The essence of giving antibiotics is to reduce the duration of diarrhoea and minimize bacterial shedding in the stool of cases (WHO, 2018b). Various antibiotics have been proposed for cholera therapy including; furazolidone, erythromycin, ampicillin, tetracycline, ciprofloxacin, and azithromycin (Khan, Saha, Ahmed, Salam, & Bennish, 2015; Leibovici-Weissman et al., 2014; Pietroni, 2020). However, the choice of antibiotic treatment will vary depending on the antibiotic susceptibility pattern of the circulating *V. cholerae* strain (Kitaoka, Miyata, Unterweger, & Pukatzki, 2011; Pietroni, 2020). The continuous monitoring of antibiotic resistance patterns of epidemic strains is important for cholera control strategies. In children up to five years, supplementation with zinc is recommended to reduce the duration of diarrhoea as well as the number of diarrhoea episodes. Early reporting of cholera cases to the health facility with adequate management can reduce the CFR to less than 1%. Case fatality rates exceeding 1% usually reflect inadequate case management or delay in initiating treatment (GTFCC, 2017; Pietroni, 2020).

2.7.2 Prevention

Natural protection against cholera is acquired after clinical cholera and the protection can be long lasting especially in endemic areas where there is the chance of re-exposures (Weil et al., 2012). This infection-derived immunity approach led to the development of cholera vaccines to produce immunity with the administration of attenuated strains of *V. cholerae*. Currently, three oral cholera vaccines (OCV) Dukoral, Shanchol, and Euvichol have been pre-qualified by WHO and are available. These OCVs have some advantages and disadvantages. The Dukoral vaccine requires the administration of a buffer solution and is licensed only for patients aged two years and above (WHO, 2017a). Two doses of Dukoral are required to confer longer protection (WHO, 2017a). It requires refrigeration at 2 – 8^{o}C and has a shelf-life of 3 years and one month at 37^{o}C (WHO, 2017a). From 1997 to 2014, a review of vaccination campaigns showed that it cost between USD 0.53 to USD 3.66 per fully immunized person (Martin et al., 2014). Shanchol is administered in two liquid doses two weeks apart. A booster dose is also required after 2 years. In terms of cost per fully immunized person, it ranges from USD 1.13 to USD 3.99 from 2011 to 2014 (Martin et al., 2014). A third vaccine Euvichol which is comparable to Shanchol was licensed and prequalified in 2016 by WHO but is not widely used (Baik et al., 2015). Shanchol and Euvichol vaccines can be stored at 2 – 8^{o}C and have a shelf life of 2 years.

Despite the protection, OCV can give, concerns are being raised regarding the vaccines because of the cost, the feasibility of organizing large vaccination campaigns, community opinion of oral vaccines, and delay in receiving vaccines (Chaignat, 2008). As such the mainstay of cholera prevention remains; improving on the provision of clean water, providing sanitation facilities, and improving on personal hygiene practices as well as enforcement of standard sanitation laws. These were the measures adapted by most of the developed countries such as Europe and the USA that eradicated cholera. World Health Organization, recommends OCV to be used during outbreaks to quickly contain the spread of the disease.

2.8 Cholera Risk Factors Influencing Transmission

2.8.1 Host Genetic factors

Host immunity plays a role in the susceptibility of an individual to cholera. It determines whether an individual will have severe and life-threatening diarrhoea or mild to no apparent symptoms. Thus, diseases that cause immune suppression may predispose people to cholera. Mushayabasa and Bhunu (2012) used a mathematical model to demonstrate an increased association between human immunodeficiency virus (HIV) infection and cholera risk in cholera endemic communities. A case-control study conducted in cholera endemic communities in Mozambique similarly found an association between HIV infection and cholera (Semá Baltazar et al., 2017). Richterman, Cheung, et al. (2018) in their study among self-reported HIV patients in rural Haiti also found an association between HIV infection and cholera.

Another host-related factor is the differences in gastric acid level and cholera infection. In a low gastric acid condition, a lower infectious dose of *V. cholerae* is required to cause an infection. This was demonstrated in an experimental trial where bicarbonate and live *V. cholerae* were given to volunteers, and the infective dose was lowered from 10^8 to 10^4 organisms (Hornick et al., 1971). A higher risk of cholera is also found among patients who have had gastric surgery. This is shown in a study in Italy, where index cases were

5.8 times more likely to have had a history of gastric surgery (Baine et al., 1974). *Helicobacter pylori* infection is common in Africa and causes gastric hypoacidity (Adagbada et al., 2012). Gastric hypoacidity increases the risk of cholera infection and may be the reason cholera is prevalent in Africa (Smith, Fowora, & Pellicano, 2019).

Blood group is one of the genetic factors that predispose to diseases, including infectious diseases. The expression of the ABO and Lewis blood group types has been shown to be associated with a cholera risk. Individuals with blood group O for instance have a lower risk of initial colonization on exposure to *V. cholerae* infection, although on colonization have a higher risk of more severe disease than the other blood groups (A&B) (Harris et al., 2005). It is suggested that the cholera toxin exerts a more potent effect on cells expressing blood group type O-associated glycan than blood group A, thus making blood group O individuals more susceptible (Kuhlmann et al., 2016). Also, individuals expressing the Lewis blood group Le (a+b-) are more susceptible to symptomatic cholera than those with Le (a-b+) (Arifuzzaman et al., 2011). In Ghana, no epidemiological studies have been conducted to relate blood group type to cholera risk although the majority of the populace belong to blood group O.

2.8.2 Demographic Factors

The host-related risk factors of cholera, such as age and sex have varyingly been reported by many researchers. Concerning age, some studies have reported younger children to have a higher risk of cholera than adults (Cummings et al., 2012; Saha et al., 2017), whiles others have suggested the risk to be higher among adults (Colombara, Faruque, Cowgill, & Mayer, 2014; Rosewell et al., 2012). Although cholera can affect all age groups equally, the variation in the risk by age may reflect the natural immunity of the population. For instance, infants could be protected through passive immunity from the mother's breast milk (Qureshi et al., 2006). Also, previous exposure to cholera by adults living in endemic communities may decrease the risk of illness in the adult population than younger children without any episode of exposure as reported in Kenya (Mutonga et al., 2013). Naturally acquired immunity also plays a role in cholera endemic areas. A Mozambican study showed that cholera infection rarely occurred in the same affected districts for at least three years (Gujral et al., 2013). In Ghana, the lull periods observed in 2013, and from 2017 to 2020 when no cholera outbreaks occurred may be attributed to the immunity acquired by the population from the previous outbreaks than an improvement of the sanitation situation.

With regards to sex-related risk of cholera, some studies in Uganda and Bangladesh have attributed a high risk to adult women and school-age girls compared to their male counterparts (Cummings et al., 2012; Saha et al., 2017). In contrast, many outbreak investigations in districts in the Greater Accra and Brong Ahafo observed a higher risk among males than females (Dzotsi et al., 2016; Noora et al., 2017; Ohene-Adjei et al., 2017). This also agrees with the findings of Opare et al. (2012) during an outbreak investigation in the East Akim Municipality. Although both sexes were equally at risk of cholera, the variation could be attributed to socially ascribed roles in the specific environment. For instance, in some jurisdictions, women or young girls may be more associated with domestic chores and involved in the care of patients as well as handling and preparation of family meals than males. In other situations, adult males may be more vulnerable because of their engagement in eating outside foods.

2.8.3 Socioeconomic factors

Several studies have explored the relationship between cholera incidence and the household income level and socioeconomic status. Rahman et al. (2009) in a multivariate analysis among cholera-infected individuals in Matlab, found a lower socioeconomic status (SES) to be a significant predictor of hospitalization with cholera. A similar multivariate analysis conducted among children in Bangladesh demonstrated children from households with lower incomes have an increased risk of cholera compared to households with higher incomes (Colombara, Cowgill, & Faruque, 2013). Root, Rodd, Yunus, and Emch (2013) also explored the impact of SES and observed that higher SES reduces cholera risk but could not explain the mechanisms, however, suggested a combination of factors including education, hygiene knowledge and practices, better housing quality and access to clean water might have accounted for the difference. Other studies, however, did not find any association between SES or income level and cholera risk (Dureab et al., 2019; Saha et al., 2017; Stoltzfus et al., 2014).

In terms of population density and cholera, Saha and colleagues found a significantly higher risk for cholera in densely populated areas (Saha et al., 2017). Such areas are often located in urban slums where there is usually overcrowding and the people live in poor sanitary conditions (Osei & Duker, 2008a; Penrose, de Castro, Werema, & Ryan, 2010).

The role of education has been explored in many case-control studies where multivariate analyses were used to establish the associations between cholera and the educational status of victims. The findings of most of the studies suggested that educational level above secondary school or nine years of education was protective against cholera (Colombara et al., 2013; Nguyen et al., 2017; Nsagha et al., 2015). This may be because people at a certain level of education would understand the basic preventive measures to avoid contracting cholera.

In terms of occupation, highly mobile populations such as traders and fishermen who travel by water, land, and air from a cholera endemic area may contract the infection and transfer to another geographical location (Manga et al., 2008; Mari et al., 2012; Piarroux & Faucher, 2012). This is particularly important because there is no trade restriction across countries under the international health regulations (IHRs) for cholera. Such susceptible people may become infected whiles travelling and transmit the disease to their home communities to start an outbreak. For example, the index case of the Guinean 2012 cholera epidemic was caused by a fisherman who travelled from Sierra Leone, where an epidemic was ongoing at the time (Rebaudet et al., 2014). The cholera epidemics in Ghana have been speculated by Sandra and colleagues to occur as a result of the mobile population (Moore et al., 2018). This is further emphasized by a recent report of two confirmed imported cases of cholera from Nigeria, who were on transit to Kumasi but developed symptoms whiles on the journey and were admitted at the Ketu South Municipal Hospital (Ghana Health Service, 2018). If not for the timely intervention of the Ketu South Municipal Health Directorate this could have started an outbreak once the bacterium got into the environment.

2.8.4 Water-related risk to cholera

2.8.4.1 Water Source, Transportation and Handling

Issues surrounding the availability and usage of water have always been a focus of cholera epidemics. The use of unimproved water sources (e.g lakes, rivers, estuaries, irrigation canals, ponds or dams, shallow wells, street-vended sachet water) are significantly associated with cholera, while drinking from improved or treated sources are protective (Richterman et al., 2018). In the GAR, the main sources of drinking water are piped-borne (in-house, outdoor, public tap) and sachet water (Ghana Statistical Service, Ghana Health Service, & ICF International, 2015). However, because of the rapid urbanization of Accra, there is an inadequate supply of piped water as demand far exceeds the water production and many residents have increasingly relied on sachet water as their source of drinking water. Others prefer the sachet or bottled water because they have the belief that piped water is contaminated. According to Semey, Dotse-Gborgbortsi, Dzodzomenyo, and

Wright (2020) sachet water is usually packaged from boreholes or treated water sources and therefore safer. However, their study did not rule out the possibility of contamination by factory workers during production. In furtherance to this, Dzodzomenyo et al. (2018) observed that a substantial proportion of sachet water sold was not formally registered and the microbial quality of such unlicensed water was high. An indication that the shift to drinking sachet water may not necessarily be protective against cholera or diarrhoea diseases. Some previous investigations of the water network during the 2012 cholera outbreak in the Osu Klottey Sub Metropolis showed that drinking community pipe borne water was associated with contracting cholera (Davie-Teye et al., 2014). Another study also revealed unsuitable residual chlorine levels and the regular presence of faecal coliform in the Accra network water even during the dry season (Karikari & Ampofo, 2013). These studies suggest that residents in Accra could be exposed to network water contamination either directly via the tap or indirectly via the consumption of sachet water.

Water contamination may also occur at the point-of-use, as a result of dipping of hands into storage vessels (Daniel, Diener, van de Vossenberg, Bhatta, & Marks, 2020; Gizachew et al., 2020). Other researchers have implicated distance from water source and time spent in water collection to the household as a factor for drinking water contamination (Boateng, Tia-Adjei, & Adams, 2013; Colombara et al., 2013; Nygren et al., 2016). Thus, testing water source alone is not enough to determine the likely source of contamination but testing the quality of the stored water as well. In other cases, the contamination may result from vessels used in water storage as reported in a systematic review where household risk factors of cholera showed a significant association between using water stored in a bucket or open container and cholera (Richterman et al., 2018). In a related study *V. cholerae* O1 strains were isolated from some household drinking water storage containers in some communities in the Accra Metropolis before the 2014 major cholera outbreak (Yirenya-Tawiah, Darkwa, & Dzodzomenyo, 2018). This indicates household water storage contamination; however, they could not confirm whether the contamination was from the source or the household or both and could not also confirm if the *V. cholerae* isolated were of the toxigenic strains. This current study investigated the microbial quality of household drinking water from the source and storage containers in cholera endemic and non-endemic communities.

2.8.4.2 Water Treatment

Water treatment is essential in preventing contamination with waterborne pathogens. Most household drinking water is contaminated from source, during transportation, or storage. This is evidenced in a study by Clasen and Bastable (2003) who found safe water to be faecally contaminated during the collection and storage at home. A similar study by Lavanya and Ravichandran (2013) indicated that household water storage was more contaminated with *E. coli* and total coliform than the piped water. The study also revealed that there was no evidence of residual chlorine, although the over tanks and wells were chlorinated before pumping to households (Lavanya & Ravichandran, 2013). These findings raise the importance of water treatment at the point of consumption. Although there has been an improvement with access to portable water (piped water, bottled and sachet water) from 37.3% in 2013 to 44.3% in 2017 as reported in the Ghana Living Standard Survey data (Government of Ghana, 2019). The quality of household water remains in doubt. Thus, for Ghana to achieve the Sustainable Development Goal (SDG) 6, the country must ensure that the population who do not yet have access to portable water practice effective household water treatment (WHO, 2012).

Various methods of household treatment including; sedimentation, filtration, disinfection, and safe storage have been recommended by WHO/UNICEF (WHO, 2013). It has been revealed in some systematic reviews that household water treatment interventions reduce the burden of cholera and the risk of transmission by improving household stored water (Lantagne & Yates, 2018; Wolfe, 2018). The current study determined the contamination levels of household drinking water and established the water treatment attitude at homes.

2.8.4.3 Water Quality Analysis

Water is important for life and should be made available in supply, however, its health benefits are dependent on the quality (WHO, 2017c). The water quality may be affected by the presence of microorganisms such as bacteria, viruses, or parasites (helminths and protozoa). These microorganisms may occur naturally in the environment or introduced through human activities (WHO, 2017c). If the drinking water becomes faecally contaminated, that will pose a great risk for diarrhoea diseases including cholera (WHO, 2017c). The drinking water contamination may occur as a result of inadequate treatment from the source or in the water distribution network (WHO, 2014). This may lead to health hazards resulting in large-scale contamination with the potential of a large waterborne outbreak or sporadic outbreaks with low contamination levels (WHO, 2014).

It is therefore important that regular water surveillance is conducted to detect early contamination with waterborne pathogens to allow remedial action to prevent the possibility of an outbreak. In Ghana, water quality monitoring is rarely done and this research analyzed the bacteriological quality of household drinking water in both cholera endemic and non-endemic communities.

2.8.4.4 Microbial Parameters

Microbial parameters are 'indicator' organisms used to provide useful information in assessing water quality. Usually, the pathogenic pathogens (*Salmonella*, *Shigella*, *Vibrio*, or *Enteroviruses*) themselves are not used as indicator organisms because they are often present in small amounts in water and may require unique microbiological techniques which might not be cost-effective. Thus, the practical approach is to adopt and use indicator organisms that inhabit the gut in large numbers and excreted in faeces. Therefore, the indicator organisms to use must demonstrate the presence of faecal contamination as a sign of health hazard (WHO, 1997). As such, WHO (1997) identified characteristics of a good indicator organism to include; availability of the organism in faeces of humans and other warm-blooded mammals, ease of detection by simple methods, must not grow naturally in water or the water distribution network, must be able to be treated by disinfection. The essence of the indicator organism is for it to predict the presence of pathogens.

Most of the enteric viruses and protozoa (*Cryptosporidium*, *Giardia*) resist disinfection and may not be considered as good indicator organisms (Omarova, Tussupova, Berndtsson, Kalishev, & Sharapatova, 2018). However, some countries have started to include enteric viruses as indicator organisms because they can afford the cost in their investigations (Wen et al., 2020). This suggests that there is no limit to the inclusion of other organisms as an indicator organism. However, the primary indicator organisms still involve assessing water quality remains total coliforms, thermotolerant coliforms or faecal coliforms, *E. coli,* and *Enterococcus* (WHO, 2017c).

Indicator Bacteria

Total Coliform Bacteria

Total coliform bacteria (TC) comprise a large group of organisms which are; Gram-negative rod-shaped, non-sporing bacteria that can grow in media containing bile salts. They are oxidase-negative and ferments lactose at 35-37^{0}C with the production of acid, gas within 24 to 48 hours (WHO, 1997). The TC belongs to the species within the family of *Enterobacteriaceae* that are capable of growth at 37^{0}C and possessed β-galactosidase (WHO, 1997). The species belong to the genera *Escherichia*, *Citrobacter*, *Klebsiella*, and

Enterobacter. They exist naturally in water and soil. When TC is used in the assessment of water quality, care must be taken in the interpretation of the findings since they exist naturally and may not necessarily be of human origin (Payment, Waite, & Dufour, 2003). This notwithstanding, TC analysis uses cost-effective laboratory techniques and results can be obtained within 14 to 24 hours, hence often included in microbial water quality assessment (Payment et al., 2003).

Thermotolerant (faecal) Coliform

Thermotolerant coliform (TTC) are a group of total coliforms that can ferment lactose at 44 to 45^{0}C with the production of acid and gas (WHO, 1997). The majority of TTC belongs to the genus *Escherichia*, while others are *Klebsiella*, *Enterobacter*, and *Citrobacter* species (WHO, 1997). Most TTC exists naturally in the soil and decaying water plants and may not necessarily be from faecal origin. Nonetheless, their presence nearly always indicates faecal contamination especially in the temperate countries where 95% of TTC isolated from water are *E. coli*, a prove of faecal contamination (Bartram & Pedley, 1996). In tropical countries, the proportion of *E. coli* present in TTC may be lower. This implies that caution must be taken in interpreting the results of the analysis, and there may be the need to include other additional tests to confirm the presence of *E. coli*. Thermotolerant coliform analysis can be performed using simple cost-effective techniques and results can be obtained within 14 to 24 hours (Payment et al., 2003).

Escherichia coli

Escherichia coli is a TTC that possesses the enzymes β-galactosidase and β-glucuronidase (Payment et al., 2003). They usually grow at 44 to 45^{0}C with acid and gas production and also produce indole from tryptophan (Payment et al., 2003). However, some strains of *E. coli* grow at 37^{0}C and do not produce gas. Although they are mostly non-pathogenic, the Enterohaemorrhagic *E. coli* serotype (O157:H7) are pathogenic (Payment et al., 2003).

Escherichia coli is present in abundance in humans and animal faeces. It is present in water and soil that has been recently contaminated with faeces. It has been suggested that *E. coli* can grow and multiply in tropical waters and not only present from human faecal contamination (WHO, 1997). This is attributed to other faecal contamination by animals other than humans. However, if *E. coli* is present in water it should not be ignored because the presumption remains that the water has been faecally contaminated with the possibility of the presence of pathogens and requires treatment (Payment et al., 2003). As such, *E. coli* is still considered as the most preferred indicator of faecal contamination than the faecal coliforms for assessing water quality (Odonkor & Ampofo, 2013). *Escherichia coli* is also easy to detect with simple laboratory techniques and sensitive to disinfection (Payment et al., 2003).

Streptococci and Enterococci

Faecal streptococci are Gram-positive cocci-shaped, non-sporing bacteria that belong to the genus *Streptococcus*. They are catalase-negative and grow optimally at 35 to 37^0C in a medium containing bile salts and sodium azide. The faecal enterococci (e.g *E. faecalis, E. faecium, E. avium, and E. gallinarium*) can be differentiated from other faecal *streptococcus* by growth in the presence of 6.5% sodium chloride at 45^0C (Cabral, 2010). The faecal enterococci are present in human and animal faeces. Humans' faeces almost always have only the two faecal *enterococci* strains (i.e *E. faecalis* and *E. faecium*) (Cabral, 2010). The other species of faecal *enterococci* (e.g *E. avium, E. ceocorum, E. durans, E. gallinarum, and E. hirae*) occur in the faeces of other animals including birds (WHO, 1997). However, faecal *enterococci* species exhibit similar phenotypic characteristics and are difficult to differentiate. In terms of the magnitude, faecal enterococci are present in lower levels in the gut than *E. coli*. Faecal enterococci are also more resistant to drying and chlorination and survive longer than *E. coli* and are a useful 'indicator' organism (Payment et al., 2003). However, caution must be taken in the interpretation of water analysis, since faecal *enterococci* can also be present in food of animal origin. Some crops and agricultural soils may also contain faecal *enterococci* resulting from the manure used on the farm. Nonetheless, in drinking water faecal *enterococci* should not be present to demonstrate water treatment efficiency (WHO, 1997). Faecal *enterococci* are easily detectable by simple inexpensive cultural techniques used in routine bacteriology laboratories (Payment et al., 2003).

Vibrio species

Vibrio are Gram-negative curved-rods that can grow at 35 to 37^{0}C in TCBS medium. They are mostly found as free-living in an aquatic environment. The distribution of the species is dependent on the sodium concentration and water temperature. They are pathogenic in humans if ingested in food or water.

Vibrio is considered a good 'indicator' organism because of the high loads of recovery from surface water and the ease of isolation using basic bacteriological cultural techniques (Okpokwasili & Akujobi, 1996). However, not usually included as an 'indicator' organism because they are mostly pathogenic and their presence can be predicted by the presence of faecal coliform or *E. coli*.

2.8.5 Food-related risk factors for cholera

Exposure to raw or undercooked seafood including shellfish, crabs, oysters, clams, dried fish, seafood, and salad can increase the risk of cholera infection (Iwamoto, Ayers, Mahon, & Swerdlow, 2010; Kim et al., 2018; Nguyen et al., 2014). This is because *Vibrio* species are found to be naturally present in the gastrointestinal tracts and gills of these seafoods, where they are protected from unfavourable conditions (Halpern & Izhaki, 2017; Iwamoto et al., 2010). *Vibrio cholerae* has also been found to survive in a wide variety of foods including rice, tea, coffee, millet gruel, as well as bakery, poultry, and dairy products and could pose a risk of cholera when contaminated with the bacterium (Hounmanou et al., 2016; Mrityunjoy et al., 2013; Tang et al., 2013). Fruits (except sour fruits) and vegetables when eaten raw or unwashed have also been associated with an increased risk of cholera (Moradi, Rasouli, Mohammadi, Elahi, & Barati, 2016).

The contamination of the vegetables or fruits may occur by contact with sewage or manure from the human origin on the farm. This has been reported in Lusaka, Zambia between November 2003 and February 2004, where an outbreak was linked to the consumption of raw vegetables (DuBois, Sinkala, Kalluri, Makasa-Chikoya, & Quick, 2006). A case-control study conducted by Dinede, Abagero, and Tolosa (2020) in Addis Ababa, Ethiopia similarly found the consumption of raw vegetables to be associated with cholera outbreaks. In the same study, drinking of 'holy water' at an orthodox church was also attributed to the outbreak (Dinede et al., 2020). The 'holy water is water blessed by the priest for religious purposes and fetched by the congregants and could have been contaminated in the process. Unhygienic practices of food handlers either at home or outside of the home may also result in the contamination of food. These poor hygienic practices are particularly common in developing countries where public health measures with regard to food are not adhered to. A cholera outbreak in Northwestern Thailand in 2010 was associated with the Hainanese chicken rice which was contaminated by the food handlers during food preparation and handling (Swaddiwudhipong, Hannarong, Peanumlom, Pittayawonganon, & Sitthi, 2012). The unhygienic handling of food is particularly more pronounced among food vendors on the streets who sell ready-to-eat foods.

Accra is highly urbanized with most of the population relying on food vendors for cheap and ready-to-eat foods. It is estimated that about 117 people eat from a vendor in a day in

Accra (Donkor, Kayang, Quaye, & Akyeh, 2009). Unfortunately, the majority of the food vendors are unlicensed and challenged in the practice of food safety. It is therefore not surprising that most residence in Accra contract cholera through the eating of food or drinking water from street vendors (Ohene-Adjei et al., 2017). This is further emphasized by Feglo and Sakyi (2012) who found higher than acceptable counts of enteric bacteria in ready-to-eat foods in Kumasi. Therefore, eating food from outside the home may play a major role in contracting cholera.

On occasions such as funerals, weddings, meetings, or conferences where there is public gathering cholera outbreaks can occur if the food served is contaminated. Acquah et al. (2016), demonstrated this during an outbreak in Medinya, in the Western region of Ghana where most of the people who ate 'fufu' and groundnut soup served at the wedding had contracted cholera. In times, where leftover foods are poorly stored and eaten unheated may increase the risk of cholera infection (Benjamin, 2018).

Vibrio cholerae can survive longer under refrigeration (<10^0C) than in ambient temperature. A study by Waturangi, Amadeus, and Kelvianto (2015) showed that *V. cholerae* survived in foods and beverages for more than two months at room temperature, more than three months under refrigeration, and even longer under frozen conditions (-20^0C). Therefore, refrigerating or freezing contaminated foods is not a guarantee of protection from cholera transmission. This is particularly critical when importing frozen foods from cholera endemic countries. In this current study, the food eating habits of people living in both cholera endemic and non-endemic communities were studied.

2.8.6 Environmental Sanitation

Cholera epidemics in Accra have been linked to improper sanitation including the poor management of waste (Anaman & Nyadzi, 2015; Ohene-Adjei et al., 2017). In Ghana, large tons of waste are produced daily due to the high population and rapid urbanization of our cities (Anaman & Nyadzi, 2015). In most communities in Accra, the waste companies are unable to cope with the large quantities of solid waste generated. Hence, oftenwaste dumpsites are left to overflow and if waste is not removed regularly, produces an odour that attract flies (Addo, Adei, & Acheampong, 2015).

Exposure of foods to these flies from the waste dumpsites could aid in the spread of diseases including cholera. Osei and Duker (2008b) used spatial statistical modelling to establish a direct relationship between cholera prevalence and proximity to refuse dumpsites. They suggested that waste dumpsites should be sited beyond a 500m radius from the households. In deprived communities where households cannot afford the high charges of waste collection companies, households resort to burying, burning, or indiscriminately dumping their waste in the gutters or along the roads (Anaman & Nyadzi, 2015). The result is that the non-biodegradable wastes are washed into the drains by rains to choke the gutters with the consequences of flooding. These floodwaters have the potential of contaminating water sources and could result in cholera outbreaks.

In the deprived communities in Accra, the majority of households lack access to a toilet in their homes and rely on public toilets or practice open defecation (Ghana Statistical Service, 2013). Yet others, are forced to use pan latrines or defecate into a plastic bag and throw ('flying toilets') into the environment. These are washed following heavy rains into the gutters with the possibility of contaminating the water net connection if the pipelines are broken. This study conducted an environmental assessment to understand the reasons for cholera persistence in the endemic communities.

2.8.7 Climatic factors

There is a strong link between climate change and human infectious diseases (Khan, Thi Vu, Lai, & Ahn, 2019; Wu, Lu, Zhou, Chen, & Xu, 2016). The commonly used climatic variables that significantly affect infectious disease transmission include; temperature, precipitation, relative humidity, and wind (Parham, Christiansen-Jucht, Pople, & Michael, 2011). These variables have been used extensively in vector and water-borne infections to make predictions and responses to control infectious diseases (Fouque & Reeder, 2019). However, their impact is not easily understood because of the other influencing factors besides the climatic factors. Nevertheless, mathematical models are developed based on climatic data and used to predict early warning signs to prevent outbreaks (Sofaer et al., 2017). A review of literature from 1974 to 2005 demonstrated the emergence of cholera outbreaks with varying seasonal patterns which increases the sea level and sea surface temperature (Emch, Feldacker, Islam, & Ali, 2008). Their findings support an earlier study in the Bengal region by Colwell (1996) who found warmer temperatures to increase the abundance of phytoplankton and therefore of zooplankton which serves as a reservoir for *V. cholerae*. de Magny et al. (2011), also found a relationship between increased zooplankton population and *V. cholerae*, hence the incidence of cholera. In that study, they associated the detection of *V. cholerae* with zooplankton such as rotifers, cladocerans, and copepods. Numerous studies also identified cholera variability to be related to the inter-annual climate oscillations (e.g., El Niño) (Constantin de Magny, Guegan, Petit, & Cazelles, 2007; De Magny, Cazelles, & Guégan, 2006). The inter-annual climate oscillations were explored by studying a 20-year monthly cholera time series data for five West Africa coastal countries including Ghana, Togo, Côte d'Ivoire, Benin and Nigeria. The researchers further examined the role of climate inter-annual variability on both global and local scales. In their study they found a significant association between cholera incidence and climate variability (Constantin de Magny et al., 2007).

Although rainfall periodically appears to be associated with cholera incidence in Ghana, this correlation is not spatially and temporally consistent. For example, Moore et al.

(2018) observed an extended lull period in cholera incidence from the climatic and cholera data analyzed from 2011 to 2014, despite the typically high temperatures and rainfall recorded along the coastal belt of the country. This work explored the risk of cholera transmission by analyzing the seasonal variation in water contamination levels in the drinking water and the determination of *V. cholerae* O1/O139 toxigenic strains in the aquatic environment in the selected communities.

2.8.8 Hygiene

Basic hygiene practices including handwashing are an important preventive measure against the transmission of infectious diseases. Handwashing with soap before meals and after defecation for instance has been found to be protective against cholera (Wolfe, 2018). However, there is low access to basic handwashing facilities at homes, schools, or public toilet facilities to promote this (Appiah-Brempong, Harris, Newton, & Gulis, 2018; Peprah et al., 2015). A study to assess the hand hygiene habits of 254 youth in Accra, Oppong, Yang, Amponsem-Boateng, and Duan (2019) found that only 22% of the youth practice handwashing after outings while 51.6% washed after using the bathroom.

In the absence of flowing tap water in households or school environments a 'bowl of water is provided for hand washing (Tetteh-Quarcoo et al., 2016). This practice is common and not protective since it creates the opportunity for the transmission of microbes washed into the water.

Among some individuals, the preference is to wash with soap after eating or defecation. These were the findings of a study of some communities in Bangladesh where handwashing with soap before eating and after defecation was much lower (Rabbi & Dey, 2013). It was also observed in a multivariate analysis that handwashing with soap was strongly associated with socioeconomic factors such as; education of household heads, availability, and access to water and soap (Rabbi & Dey, 2013). In this study, handwashing habits were studied in the communities.

2.8.9 Water, Sanitation and Hygiene Interventions related to Cholera

Water, sanitation and hygiene (WASH) is a strategy adopted by WHO to reduce the burden of cholera in the endemic or hotspot areas (WHO, 2018d). While sufficient WASH is acknowledged as being essential to avoiding cholera, it is frequently viewed as a long-term, expensive intervention that poor countries cannot quickly implement (WHO, 2018d). In order to quickly control the disease during an outbreak, some nations have relied on temporary treatments like the use of oral cholera vaccine which are costly and unsustainable (Chaignat, 2008).

Adopting long-lasting WASH measures is crucial for the elimination of cholera (Lantagne, Bastable, Ensink, & Mintzd, 2015). Accordingly, the World Health Assembly (WHA) decided in 2011 to collaborate with the Global Task Force for Cholera Control (GTFCC) to eliminate cholera, particularly in cholera hotspot areas (WHO, 2018d). The WHO Global Cholera Elimination Plan, which seeks to reduce cholera mortality by 90% by 2030, has over 50 partners as of October 2017 (WHO, 2018d). Their agenda is to help nations accomplish the Sustainable Development Goals (6.1 and 6.2) that call for achieving equal and universal access to clean water and sanitation (WHO, 2018d).

The main WASH programmes involved enhancing access to "improved" water supplies, assuring microbiological water safety, lowering inequities, maintaining sustainability in households and institutions, and ending open defecation (Lantagne et al., 2015). In the meanwhile, a comprehensive plan to stop the spread of cholera must include WASH initiatives at the community and household levels (Lantagne et al., 2015). To fully utilize the initiative, regular monitoring and evaluation of the WASH interventions is required. The current study investigated the risk factors for cholera in endemic and non-endemic communities and offered recommendations in conjunction with WASH to prevent cholera and other diarrhoeal disease epidemics.

2.9 Spatial distribution of cholera

Geographic Information System technology is a tool that is used to process, analyze and visualize spatial data in environmental health, disease ecology, and public health (Kistemann, Dangendorf, & Schweikart, 2002). This concept of spatial distribution was first demonstrated by John Snow during the London cholera outbreak in 1855 (Brody, Rip, Vinten-Johansen, Paneth, & Rachman, 2000). In his study, Snow was able to use spatial patterns of cholera cases which he represented with dots to illustrate how the cases clustered around the contaminated water sources. Thus, was able to determine the cause of the outbreak. This happened before the discovery of the cholera bacterium by Robert Kock in 1884. After the discovery of *V. cholerae*, most studies on cholera were related to the biological and pathogenic characteristics of the bacterium and could not accurately detect the individual exposures and the high-risk geographical areas. However, with the advent of the GIS technology disease incidence of cases can accurately be identified by location for immediate public health action. Geographic Information System has also been used to integrate multiple layers of spatial data in different disciplines such as health, environmental, genetic, socio-demographic risks and can establish relationships using different spatial analysis including; proximity calculation, assessment of clustering, spatial smoothing, interpolation and spatial regression (Auchincloss, Gebreab, Mair, & Diez Roux, 2012). Many studies have employed proximity measures and spatial statistical methods to establish the relationships between cholera and environmental risk. Osei and Duker (2008b) explored the proximity and density of open refuse dumps. In the study, they used spatial statistical modelling to demonstrate that proximity and density of open refuse dumps played a role in cholera transmission in Kumasi. They indicated that open refuse dumps serve as niches for cholera infection and individual households living within a 500 m radius were more at risk of cholera infection. Their findings are similar to that of Olanrewaju and Adepoju (2017), who also found cholera to occur close to waste dump sites near markets, rivers/streams, and abattoirs. In another related research, Osei and Duker (2008a) used spatial and statistical analysis to show that high cholera cases were clustered around Kumasi Metropolis and further indicated high urbanization and overcrowding to be important predictors of cholera in the Ashanti Region. Spatial analyses have also been used to predict cholera occurrence and drinking contaminated water. Olanrewaju and Adepoju (2017) demonstrated this in a composite risk cholera map where cholera 'hotspot' areas recorded high coliform counts in their drinking water source. Thus, suggesting that faecal contamination of drinking water sources predisposes communities to cholera infection.

Although GIS technology has been used to identify risks factors for cholera, this has often been done during outbreaks to show how cases clustered. This study used spatial analysis to produce composite risk maps for cholera in both endemic and non-endemic coastal communities in the GAR. By mapping the cholera risk factors in geographical space, local authorities will be able to easily identify, make a prediction of potential future outbreaks and appropriately plan with the necessary resources.

In summary, there is evidence that cholera outbreaks are linked to drinking water quality, environmental-related factors, socioeconomic and behavioral factors. Geographical Information System is a useful tool that could be used to spatially map the distribution of these factors in relationship to cholera cases for future control of outbreaks.

CHAPTER THREE

METHODS

3.1 Study Design

A community-based cross-sectional comparative design was used for this study. The study area was classified into two communities; cholera endemic and cholera non-endemic. The cholera endemic communities were cholera ‘hotspots in the Accra Metropolitan Area, Ledzokuku, and Krowor Municipal Areas which over four years (2011 to 2014) have recorded high cholera cases with high attack rates. The cholera non-endemic communities were the Ningo Prampram, Ada West, and East districts that have rarely recorded any case of cholera over the same period. Households were randomly selected and data on socioeconomic, water supply, solid and liquid waste disposal and behavioural factors about food safety were collected from the household heads or their adult representatives using a questionnaire. Drinking water from selected households was sampled for bacteriological water quality assessment. An environmental assessment was also done using an environmental health inspection guide and risk factors including; water bodies, markets, public toilets, and refuse disposal sites mapped using GIS. The water bodies were sampled for the presence of *Vibrio cholerae* O1 and O139 toxigenic strains. The study was conducted in the wet (April to August 2019) and dry seasons (January to March 2020).

3.2 Study Area

The study was carried out in the Greater Accra Region (GAR), one of the 16 administrative regions of Ghana. It is located in the south-central part of the country and bordered by the Central Region to the west, Volta Region to the east, Eastern Region to the north, and the Gulf of Guinea to the south (Figure 3.1). It is the smallest of the 16 regions, occupying a total land surface of 3,245 square kilometres or 1.4 percent of the total land area of Ghana with estimated population to be 5,055,805 (Ghana Statistical Service, 2014). The capital city of Ghana, Accra is also located in the region with a daily influx of people from within and outside the country.

The region is the most urbanized and has the highest population density in the country (Ghana Statistical Service, 2013). There are highly populated slums, squatter, and informal settlements in parts of the region due to the rapid urbanization being experienced (Accra Metropolitan Assembly and UN Habitat, 2011; Amoako, 2016). The region has 29 districts of which two are metropolitans; namely Accra and Tema and 23 municipalities including; Ablekuma Central, West and North, Adenta, Ashiaman, Ayawaso East, Central, North and West, Ga Central, East, North, South, and West, Korle Klottey, Kpone Katamanso, Krowor, La Dade Kotopon, La Nkwantanang, Ledzokuku, Okaikoi North, Tema West, Weija Gbawe and four ordinary districts; Ada East and West, Ningo Prampram, and Shai Osudoku as shown in Figure 3.1.

The region is endowed with many tourist attractions including forts, Kwame Nkrumah Mausoleum, outstanding beaches, estuaries that attract tourists to the region. There are diverse ethnic groups with the predominant group being the Akans followed by the indigenous Ga-Dangmes (Ghana Statistical Service, 2013).

With regards to occupation, the majority of the people living in the urban areas are engaged in trading, transport business with a larger concentration of professional and technical workers whereas, farming, animal husbandry, and fishing are the predominant occupation of the people in the rural communities (Ghana Statistical Service, 2013). Salt mining is common among inhabitants living along with the tributaries of the Volta River where the soil is waterlogged and salty (Ghana Statistical Service, 2013).

The region has a coastline stretching the entire southern end of the region from Kokrobite in the west to Ada in the east. The region is located in the dry equatorial belt with coastal savannah vegetation. The average temperatures of the region range between 24.7°C and 33.0° C (Ghana Statistical Service, 2014). The highest temperatures are recorded in April and the lowest in August. The region is the driest in the country with annual rainfall ranging between 635 millimetres along the coast to 1,140 millimetres in the northern parts (Ghana Statistical Service, 2014). There are two rainfall seasons, the major occurring from April to July and the minor from September to November, however peaking respectively in June and October (Ghana Statistical Service, 2014). The driest months of the year are between November and April.

The region has low-lying areas which are usually subjected to severe perennial flooding following heavy rains due to the poor drainage system and choked gutters (Ghana Statistical Service, 2013). This has the potential of contaminating water pipelines and spreading diseases. The river Densu and Volta are the two main rivers flowing through the region but have tributaries flowing into the sea through Lagoons. The most important of these are the Odorna river and Korle Lagoon which is highly polluted and located in the Accra Metropolis (Aglanu & Appiah, 2014).

The majority of the households do not have toilet facilities at their homes and use public toilets (Ghana Statistical Service, 2013). Others living along the beaches drains, refuse dumps, and open spaces engaged in open defecation (Ghana Statistical Service, 2013). Solid waste disposal is generally poor and usually dependent on the availability of vehicles to convey the waste to the field sites (Ghana Statistical Service, 2013). This usually results in solid waste overflowing and posing a health risk to the public. Clean water supply is not regular in some communities and they rely on vendor supply for their domestic chores (Ghana Statistical Service, 2013).

The GAR has been the epicenter for most cholera epidemics in the country since the first case of cholera was reported in 1970. Since 2004 the Accra Metropolitan Assembly (AMA) and Ledzokuku Krowor Municipal Assembly (LEKMA) have persistently reported cholera cases almost every year (Ghana Disease Surveillance Department, 2015). The Disease Surveillance Department of the Ghana Health Service identified communities where the outbreaks usually begin with the highest recorded cholera cases and attack rates as cholera 'hotspot' communities. These communities are in AMA and LEKMA were selected as the cholera endemic sites for the study while communities from Ningo Prampram, Ada West, and East districts which rarely recorded a case of cholera and also located along the coastal belt selected as non-endemic communities.

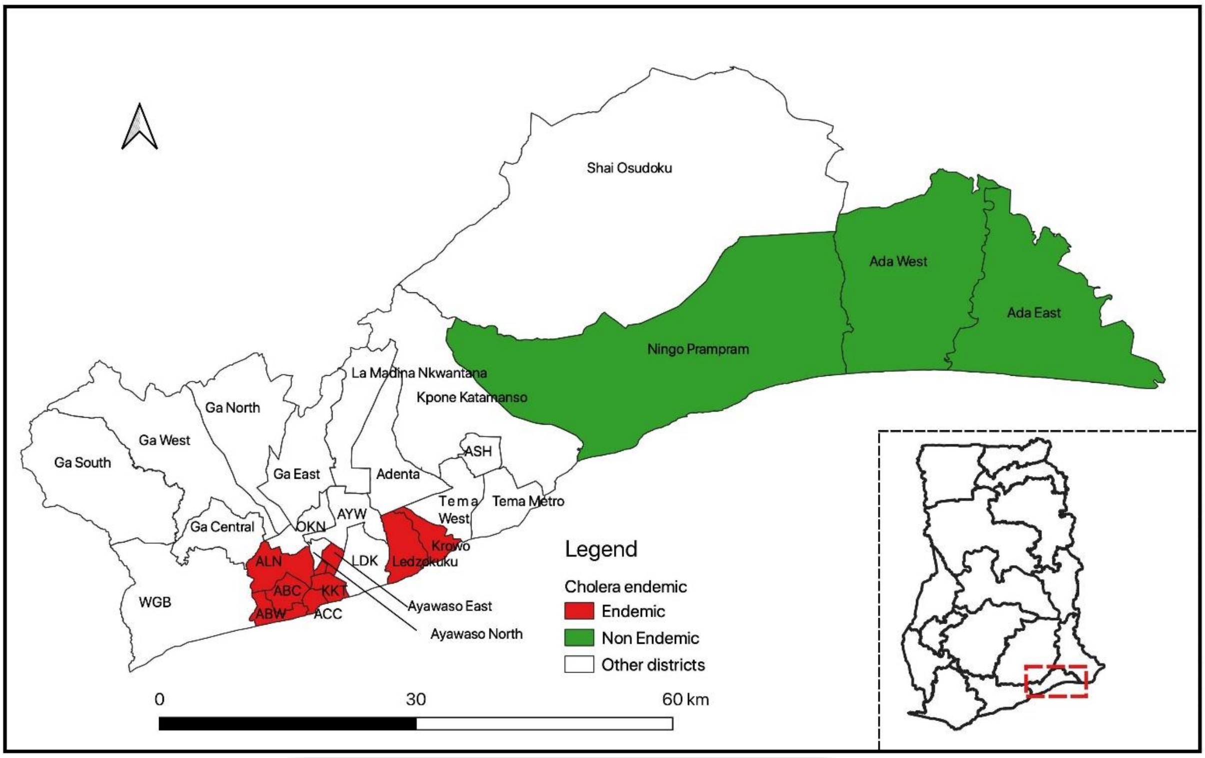

Figure 3.1: A Map showing the endemic and non-endemic areas in the Greater Accra Region

ALN-Ablekuma North, ABC-Ablekuma Central, ABW-Ablekuma West, ACC-Accra Metropolis, KKT-Korle Klottey, WGB-Weija Gbawe, OKN-Okaikoi North, AYW-Ayawaso West, LDK-La Dade Kotopon, ASH-Ashaiman

3.2.1 Cholera Endemic Communities

Twelve (12) communities were identified from the AMA and LEKMA based on the regular high burden of cholera with evidence of local transmission over the past four years (2011 to 2014) (Ghana Disease Surveillance Department, 2015; WHO, 2018c). These communities are; Agbogbloshie and James Town in the Ashiedu Keteke Sub Metropolis; Adabraka in the Korle Klottey Sub Metropolis; Kaneshie in the Okaikoi South; Maamobi and Nima in the Ayawaso Sub metropolis; Chorkor and Mamprobi in the Ablekuma South Metropolis; Agege and Dansoman in the Ablekuma West Sub Metropolis; Teshie in the Ledzokuku Municipality and Nungua in the Krowor Municipality (Figure 3.2).

Figure 3.2: Cholera ‘hotspots’ or endemic communities in the Greater Accra Region

3.2.2 Cholera Non-endemic Communities

The non-endemic communities were communities selected from Ningo Prampram, Ada West, and East districts which rarely recorded a case of cholera with no evidence of local transmission for the past four years (2011 to 2014)(WHO, 2018c). These communities were 12 in number, viz. Dawhenya North, Kofikope, Awhiam, and Dawa in the Ningo Prampram district; Addokope, Dogobom, Anyaman East, Adjumanikope in the Ada West district; Adedetsekope, Angomya-Ada, Kophemm Kasseh and Asigbekope in the Ada East district.

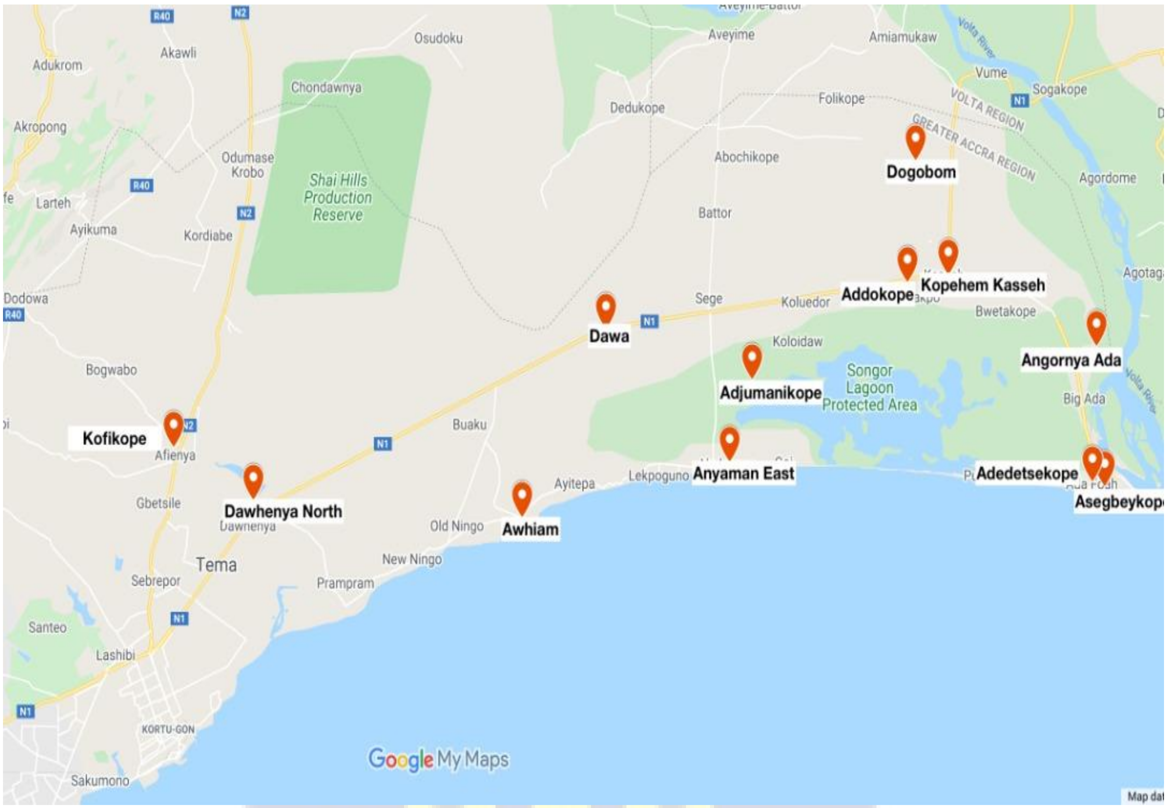

Figure 3.3: Cholera non-endemic communities in the Greater Accra Region

3.3 Study Population

The study population was the population of households living in the Greater Accra Region at the time of the study.

3.4 Study Variables

The dependent or outcome variable was 'cholera' defined based on the surveillance suspected or confirmed case definition as in table 3.1(a) and reported by household members past experience. 'Cholera' which is binary was recorded as 'Yes' if household member ever had cholera and 'No' if household member never had cholera. The independent variables were categorized into three main areas and described as: socioeconomic factors (age, sex, ethnicity, religion, occupation, educational level, income level, wealth index and house occupancy, residential unit); behavioural factors (solid and liquid waste disposal practices, food and water safety habits); environmental factors (availability of aquatic reservoir, toilet facilities, waste dumpsites, markets, and abattoir) and water sanitation factors (water source, water quality, water distribution network). All the variables are summarized in Table 3.1(a,b,c).

Table 3.1a: Operationalization of the Key study variables

Variables	Operational Definition	Level of Measurement	Categories
Dependent variable			
Cholera	A patient 2 years and above with acute watery diarrhoea with or without vomiting and severe dehydration or dies of acute diarrhoea or a patient of any age with acute watery diarrhoea from whom *V. cholerae* (O1 or O139) was isolated from faeces during illness by culture or PCR.	Dichotomous	Yes No
Independent variables			
Age	Age of respondent in completed years at the time of the study	Discrete but was categorized	18-27, 28-37, 38-47, 48-57, 58-67, >68
Sex	Respondent being identified as male or female	Dichotomous	Male, Female
Marital status	Respondent relationship with a significant other	Nominal	Single, Married, Cohabit, Separated, Divorced, Widowed
Religion	Religious affiliation of the respondent	Nominal	No religion, Christianity, Islam, Traditionalist, others
Ethnicity	Identified group with similarities such as culture, language or history that the respondent belongs	Nominal	Akan, Ewe, Ga Dangme, Guan, Mole-Dagbon, Others
Employment	Refers to the current work position of a respondent	Nominal	Unemployed, Retired worker, Formal employment, Informal employment
Level of Education	The highest level of formal school that a respondent ever attended or was attending	Nominal	No formal Education, Primary Education, JSS/Middle School Education, Senior High Education, Tertiary Education
Income level (ranked)	All money received from salary, wages or own business as well as money from additional work activities, remittances from family elsewhere, pensions or grants	Ranked or ordinal	Low income (≤ GhȻ300), Medium income (> GhȻ300 - ≤ GhȻ1000), High income (> GhȻ1000 - ≤ GhȻ2000), Highest income > GhȻ2000

Table 3.1b: Operationalization of the Key study variables

Variables	Operational Definitions	Level of Measurement	Categories
Residential unit	Refers to the various types of dwelling	Nominal	Separate house, Semi-detached, Flat/apartment, Compound house (rooms), Tent, Improvised house (Kiosk/container) Living quarters attached to office/shop, Uncompleted building, others
Household occupancy	Number of people living in the household	Discrete and categorized	1-3, 4-6, ≥7
Drinking water source	Source of water for drinking and domestic chores	Nominal	Private pipe, public pipe, borehole, protected well, unprotected well, water-carrier/tanker/vendor, surface water (dam/river/stream/spring), Other water sources
Drinking water quality	Drinking water that is free from harmful microorganisms	Nominal	Private pipe, public pipe, borehole, protected well, unprotected well, water-carrier/tanker/vendor, surface water (dam/river/stream/spring), Other water sources
Water safety	Water handling and storage habit	Nominal	water treatment at the point of used, water storage in clean containers, storage vessels covered
Food safety	Food handling, storage and eating habit	Nominal	Treatment of leftover food before eating, handwashing before meals, washing of fruits before eating, storage of cooked food, eating home prepared food
Household waste disposal	Measures taken for the safe transportation and disposal of household waste	Nominal	Waste collected once a week, Waste collected irregularly, burned by households, public dump (container), public dump (open space), dumped indiscriminately, Buried by household, Others
Human waste disposal	Measures taken for the safe collection of by-products of digestion such as faeces and urine	Nominal	No toilet facilities, Water closets, Pit latrine, KVIP, Bucket/Pan, Public toilet (WC, KVIP, Pit pan/etc), others

Table 3.1c: Operationalization of the Key study variables (cont'd)

Variables	Operational Definitions	Level of Measurement	Categories
Liquid waste disposal	Measures taken for the safe disposal of liquid waste	Nominal	Through the sewage system, Through the drainage system into the gutter, through drainage into a pit (soak away), Thrown onto the street/outside
Aquatic water reservoir	The availability and closeness of water bodies (river, stream, sea, others) to the community	Dichotomous	Proximity to cholera cases, No proximity to cholera cases
Public refuse dumpsite	The availability and closeness of refuse disposal site to the community	Dichotomous	Proximity to cholera cases, No proximity to cholera cases
Markets	The availability and closeness of markets to the community	Dichotomous	Proximity to cholera cases, No proximity to cholera cases
Abattoirs	The availability and closeness of an abattoir to the community	Dichotomous	Proximity to cholera cases, No proximity to cholera cases

3.5 Sample Size Determination for households

The sample size was calculated using Cochran's formula given (Cochran, 1963).

$$N = \frac{z^2 \, p(1-p)}{e^2}$$

where,

N = desired sample size of the households to be interviewed

p = proportion of households that reported a case of cholera in a pilot study conducted at Ashongman community in the Ga East Municipality =23.0%

1-p = the proportion of households that did not report a case of cholera

z = the standard normal deviation (set at 1.96 corresponding to the 95% CI)

e = Precision or degree of accuracy desired (set at 0.05)

$$N = \frac{(1.96)^2 \times 0.23(1-0.23)}{(0.05)^2}$$

N= 272.02

A design effect of 1.5 was assumed because there is no information in the study area. It is meant to produce a larger sample size than what will be expected with a simple random sampling method.

272.02 X 1.5 = 408.03

However, adjusting for a non-response rate of 10% (Awalime, Davies-Teye, Vanotoo, Owoo, & Nketiah-Amponsah, 2017)

The total sample size was therefore estimated as follows;

N= 408.03/ (1-0.10) = 453.37 = 453.37. This was rounded up to 453 as the minimum sample size of households selected for the endemic communities.

The sample size for each community was then calculated based on proportional representation as indicated in Table (3.2 & 3.3)

where,

'n' represents the sample size of the households for the study area

'Nh' represent the household population of each community population

'N' the total household population in the study area

'n_1' represents the sample size of the households to be selected

i.e $n_1 = \frac{Nh \times n}{N}$

Table 3.2: Population of households as of 2014 in the endemic communities

Community	Household Population	Sample size (proportionate to size)
Agbogbloshie	43, 121	32
James Town	17, 936	13
Adabraka	40, 369	30
Kaneshie	34, 433	25
Maamobi	68, 248	50
Nima	89,388	66
Chorkor	29, 694	22
Dansoman	62,593	46
Agege	10, 162	7
Mamprobi	28,585	21
Teshie	111, 785	82
Nungua	80, 796	59
Total	617,110	453

Source Ghana Statistical Service, 27th June, 2018

The sample size for the non-endemic communities were similarly calculated as shown in table 3.3.

Table 3.3: Population of households as at 2018 in the non-endemic communities

Community	Household Population	Sample size (proportionate to size)
Ningo-Prampram District (151)		
Kofikope	5173	42
Awhiam	4155	34
Dawhenya-North	5440	44
Dawa	3876	31
	18,644	
Ada West District (151)		

Addokope	123	35
Dogobom	89	25
Adjumanikope	164	47
Anyamam East	156	44
	532	
Ada East District (151)		
Angomya-Ada	535	28
Kopehem Kasseh	785	41
Asigbeykope	868	46
Adedetsekope	691	36
	2879	
	Total	**453**

Source District Health Directorate Populations, 2018

3.6 Sample size Determination for household drinking water

To determine how many drinking water samples of the selected household to sample the Cochran's formula was used;

$$N = \frac{z^2\, p(1-p)}{e^2}$$

Where,

N = desired number of water samples to collect from the communities,

p = proportion of households drinking water contaminated with *V. cholerae* as 83.8% reported in Accra Metropolis (Yirenya-Tawiah et al., 2018),

e = Precision or degree of accuracy desired (set at 5%)

z = Standard normal deviation (set at 1.96 corresponding to the 95% CI)

$$N = \frac{(1.96)^2 \text{ X } 0.838(1-0.838)}{(0.05)^2}$$

N= 208.608

The minimum sample size was 209 for each of the communities. Therefore, for both dry and wet seasons in the endemic and non-endemic communities, total sample size was estimated to be 420.

3.7 Sampling Technique

This study employed a multistage cluster sampling approach. A cluster represented cholera 'endemic' or 'non-endemic community. A three-stage sampling technique was used in the endemic community. The first stage represented the selection of all twelve (12) clusters from the endemic communities which included; Agbogbloshie, James Town, Adabraka, Dansoman, Agege, Mamprobi, Chorkor, Kaneshie, Nima, Maamobi, Teshie, and Nungua were purposively selected based on their identification as cholera 'hotspots' (Ghana Disease Surveillance Department, 2015).

The second stage involved the listing of all the Ghana Health Service Community-based Health Planning and Services (CHPs) zones in each of the endemic communities and random selection of one CHPs zone by ballot. This was necessary because of the large size of the communities.

A systematic sampling procedure was used in the third stage to select the households. A household was defined as a person or group of people who lived in the same dwelling and shared meals during the survey period. Each of the selected clusters were mapped by drawing to show the various important landmarks such as health Centre, church, mosques, school, market, major roads or public toilet.

The centre of each cluster was located and a random direction was obtained by spinning an empty bottle. The households along the direction indicated by the bottle top were followed in a straight line, numbering the houses on both sides until the boundary of the sector was reached. A sampling interval was first determined for each community by dividing the total number of households in the CHPs zone by the number of households to sample.

From the listed number of houses obtained, a random household number was selected as the starting point for inclusion in the study and then the sample interval to locate the next household to sample. This continued until the calculated minimum sample size for each cluster was obtained. In situations where there was more than one household in a house as in a compound house or flats only one household was selected by a ballot. If there was no qualified respondent the interviewer went to the next house that has the closest door and checked for a qualified respondent. This continued until an eligible respondent was found.

In cholera ‘non-endemic communities, a two-stage approach was employed. The first stage was the selection of four communities each from the three cholera non-endemic districts (Ningo Prampram, Ada west, Ada East) in the GAR. All 12 clusters were randomly selected by a ballot using the list of all the communities from each of the three cholera non-endemic districts that rarely reported a case of cholera over the same period (2011 to 2014) (Ghana Disease Surveillance Department, 2015). The selected communities were; Addokope, Dogobom, Adjumanikope, Anyaman, Adedetsekope, Agomya-Ada, Kopehem-Kasseh, Asigbeykope, Awhiam, Dawa, Kofikope, and Dawhenya North.

A systematic sampling procedure was similarly used in the second stage to select the households as described in the endemic communities. The sampling and recruitment were done by the researcher and the research assistants.

3.8 Inclusion Criteria

Participants who were eligible for inclusion in this study were;

1. The head of households or a senior member of the family (above 18 years)
2. Members of the household living in the community before 2015 to the time of the study.

3.9 Exclusion Criteria

1. Visiting adults were not allowed to act in the absence of the head of household

3.10 Data Collection Method/Technique and Tools

The research was conducted from March 2019 to March 2020. All stakeholders including the Disease Surveillance Department, Accra Regional Health Directorate, Accra Metropolitan and Sub-metropolitan health Directorates, Accra Environmental Health, and the respective District Health Directorates that were sites for this study were engaged to solicit their inputs during the proposal development phase of this work. Site visits were also conducted to meet with some community leaders including chiefs and assemblymen/women to inform them of the objective of the study. The Public Health Nurses, Community Health Nurses and Disease Control Officers serving in the study sites served as liaisons between the researchers and the community to facilitate easy entry into the communities.

A questionnaire designed specifically for this study was used to collect data from the household members about their sociodemographic factors, water sanitation factors, solid waste, and sewage disposal factors, and behavioural factors. The socioeconomic characteristics section of the questionnaire was adopted from the Demographic and Health Survey (DHS) questionnaire and modified.

The other sections of the questionnaire were designed by the researcher with expert guidance from public health and environmental health specialist. The questionnaire comprised a series of questions to be filled in by the research assistants during the interview with the participant. The questionnaire included both open-ended and closed-ended questions. The open-ended questions allowed the participants to respond to questions in their own words while the closed-ended questions gave alternative responses for the participants to select. The questionnaire was written in English and interpreted by the researcher or research assistant in Twi, Ga, or Ewe if the respondents did not understand or write English.

During the administration of the questionnaire, the research assistants first introduced themselves to the participants and explained the purpose of the study. The participants were then assured of their confidentiality and anonymity. Consent was sought and participants signed or thump printed an informed consent form and another community member or the research assistant witnessing.

3.11 Environmental Assessment

An onsite sanitation inspection guide adopted from Cheesbrough (2006), modified and pretested at Ashongman community in the Ga East Municipality by the researcher was used for the assessment of the water supply pipelines (availability of exposed pipelines, accumulation of water around the pipelines, pipelines laid in open drains/gutters, broken pipelines); refuse disposal (availability of waste dumpsite/landfill site, refuse dump container overflowing, flies around refuse dumpsite, refuse dumpsite near a water source); Sewage disposal (choked gutters with waste, indiscriminate disposal of sewage, evidence of sewage disposed into water body); Excreta disposal (availability of public toilets, flies hovering around toilet facility, evidence of open defecation) in the environment of the households in the endemic and non-endemic communities.

In addition, water from surface water bodies including rivers/streams, dams, lagoons, and estuaries located in the selected communities was also collected for microbial analysis.

The GIS coordinates and photographs of potential environmental cholera risk factors were taken.

3.12 Water Sample Collection

Households' drinking water samples were collected for bacteriological quality analysis. The households from which samples of water were collected were randomly selected by listing all the households in each of the communities and balloting to select nine households for each of the 24 sites in the endemic and non-endemic communities. One additional sample was purposively collected from the main drinking water source point (e.g public tap, pipe, or dam) as a control sample in each community. The water samples were collected in the wet season (April to August 2019) and repeated in the dry seasons (January to March 2020) to determine the temporal variation in water quality.

About 500 ml of the stored household drinking water was sampled using the container usually used to scoop water from the storage container into a sterile plastic bottle. In case the household drank sachet water they were collected and a selected edge of the sachet water decontaminated by cleaning with 70% ethanol, then cut aseptically with sterile scissors and the water poured into the sterile plastic bottle. On the other hand, if the household drank bottled water, they were only collected and labelled. The water samples from the source point were directly collected from the pipe, standpipe, dam, or well.

The collection of water from the tap or pipe water was done by cleaning the outside of the tap with 70% ethanol and allowing flow at maximum for about a minute before directly collecting into the sterile plastic bottle (Cheesbrough, 2006). A drop of sodium thiosulphate (0.5 ml of 10% solution) was added to the pipe-borne water to neutralize residual chlorine before transporting it to the laboratory (Cheesbrough, 2006).

Environmental water samples were collected from the water bodies (lagoons, estuaries, rivers or streams, and dams) located in the selected communities. These environmental water samples were collected manually by attaching a string to a 500 ml Wheaton sterile glass bottle and directly lowering it to a depth of about 30 centimetres in the water body and allowed to fill before redrawing as described by Cheesbrough (2006). The river or streams water samples were collected in the up and down streams. The dams and lagoons water samples were collected at multiple points, whiles the estuarine water was sampled from where the lagoon or river meets the seawater. Coordinates of these sampling points were recorded by GPS to ensure the water samples were collected at the same point in both the dry and wet seasons.

The water samples collected from the field were transported in insulated boxes at 4^0C to the laboratory within six hours. In case of a delay, the water samples were stored in the refrigerator (2^0C to 8^0C) and processed the next day (within 24 hours).

3.13 Laboratory Analysis

3.13.1 Bacteriological Quality of water

3.13.1.1 Membrane Filtration Method

The water samples were analyzed in accordance with the American Public Health Association (APHA) standard methods for examining drinking and wastewater using the membrane filtration method as described by Eaton et al. (2005).

3.13.1.2 Media Preparation and Selection

The appropriate media including; Eosin Methylene Blue (EMB) agar, Violet Red Bile (VRB) agar, Plate Count Agar (PC), Thiosulfate-Citrate-Bile-Sucrose (TCBS) agar, Tryptone Soy Agar (TSA), MacConkey Agar (MAC), were prepared following the manufacturer's instructions. The prepared media was dispensed into 25 ml sterile plastic disposable petri dishes and allowed to set. They were dried in a hot air oven set at 55 to

60^0C for about 30 minutes to sufficiently dry before being used. Buffered peptone water was also prepared and sterilized in bottles for use in the dilution of the water samples where the growth was uncountable.

3.13.1.3 Set up of the membrane filtration system

The membrane filtration system consists of a funnel or filtration apparatus connected to a vacuum source/pump and a suction flask. The apparatus was sterilized with 70% ethanol and to ensure sterility, placed or mounted in the laminar flow hood. A membrane filter of 0.45µm was aseptically removed from the sterile package and place over the porous plate of the filtration receptacle with the grid side up. The funnel unit was carefully placed over the receptacle and locked with a clamp. About 100 ml of water was poured directly into the filtration funnel. In instances that the growth was heavy and uncountable (i.e greater 200 colony count), 10 ml, 1 ml, 0.1 ml, or 0.01 ml of the water was used and diluted with buffered peptone water to make up the 100 ml before filtration. The pump was turned on to filter the sample under partial vacuum with the filter still in place and sample allowed to be drawn completely but retaining any bacteria on its surface. Forceps were flamed and aseptically used to transfer the filter unto a well labelled sterile petri dish with the appropriate media (PC agar, VRB agar, EMB agar, and TCBS). The filtration funnel was decontaminated with 70% ethanol and the vacuum turned on to allow the liquid to be drawn completely out before repeating with another sample.

The inoculated PC and VRB agar were incubated at 36^0C±1, while the EMB and TCBS were incubated at 44^0C±1. The plates were observed after 24 hours and 48 hours. The plates for PC and VRB were counted and estimated respectively for the total viable count and total coliform. The EMB was estimated for faecal coliforms (thermotolerant coliforms) and TCBS for suspected *Vibrio* species.

3.13.1.4 Counting of colonies on the Plates

After the incubation period, the grid pattern of the sterile membrane filter was used as a guide for rapid counting of the colonies with the aid of a hand lens. The colony density was estimated using the formula;

$$\text{Colonies/100 ml} = \frac{\text{Number of colonies counted}}{\text{mL of sample filtered}} \times 100 \text{ ml}$$

The ‘mL of sample filtered’ refers to the actual sample volume and not the diluted.

3.13.1.5 Sub-culturing of colonies

The morphological characteristics of the colonies on the filter membrane were described. Colonies that appeared as; large, smooth, yellow, slightly flattened, and translucent peripheries on the TCBS medium were presumptively identified as *Vibrio cholerae*. These colonies were sub-cultured onto another TCBS plate for purification. The suspected *Vibrio* colonies were further sub-cultured on TSA (a non-selective media) for biochemical testing and serotyping. Other suspected coliform bacteria growing on the PC agar, VRB, EMB were sub-cultured on MAC plate for purification. Distinct colonies were sub cultured on TSA for presumptive identification and biochemical testing.

3.13.1.6 Confirmation of *V. cholerae* serotype

To confirm the *V. cholerae*, the suspected isolate was first serotyped with polyvalent antisera type-specific O1 and O139 (Denka Seiken Co., Ltd, Tokyo, Japan). The agglutination test was done by picking the suspected *V. cholerae* colonies and emulsified them in a drop of sterile distilled water on clean glass slides. The slide was then thoroughly mixed by tilting back and forth for 30 seconds. The suspension was then examined to ensure the suspension is smooth and without any agglutinins. A drop of polyvalent O1 antisera was added to the suspension and mixed on the slide by tilting back and forth and observed for the presence or absence of agglutination. For a positive reaction, strong clumps appeared within 30 seconds to 1 minute. This process was repeated with polyvalent antisera for O139. If polyvalent O1 antiserum was positive, the specific serotype was confirmed using monovalent Inaba and Ogawa antisera.

3.13.1.7 Biochemical testing of colonies

In addition to a Gram stain test that was performed on the pure culture isolates, the conventional method of bacteria identification was carried out by testing isolates for catalase, oxidase, motility, Indole, and ornithine decarboxylase activity as well as carbohydrate fermentation on Triple Sugar Iron (TSI) agar. The Gram-Negative bacteria which could not be identified by the conventional biochemical test were subsequently identified using Mini Antigenic Profile Index for Enterobacteriaceae (API 20E).

3.13.1.8 Processing of the Antigenic Profile Index 20E Biochemical Test

The Antigenic Profile Index (API) 20E is a standardized identification system for Enterobacteriaceae and other non-fastidious, Gram-Negative rods which uses 21 miniaturized biochemical tests and a database (bioMérieux, France) was used. It consists of 20 microtubes containing dehydrated substrates. A single test isolate was emulsified in an ampule of 5 ml of API sodium chloride (0.85%) medium to achieve a homogeneous bacterial suspension. The bacterial suspension was distributed with a sterile pipette into the 20 microtubes on the API strip and incubated at 36 ± 2^{0}C for 18 to 24 hours. The strip was read with the reading table. All the microtubes that showed spontaneous reactions were recorded and those microtubes that required the addition of reagents to determine; tryptophan deaminase (TDA), indole (IND), and acetoin (VP) production were added and also read.

The tests were separated into three groups and a value of 1, 2, or 4 was indicated for each. By adding together, the values corresponding to positive reactions within each group, a 7-digit profile number was obtained for the 20 tests on the API strip. The oxidase test which was performed first constitutes the 21st test and had a value of 4 if it was positive. The identification was performed by keying in the 7-digit numerical profile in the API 20 E database (bioMérieux, France) and bacteria identified.

3.13.2 Genomic DNA Extraction

All strains of *V. cholerae* isolated from the culture and directly from the environmental water samples collected from rivers, dams, lagoons, and estuaries were identified and confirmed for the presence of *omp*W, O1*rfb*, O139*rfb*, and *ctx*A genes.

3.13.2.1 Extraction of *V. cholerae* DNA from Environmental water samples and broth Culture

The environmental water samples were concentrated by filtering 250 ml of the samples aseptically through a 0.22 µm pore size bacteriological membrane filter (Millex GP Syringe filter Unit). The retained contents of the membrane were placed in a 2 ml sterile microcentrifuge tube and 200 µl of phosphate-buffered saline (pH 8.0) was added. It was vortexed for 5 minutes at full speed to wash the filter paper and the DNA extraction was carried out using the Zymo Research DNA extraction kit (D3024, USA). The filter paper was removed and 800 ml genomic lysis buffer was added to the tube (4:1, i.e 800 µl genomic lysis buffer to 200 µl of phosphate-buffered saline (PBS)). It was vortexed again at full speed for 10 minutes and incubated for 10 minutes at room temperature. The contents were carefully transferred into the Zymo-Spin Columns in a collection tube without wetting the rim. The tubes were centrifuged at 10,000 x g for a minute (Thermo Scientific, max. 14,800 rpm or 24 x 4g, UK). The collection tube was discarded and the Zymo-Spin Column was transferred into a new 2 ml collection tube. The mini spin columns were carefully opened and 200 µl of DNA Pre-wash buffer was added. The mini spin columns were centrifuged at 10,000 x g for a minute. The mini spin columns were transferred into new 2 ml collection tubes. About 500 µl of DNA wash buffer was carefully added to the mini spin columns and centrifuged at 10,000 x g for a minute. The spin columns were transferred into sterile 1.5 ml microcentrifuge tubes and 50 µl DNA elution buffer was added. It was then incubated at room temperature for 5 minutes and centrifuged at top speed (10,000 x g) for 30 seconds to elute the DNA. The DNA quantity and quality were determined using NanoDrop One (Thermo Fisher Scientific Inc, USA). The DNA extract was divided into 25 aliquots, labelled, and stored at -20 ^{0}C until tested.

Overnight cultures (18 to 24 hours) of *V. cholerae* isolates were made in Luria broth and incubated at 37 ^{0}C. The extraction of the broth cultures was also carried out using Zymo kit (D3024, USA) for DNA purification. About 1 ml of broth culture was centrifuge at 10,000 x g for 2 minutes. The supernatant was discarded and cells resuspended in 200 ml of PBS and the procedure continued as described by protocol from the Zymo kit for extraction of DNA from cell cultures.

3.13.2.2 Polymerase Chain Reaction Analysis

Polymerase Chain Reaction detection (PCR) of *V. cholerae* was based on the protocol described previously by Alam et al. (2006) with few modifications. The detection of *V. cholerae* was done by amplification of the *omp*W gene which targets the outer membrane protein; *ctx*A which targets the cholera toxin gene subunit A; and the O1*rfb* and O139*rfb* genes specific for serogroup O1 and O139 strains, in a uniplex PCR assays. The primers used were;

Table 3.4: PCR primers used in the study

Primer no.	Primer name	Primer sequence	Amplicon size(bp)
1	*ctx*AF	5'-CTCAGACGGGATTTGTTAGGCACG-3'	310
2	*ctx*AR	5'-TCTATCTCTGTAGCCCCTATTACG-3'	310
3	O1*rfb*F	5'-GTTTCACTGAACAGATGGG-3'	200
4	O1*rfb*R	5'-GGTCATCTGTAAGTACAAC-3'	200
5	O139*rfb*F	5'-AGCCTCTTTATTACGGGTGG-3'	450

6	O139*rfb*R	5'-GTCAAACCCGATCGTAAAGG-3'	450
7	*omp*WF	5'-CACCAAGAAGGTGACTTTATTGTG-3'	310
8	*omp*WR	5'-GGTTTGTCGAATTAGCTTCACC-3'	310

PCR amplification of *V. cholera* genes

Four uniplex PCR assays were carried out in 12.5 µl reaction volumes consisting of 6.25 µl One *Taq* Quick-Load 2X Master Mix with Standard Buffer (Biolabs, New England), 4.75 µl of nuclease-free water, 1µl of the extracted DNA template, and 0.25 µl of 10 µM of each specific primer for each gene (Inqaba Biotec, West Africa Ltd). Each reaction was thoroughly mixed by vortexing and flush centrifugation. This was then put in the PCR Biometra TRIO thermocycler (Analytik Jena, Germany). The conditions used in the PCR amplification consist of an initial denaturation at 94 ^{0}C for 1 min followed by 30 cycles of 1 min denaturation at 94 ^{0}C, 1 min annealing at 51^{0}C for *omp*W and O139*rfb* genes, 52 ^{0}C for *ctx*A genes, and 45 ^{0}C for O1rfb genes followed by 1 min extension at 68 ^{0}C. A final extension was performed at 68 ^{0}C for 5 min.

Agarose gel electrophoresis of amplified genes

After the amplification, 10 µl of the amplicon was taken and electrophoresis was carried out in 1X Tris Acetate EDTA buffer at 250v for about 45 minutes on 1.5 % agarose gel containing 0.5 µg/ml ethidium bromide. The gel was visualized with a UV transilluminator and images digitized with a gel documentation system (UVITEC, Cambridge). The bands were estimated by comparing to a 50 base pair ladder.

3.15.2 Wealth Index

A total of 26 items were selected to determine the wealth index of the households using the principal components analysis (PCA). These items included demographic health survey variables such as access to electricity, ownership of television, ownership of refrigerator, cooking fuel (LPG, charcoal, wood), type of dwelling (detached house, semi-detached house, flat/apartment, compound house), and materials used for building (cement, mud). Fourteen (14) of the variables had frequencies less than 5% and greater than 95% and were excluded from the analysis. The remaining 12 variables (electricity, television, fridge, LPG, charcoal, wood, detached house, semi-detached house, flat/apartment, compound house, cement, and mud) were included in the PCA analysis. The variables were changed into binary as yes (present) or no (absent) and coded as '1' and '0' respectively. The wealth index score was then produced into three equal parts (poor, medium, and wealthy).

3.15.3 Sanitation Assessment

In determining the community sanitation score index, a total of 14 items were selected to cover the conditions of the water supply pipelines, refuse and sewage disposal practices of the households. A total of 4 variables had frequencies less than 5% and greater than 95% and were excluded from the analysis. The remaining 10 items (variables) included in the PCA were; availability of exposed pipelines, accumulation of water around the pipelines, refuse dumpsite in the neighbourhood, refuse dump overflowing, house flies hovering around the dumpsite, refuse dumpsite located near a water source, evidence of open defecation, availability of public toilets, choked gutters or drains, indiscriminate disposal of solid waste, discharge of liquid waste or sewage into a water body. The variables were changed into binary as yes (present) or no (absent) and coded as '1' and '0' respectively. The principal component generated was used to assign to each of the households a sanitation score in relation to the other households in the sample. The sanitation assessment index score was then produced into three equal parts (poor, moderate, and good sanitation condition). The reliability of the sanitation index was found to be 0.76 and above the Cronbach's alpha value of above 0.7 and acceptable.

3.14 Spatial Data Collection

3.14.1 Cholera data abstraction

Cholera incidence data for Greater Accra Region (GAR) from 2012 to 2015 obtained from the Greater Accra Regional Health Directorate was used for the study. Linelist from outbreaks that occurred within the period was obtained from Greater Accra Regional Health Directorate. The data were entered into Microsoft Excel 2016 and saved in a GIS usable format. The variables included in the abstraction tool included: year, district, date of onset, the town of residence, and geographic position. Garmin Hand Held GPS device (eTrex 10, Taiwan) was used to capture the spatial coordinates (accuracy of 12 metres) for the location of the cases and added to the cholera data abstracted.

3.14.2 Other spatial data

A digitized boundary map of Ghana showing the regions, and districts (GHA_ADM 0,1 and 2) shapefile, a digitized elevation (raster), and Ghana town location was obtained from Land Use and Spatial Planning Authority-Ghana. The shapefiles were imported into ArcGIS and the query tab was used to subset it to GAR. This was used to generate shapefiles for district boundaries (vector), elevation (raster), and town location in GAR.

3.15 Data Analysis

3.15.1 Data processing and analysis

The data collected from the survey questionnaire were coded and double entered in EPI Data version 3.1. It was exported into STATA software version 14.0 (State Cooperation, USA), cleaned, and analyzed.

Descriptive statistics such as means and standard deviation for continuous variables and frequencies and percentages for categorical variables were used to summarize and present the background characteristics of the study participants.

For categorical data (nominal and ordinal variables) such as; sex, marital status, religion, ethnicity, employment status, level of education, residential unit, water source, water quality, piped water supply, water safety, and food safety results were presented by summary tables of counts and percentages or frequencies.

For continuous variables such as; age, income level, household occupancy, duration to seek care, distance to a health facility, summary tables of means, median, and or ranges, and standard deviations were presented. For continuous variables that were skewed, the median and interquartile ranges were presented.

Inferential statistics were applied to estimate parameters of the population based on the sample data. Kruskal Wallis rank test, a non-parametric equivalent of the Analysis of Variance (ANOVA) test was performed to determine whether there were any differences between the means of the log of bacteria counts among some selected factor variables. Chi-square/Fisher exact test was used to perform a test of association between cholera and other independent variables. All independent variables with p-values less than or equal to 0.2 significance level were retained in a multivariable penalized logistic regression model using forward stepwise deletion.

A penalized logistic regression model was specified as the most appropriate statistical tool for the data because of the sparse data (small number of cholera cases relative to the sample size). The penalized logistic regressions model for a binary response was used to estimate the odds of cholera as a function of independent variables. To understand the effect of the independent variables on the dependent variable (cholera), the parameter estimated was interpreted in terms of odds ratios. The p-value less than 0.05 was interpreted as significant.

A multicollinearity test of the regression model was conducted using variance inflation factors (VIFs). The results from the socioeconomic characteristics, behavioural and environmental risk factors model showed the mean VIF values to be 1.53, 1.44 and 1.75 respectively. All the individual VIF values were less than 2.32 (lower than the widely used cutoff value of 5.0) as reported by Craney and Surles (2002) which suggest that multicollinearity was not of a concern.

Goodness of fit test was conducted using the McFadden R-squared value. Models on socioeconomic characteristics, behavioural, and environmental risk factors were found to have an R-squared values of 0.102, 0.055, and 0.130 respectively. According to McFadden, Tye, and Train (1977), McFadden R-squared value between 0.20 to 0.40 tested represents an excellent fit. Based on this it was concluded that the models did not fit the data well.

3.15.4 Spatial data processing and Analysis

The Geographic Positioning System (GPS) waypoint data was imported into ArcGIS software version 10.2 by connecting the Universal Serial Bus (USB) port of the computer with the Garmin data cable. The data from data collectors were cleaned using Microsoft excel. The Geographic Information System (GIS) maps were developed to show the distribution of cholera cases from 2012 to 2015. Spatial point pattern analysis was employed to show the distribution of the cholera cases in GAR.

3.15.5 Spatial point pattern analysis

Firstly, the outline shapefile of GAR was imported and used as an outline map. This was done through the data menu and navigation to the location of the file. The cholera case location shapefile for each of the four years (2012 to 2015) was imported into the ArcGIS and laid on the outline map of GAR to generate a map showing the distribution of cholera in GAR for the respective years. Case magnitude was visualized using cell size graduated symbol where larger cell size denotes a high number of reported cases and vice versa. However, to detect any change in the distribution pattern of the cholera cases over time for the four years, the cholera case location shapefile as graduated was computed for each of the four years and visualized. Geographic coordinates for significant environmental risk factors such as toilets, dumping sites, and big gutters were obtained. Each risk factor was imported into the GAR boundary map with the reported cases to show the proximity of risk factors to the reported cases. Subsequently, a shapefile of combined risk factors was imported onto the GAR boundary map to show the combined effects of the risk factors of the cholera incidence in the communities. Point pattern descriptors were used to show the distribution of cholera risk factors in endemic and non-endemic communities.

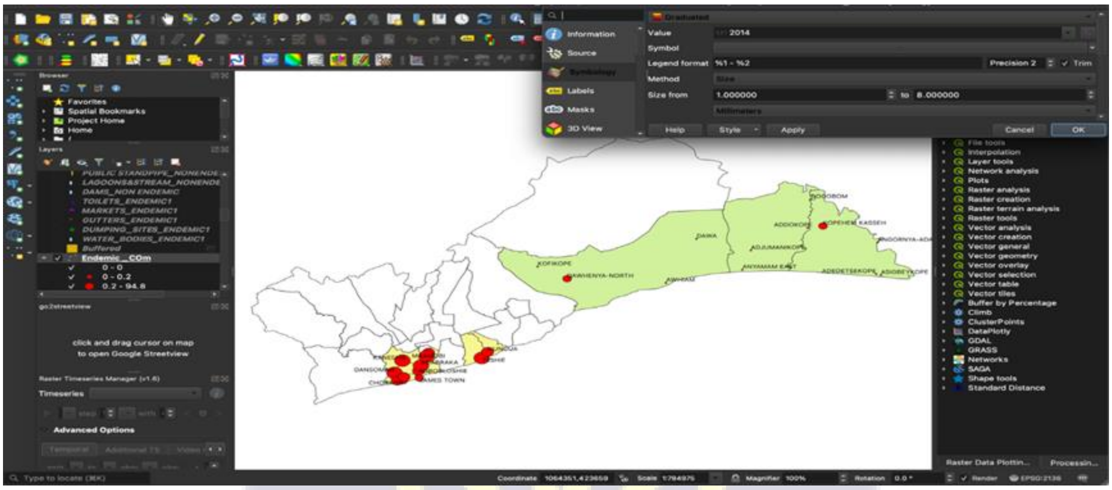

Figure 3. 4: Screenshot of spatial pattern analysis

3.15.6 Proximity analysis

The spatial analysis tool of proximity function in ArcGIS was used to determine the relationship between the cholera cases in the community and the identified features particularly the environmental factors (toilets, big gutters, dumping sites, markets, and water bodies) associated with cholera. A 500m buffer distance was created for each risk factor to examine its relationship with the distribution of cases. Shapefile for all the risk factors (toilets, big gutters, dumping sites, markets, and water bodies) were imported onto the classified GAR boundary map and the cholera outbreak distribution map at 500m buffer to examine the combined effects of the cholera outbreak.

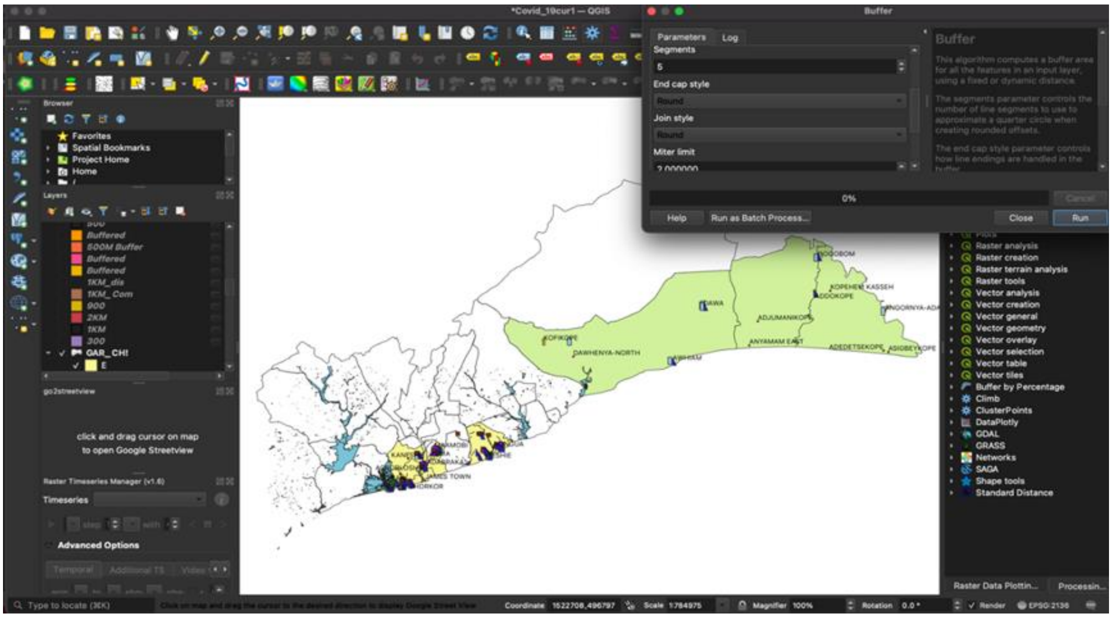

Figure 3. 5: Screenshot of proximity analysis

3.16 Quality Assurance

3.16.1 Questionnaire Development and Data Collection

3.16.1.1 Training of Research Assistants

Research assistants (8) for this study consisted of three field coordinators at the Dodowa Research Centre of the Ghana Health Service, one disease control officers, and four national service personnel. A one-day training was conducted at the School of Public Health, University of Ghana lecture Room G2. They were trained on the objectives of the study, data collection techniques as well as questionnaire administration. They were also trained on the use of the GPS receiver to collect the geographic coordinates, perform an accuracy checkpoint (ACP) and verify if the set-up was correct.

3.16.1.2 Pilot Study

A pilot study was conducted to assess the practicality of the instrument to prevent divergences between research assistants and the attributes to be measured. The pilot study was conducted in the Ashongman community in the Ga East Municipality of the GAR with similar population dynamics as the Accra Metropolis or LEKMA. The questionnaire was administered to 25 households and the outcome was used to make modifications to the questionnaire items before being used for all respondents during the actual fieldwork. The data was not included in the main study.

3.16.1.3 Validity

The questionnaire content was reviewed by environmental and health experts to ensure it measured items by the concept of what was intended.

3.16.1.4 Reliability

Since lack of reliability may arise from divergences between research assistants or the questionnaire of measurement or the instability of the attributes to be measured, the questionnaire was piloted, revised, and the same set of questionnaires administered to all respondents.

3.16.1.5 Data Entry

All questionnaires were cross-checked before entering into the database. The cross-checking of questionnaires was to ensure data completeness and logical consistency. The questionnaire found to be incomplete or with errors were either taken back to the field or rectified by calling the participants within 24 hours. Data entry clerks were trained on the creation of the data entry screen and coding of the variables. Checks and skip patterns were created to ensure required variables are captured to eliminate data incompleteness during data entry.

3.16.2 Spatial Data collection

A positional accuracy check was conducted throughout the data collection. The maximum error distance allowed for taking the coordinate was 6 metres. The coordinates collected were plotted on a google map to validate the data obtained from the field.

3.16.3 Laboratory Analysis

All the containers used for the collection of water samples from the field were sterilized by autoclaving and checking with an autoclave tape to ensure sterilization. The water samples were collected under strict aseptic techniques following the protocol and immediately stored in an insulated box at 4^0C and transported immediately to the laboratory. In case of a delay, the samples were stored in a refrigerator at 2 to 8^0C and transported within a day (within 24 hours) to the laboratory.

All the bacteriological media were prepared in accordance with the manufacturer's instructions and dispensed into disposable pre-sterilized plastic Petri dishes. A sterility test was performed on each batch of media prepared by incubating a plate at 35 to 37^0C overnight (18 to 24 hours) and examined for any contamination. A media performance test was also conducted by culturing in-house or reference bacteria strains obtained from the National Public Health and Reference Laboratory, Korle-bu to assess the growth characteristics of the media. The reference strains and in-house control strains were used as reference points to validate the results of the biochemical tests.

Blank membrane filtration was carried out after every fifth sample. This was to ensure growths on the filter membrane were not influenced by the laboratory conditions. The filtration techniques were carried out in a laminar flow hood to avoid contamination as much as possible.

Vibrio cholerae O1 toxigenic in-house strains were used as the positive controls and included each time during the extraction and amplification procedure of the DNA. The nuclease-free water and the master-mixed were used as the negative controls. The controls were read first in all cases to validate the test.

3.17 Ethical Consideration

3.17.1 Ethical Approval

Ethical clearance was obtained from the Ghana Health Service Ethical Review Committee (GHS-ERC) with approval number GHS-ERC 006/01/19 before the commencement of this study.

3.17.2 Study Area Approval

Permission was obtained from the Greater Accra Regional Health Directorate, Accra Metro Health Directorate, Ledzokuku-Krowor Municipal Health Directorate, Ningo Prampram, Ada West and Ada East District Health Directorates and the Accra Metropolitan Environmental and Sanitation Directorate to have access to the study area and their facilities before the field activities started.

3.17.3 Informed consent

Informed consent was obtained from potential study participants before they took part in the study. It was obtained without coercion, undue influence, or misrepresentation of the potential benefits and risks that might be associated with participation in the study. Before participation in this study, the research assistants reviewed the informed consent form with the participant in a language that was understandable to the participant. They informed them of the purpose, rationale of the study, how they were selected, the procedures, and the length of the interview. Those who agreed to participate consented by signing or thump printing two consent forms. A copy of the signed or thump printed consent form was given to the participant.

3.17.4 Risk of the study

No foreseen risks were associated with this study except for the time they spent responding to the questionnaire.

3.17.5 Benefits

Participants did not benefit directly from the study but the results from the study would help identify cholera risk factors in the communities and make recommendations for policymakers to improve on how to prevent cholera outbreaks.

3.17.6 Right to withdrawal

Participants were not obliged to take part in the study and were allowed to freely opt-out at any stage of the study without any punishment, intimidation, losing any benefit whatsoever.

3.17.7 Confidentiality/Anonymity

Data were handled anonymously and confidentially. Extensive effort was made to minimize the physical movement of data and or devices containing them. Data were entered into electronic devices to avoid unauthorized persons having access to them. Efforts were also made to minimize the transfer of identifiable data in physical or electronic form. Anonymity was ensured by using other identifiers including codes instead of the names of participants in data collected and analyzed. GPS coordinates of household locations were converted to distances so that the individual locations are not revealed. The maps were also created from aggregated data of larger areas.

3.18 Safety Precautions

Universal safety measures were employed throughout sample collection and testing. Gloves and laboratory coats were worn during all laboratory procedures. The bench tops and hoods were regularly decontaminated with 5% hypochlorite. The sharps which included the used pipette tips were placed in puncture-resistant containers and the used media were decontaminated with 5% hypochlorite and placed in biohazard bags. They were autoclaved before incineration.

CHAPTER FOUR

RESULTS

4.1 Socioeconomic characteristics of households in the Greater Accra Region

Table 4.1(a & b) provides descriptive information on the socioeconomic characteristics of the households. A total of 906 household respondents were interviewed with a 100% response rate to the survey questionnaire. Of these 453 households, were sampled from both cholera endemic communities and non-endemic communities. The mean age of the respondents was 41.52 ± 15.66 years in the endemic communities and 42.58 ± 14.29 years in the non-endemic communities. The female respondents were 343 (75.7%) and 269 (59.4%) in the endemic and non-endemic communities respectively.

With regards to the marital status of the respondent, the majority were married 215 (47.5%) of the respondents been from the endemic communities and 307 (67.8%) in the non-endemic communities. The majority of the respondents were Christians in both endemic 312 (68.9%) and non-endemic 392 (86.5%) communities.

The respondents were predominantly 902 (99.6%) Ghanaians with 165 (36.4%) and 391 (86.4%) of them belonging to the Ga-Dangme ethnic group in the endemic and non-endemic communities respectively.

More than three-quarters of the respondents in the endemic communities 390 (86.1%) and non-endemic communities 369 (81.5%) had primary education and above (basic education) whereas 63 (13.9%) and 84 (18.5%) had no formal education respectively.

Concerning the employment status of respondents, more than two-thirds were employed in the informal sectors in both the endemic 329 (72.6%) and non-endemic communities 318 (70.2%). However, less than a quarter of the respondents in the endemic communities 102 (22.5%), and the non-endemic communities 82 (18.1%) were unemployed. Most of the respondents in the endemic communities 318 (70.2%) were in the low-income brackets of less than GH¢300 in average monthly earnings, whereas 200 (44.2%) of them were in the non-endemic communities earned a medium-income (GH¢300 - GH¢1000).

In terms of wealth, the majority 218 (48.1%) of the households in the endemic communities were medium compared to households in the non-endemic communities who were poor 223 (49.3%). With regards to the household size, about half of the respondents had a household size of 4 to 6 in both communities.

Table 4.1a: Socioeconomic characteristics of household respondents in cholera endemic and non-endemic communities – (N=906)

Variable	Endemic		Non-Endemic	
	No.	%	No.	%
Age (mean ± SD) in years	41.52 ± 15.66		42.58 ± 14.29	
Age Group				
18-27 years	93	20.5	52	11.5
28-37 years	110	24.3	132	29.1
38-47 years	103	22.7	127	28.0
48-57 years	75	16.6	66	14.6
58-67 years	43	9.5	45	9.9
68+ years	29	6.4	31	6.8
Length of time living in the community (years) Median (IQR)	15 (7-29)		14(8-25)	
Gender				
Male	110	24.3	184	40.6
Female	343	75.7	269	59.4
Marital Status				
Single	100	22.1	40	8.8
Cohabiting	107	23.6	53	11.7
Married	215	47.5	307	67.8
Separated	8	1.8	12	2.6
Divorced	2	0.4	14	3.1
Widowed	21	4.6	27	6.0
Religion				
No Religion	1	0.2	7	1.6
Christian	312	68.9	392	86.5
Islam	139	30.7	16	3.5
Traditional	1	0.2	38	8.4
Nationality				
Ghanaian	452	99.8	450	99.6
Non-Ghanaian	1	0.2	3	0.4
Ethnicity				
Akan	98	21.6	26	5.7
Ewe	62	13.7	26	5.7
Ga-Dangme	165	36.4	391	86.4
Guan	6	1.3	0	0
Mole-Dagbon	121	26.7	8	1.8
Others	1	0.2	2	0.4
Employment				
Unemployed	102	22.5	82	18.1
Retired Worker	10	2.2	7	1.5
Formal Employment	12	2.7	46	10.2
Informal Employment	329	72.6	318	70.2

Table 4.1b: Socioeconomic characteristics of household respondents in cholera endemic and non-endemic communities – (N=906)

Variable	Endemic		Non-Endemic	
	No.	%	No.	%
Educational Level				
No Formal Education	63	13.9	84	18.5
Primary Education	112	24.7	79	17.4
JSS/Middle School Education	172	38.0	144	31.8
Senior High Education	84	18.5	102	22.5
Tertiary Education	22	4.9	44	9.7
Monthly income (earnings)				
Low income(<Gh¢300)	318	70.2	198	43.7
Medium income (>Gh¢300 - Gh¢1000)	130	28.7	200	44.2
High income (>Gh¢1000 - Gh¢2000)	5	1.1	45	9.9
Highest income(>Gh¢2000)	0	0	10	2.2
Wealth index				
Poor	84	18.6	223	49.3
Medium	218	48.1	146	32.2
Wealthy	151	33.3	84	18.5
Household size				
1-3	193	42.6	91	20.0
4-6	227	50.1	224	49.5
7+	33	7.3	138	30.5

Table 4.2 (a & b) shows the drinking water sources and handling of households surveyed in both endemic and non-endemic communities.

With respect to the source of drinking water as in table 4.2(a), households in the endemic communities 367 (81.0%) mostly drank sachet water, followed by 49 (10.8%) public pipe, 36 (8.0%) private pipe, and 1(0.2%) borehole water. In the non-endemic communities, on the other hand, the majority of the household drank from water sources including; 171 (37.7%) public pipe, 133 (29.4%) sachet water, 78 (17.2%) private pipe, 37 (8.2%) unprotected dug well, 33 (7.3%) surface water, and 1 (0.2%) borehole water. Apart from sachet water that was purchased from vendors, the majority of the households in the endemic 49 (57.0%) and non-endemic communities 243 (75.9%) had their drinking water sources located from outside their house dwelling or yards. The mean distance in minutes to the drinking water source and back was 7.82 ± 3.85 in the endemic community and 10.03 ± 5.53 in the non-endemic communities.

Of the 406 households studied, only 8 (9.3%) in the endemic communities and 38 (11.9%) in the non-endemic communities treated their drinking water. Of the households that treated their drinking water in the endemic communities, half 4 (50.0%) boiled, 1 (12.5%) allowed the water to settled, and 3 (37.5%) others added camphor or naphthalene. In the non-endemic communities, 14 (36.8%) treated with bleach/chlorine/alum, 10 (26.3%) strained through a cloth/strainer, 8 (21.1%) allowed the water to settled, 8 (21.1%) boiled, 7 (18.4%) applied camphor/naphthalene, and 1 (2.6%) filtered. The households in the endemic communities mostly stored their water as sachet 367 (81.0%) and in plastic containers 84 (18.5%) with a few others storing in pot/earthenware vessels 2 (0.4%), metal 1(0.2%) and elevated polytanks 1 (0.2%). In the non-endemic communities, their drinking water was mostly stored in (217, 47.8%) plastic containers, 133 (29.4%) sachet, 121 (26.7%) pot/earthenware vessels, and a few 6 (1.3%) in metal and 2 (0.4%) elevated polytanks.

In respect to the covering of the household drinking containers, the majority in the endemic 83 (96.5%) and non-endemic communities 285 (89.1%) were covered.

Table 4.2a: Water sources and handling factors among households in cholera endemic and non-endemic communities – (N=906)

Variable	Endemic		Non-Endemic	
	No.	%	No.	%
Main source of drinking water				
Private pipe	36	8.0	78	17.2
Public pipe	49	10.8	171	37.7
Tube well or borehole	1	0.2	1	0.2
Unprotected dug well	0	0.0	37	8.2
Surface water	0	0.0	33	7.3
Sachet water	367	81.0	133	29.4
Location source of drinking water (n=406)				
In own house/dwelling	35	40.7	68	21.3
In own yard	2	2.3	9	2.8
Elsewhere	49	57.0	243	75.9
Distance to drinking water and back (Mean±SD) in minutes	7.82±3.85		10.03±5.53	
Treatment of drinking water (n=406)				
Treated	8	9.3	38	11.9
Untreated	78	90.7	282	88.1
Water treatment methods used(n=46) **				
Boil	4	50	8	21.1
Add bleach/chlorine/alum	0	0	14	36.8
Strain through a cloth/strainer	0	0	10	26.3
Use water filter	0	0	1	2.6
Stand and settle	1	12.5	8	21.1
Camphor/Naphthalene	3	37.5	7	18.4
Storage of drinking water **				
Plastic Container	84	18.5	217	47.8
Pot/earthenware vessels	2	0.4	121	26.7
Metal container	1	0.2	6	1.3
Elevated polytanks	1	0.2	2	0.4
Bottled/Sachet	367	81.0	133	29.4
Drinking water storage vessels (n=406)				
Covered	83	96.5	285	89.1
Not covered	3	3.5	35	10.9

**Multiple responses

There were alternative water sources also used for other domestic purposes as shown in table 4.2 (b). Among these water sources, a public pipe was the most commonly used in both 270 (59.6%) endemic and 217 (47.8%) non-endemic communities.

In terms of pipe-borne (private and public pipe) water supply, the majority of the households in the endemic communities reported water shortages sometimes 368/446 (82.5%) with only a few households that never experienced a shortage 3/446 (0.7%). In the non-endemic communities' majority of the households reported water shortages sometimes 247/348 (71.0%) and non-reported never having any water shortages in the past.

Table 4.2b: Water sources and handling factors among households in cholera endemic and non-endemic communities – (N=906)

Variable	Endemic		Non-Endemic	
	No.	**%**	**No.**	**%**
Alternate sources of water for domestic purposes**				
Private pipe	176	38.8	131	28.8
Public pipe	270	59.6	217	47.8
Borehole	4	0.9	31	6.8
Protected dug well	1	0.2	40	8.8
Unprotected dug well	0	0	67	14.8
Unprotected spring	0	0	3	0.7
Rainwater	3	0.7	180	39.6
Tanker truck	5	1.1	2	0.4
Surface water	0	0	91	20.0
Water shortage experience				
Always	18	4.0	7	2.0
Often	57	12.8	94	27.0
Sometimes	368	82.5	247	71.0
Never	3	0.7	0	0.0

**Multiple responses

Table 4.3 (a & b) shows the food eating practices of households surveyed. Over three-quarters of the households in the endemic 331 (73.1%) and non-endemic 372 (82.1%) ate food cooked at home and outside the home as shown in table 4.3(a). In addition, only 4

(0.9%) of the households in the endemic community ate food from outside the home, whereas 118 (26.0%) only ate food cooked at home. In the non-endemic communities, 81 (17.9%) only eat food cooked at home and no household ate food from outside the home.

In terms of the household food cooking habit, 121 (27.0%) of them always cooked at home, 160 (35.6%) often cooked at home, and 168 (37.4%) sometimes cook at home in the endemic communities. In the non-endemic communities, on the other hand, 112 (24.7%) always cooked at home, 236 (52.1%) often cooked at home, and 105 (23.2%) sometimes cooked at home. More than half 228 (50.8%) of households that cook at home in the endemic communities do the cooking outside, and 193 (43.0%) others cook from inside the house/dwelling. It was also observed that the majority 169 (37.3%) of the households in the non-endemic communities also cook outside, with others cooking inside the house dwelling 147 (32.5%) and in separate buildings 137 (30.2%).

With the treatment of raw vegetables before eating, more than half 304 (67.1%) of the households in the endemic communities washed in only water, 116 (25.6%) washed in saltwater, 3 (0.7%) others heat steam or parboiled, 8 (1.8%) washed in water and heat steam, 15 (3.3%) washed in vinegar, whiles 2 (0.4%) ate without any treatment. In the non-endemic communities, raw vegetables were mostly 364 (80.4%) washed with only water, while 83 (18.3%) were washed in saltwater. In addition, only one (1) other household did not treat their vegetables before eating.

It was revealed that the majority of the households in the endemic 441 (97.4%) and non-endemic 448 (98.9%) communities ate leftover foods as in table 4.3(a).

Table 4.3a: Household food eating practices in the cholera endemic and non-endemic communities – (N=906)

Variables	**Endemic**		**Non-Endemic**	
	No.	**%**	**No.**	**%**
Food eating habit				
Only eat food cooked at home	118	26.0	81	17.9
Eat food cooked from home and outside home	331	73.1	372	82.1
Only eat food from outside home	4	0.9	0	0
Food cooking at home (n=902)				
Yes, always	121	27.0	112	24.7
Yes, often	160	35.6	236	52.1
Yes, sometimes	168	37.4	105	23.2
Where cooking is done (n=902)				
Inside the house/dwelling	193	43.0	147	32.5
In a separate building	28	6.2	137	30.2
Outdoors (in the yard)	228	50.8	169	37.3
Washing of raw vegetables before eating				
Washed in water only	304	67.1	364	80.4
Washed in water and heat steam	8	1.8	2	0.4
Washed in salt water	116	25.6	83	18.3
Washed in salt water and heat steam	0	0	2	0.4
Heat steam or parboiled only	3	0.7	0	0
Washed in vinegar	15	3.3	0	0
Washed in vinegar and heat steam	1	0.2	0	0
Others (heat steam with salt/without salt)	4	0.9	1	0.2
Does nothing to it	2	0.4	1	0.2
Leftover foods				
Eaten	441	97.4	448	98.9
Not eaten	12	2.6	5	1.1

Of the 889 households where leftover foods were eaten, the majority 255 (57.8%) of them in the endemic communities stored their leftover foods in a refrigerator/fridge, and a few 13 (2.9%) kept them frozen as shown in table 4.3(b). Among households in the non-endemic communities, 310 (69.2%) stored their leftover foods covered in the room/kitchen, 91 (20.3%) stored in refrigerators whiles 47 (10.5%) others kept them frozen.

It was also found that the majority of households in both endemic 413 (92.2%) and non-endemic 438 (99.3%) communities treated their leftover stored foods by reheating to boil before eating. However, 22 (4.9%) of the households in the non-endemic communities ate the leftover foods cold without heating.

In terms of handwashing practice of the households before meals, 268 (59.2%) washed their hands in a bowl of water, 157 (34.7%) washed with soap in a bowl of water, 21 (4.6%) washed under running water and only 7 (1.5%) washed with soap under running water in endemic communities. In the non-endemic communities, 288 (63.6%) were washed in a bowl of water, 75 (16.5%) washed with soap in a bowl of water, 63 (13.9%) washed under running water and 27 (6.0%) washed with soap under running water.

With the washing of fruits before eating such as mangoes, oranges, watermelon, apple most of the households 310 (68.4%) in the endemic communities washed their fruits in a bowl of water, 112 (24.7%) washed with safe running water, 16 (3.5%) in a bowl of water and soap and 6 (1.3%) did not wash before eating. In the non-endemic communities, 343 (75.7%) were washed with water in a bowl, 75 (16.6%) were washed with safe running water, 25 (5.5%) were washed with water and soap in a bowl and 6 (1.3%) did not wash before eating as in table 4.3(b).

Table 4.3b: Household food eating practices in the cholera endemic and non-endemic communities – (N=906)

Variables	Endemic		Non-Endemic	
	No.	%	No.	%
Storage of leftover foods at home (n=889)				
Left uncovered in the room/kitchen	24	5.4	0	0.0
Covered and left in the room/kitchen	149	33.8	310	69.2
Put in the refrigerator/fridge	255	57.8	91	20.3
Put in a freezer	13	2.9	47	10.5
Treatment of leftovers before eating (n=889)				
Eaten cold in the next meal	0	0.0	22	4.9
Reheated to boil before eating	438	99.3	413	92.2
Warmed in the microwave before eaten	3	0.7	13	2.9
Handwashing practice before eating				
Wash hands in a bowl of water	268	59.2	288	63.6
Wash hands with soap in a bowl of water	157	34.7	75	16.5
Wash hands under running water	21	4.6	63	13.9
Wash hands with soap under running water	7	1.5	27	6.0
Washing of fruits before eating				
They are washed with soap under safe running water	9	2.0	4	0.9
They are washed with safe running water	112	24.7	75	16.6
They are washed with water and soap in a bowl	16	3.5	25	5.5
They are washed with water in a bowl	310	68.4	343	75.7
They are not washed	6	1.3	6	1.3

Table 4.4 (a & b) shows the solid waste disposal practices among households in the endemic and non-endemic communities.

Out of the 321 (70.9%) households that did not have toilet facilities in the endemic communities, 312 (68.9%) used public toilets, whiles 9 (2.0 %) practice open defecation as shown in table 4.4(a). Of the households with toilet facilities in the endemic communities, majority 73 (16.1%) used flush to piped sewer system, followed by 30 (6.4%) which used flush to septic tank and 2 (0.4%) used bucket as in table 4.4(a). In the non-endemic communities, 80 (17.7%) of the households used public toilets while 154 (33.9%) practice open defecation in the bushes. Of the households with toilet facilities majority 59 (13.0%) used flush to septic tank, followed by 52 (11.5%) used flush to pit latrine, no household was engaged in the use of a bucket toilet as in table 4.4(a). Out of the total of 351 respondents who indicated that their households had toilet facilities, more than two-thirds in the endemic communities 91/132 (68.9%) and non-endemic communities 171/219 (78.1%) shared their toilet facilities with other households. It was observed that 37/132 (28.0%) of households in the endemic communities did not share their toilet facility compared to 29/219 (13.2%) in the non-endemic communities. A few other households in the endemic 4/132 (3.0%) and non-endemic 19/219 (8.7%) communities shared their toilet facilities with the general public. Of those households that did not have toilet facilities and used public toilet facilities, a mean time of 12.17 ± 6.47 and 9.50 ± 7.08 minutes round trip to use the toilet facility was observed in the endemic and non-endemic communities respectively.

In respect to handwashing at household and public toilet facilities, more than three-quarters 366/444 (82.4%) of the facilities had handwashing devices in the endemic communities and less than half 125/299 (41.8%) in the non-endemic communities.

The most common storage medium used to temporally store refuse in the households were sacks 297 (65.6%) in the endemic communities and rubbish bins 146 (32.2%) in the non-endemic communities as shown in table 4.4(a).

Table 4.4a: Solid waste disposal practices among households in cholera endemic and non-endemic communities – (N=906)

Variable	**Endemic**		**Non-Endemic**	
	No.	**%**	**No.**	**%**
Type of toilet facility used				
Flush to piped sewer system	73	16.1	3	0.7
Flush to septic tank	30	6.4	59	13
Flush to pit latrine	19	4.2	36	7.9
Flush don't know where it goes	2	0.4	0	0
Ventilated improved pit latrine	1	0.2	20	4.4
Pit latrine	3	0.6	52	11.5
Pit latrine with slab	2	0.4	29	6.4
Pit latrine without slab/open pit	0	0	20	4.4
Bucket toilet	2	0.4	0	0
No toilet facility used bush/field	9	2.0	154	33.9
No toilet facility used public toilet	312	68.9	80	17.7
Toilet facility in households (n=351)				
Yes, shared with other households only	91	68.9	171	78.1
Yes, shared with public	4	3.0	19	8.7
No, used by household only	37	28.0	29	13.2
Number of households using toilet Median (IQR)	5(3-6)		3(2-4)	
Location of toilet facility in households (n=335)				
In own house/dwelling	49	37.1	90	41.1
In own yard	83	62.9	113	51.6
Distance from household to public toilet facility and back in minutes (n=392)	12.17±6.47		9.50 ± 7.08	
Hand washing facility at the household and public toilet facilities (n=743)				
Available	366	82.4	125	41.8
Not available	78	17.6	174	58.2
Medium of storage of refuse				
Plastic bags	46	10.1	133	29.4
Cardboard boxes	13	2.9	7	1.5
Rubbish bin	88	19.4	146	32.2
Sacks	297	65.6	64	14.1
No storage-direct disposal	9	2.0	90	19.9
Others	0	0	13	2.9

With regards to household's solid waste disposal practices as in table 4.4(b), it was observed in the endemic communities that the majority 335 (73.9%) of their refuse were collected mostly by motorized tricycle operators (290, 86.6%) popularly known locally as 'Aboboyaa'. For those whose refuse was not collected by waste companies, they were disposed at public dumpsites covered or uncovered (69, 15.2%), burnt, 23 (5.1%), buried, 1(0.2%) or dump indiscriminately, 25 (5.5%). In the non-endemic communities, more than half 265 (58.5%) burnt their households solid waste, 58 (12.8%) dump waste indiscriminately, whiles 46 (10.2%) had their households waste collected by waste companies. Among the waste companies, Zoomlion company was the most common (32, 69.6%) in the non-endemic communities.

The duration of waste collection by the companies in the endemic communities depended mostly on when the waste bin gets full 182 (54.3%), whereas that in the non-endemic communities were collected weekly 46 (97.9%). The most common reasons given by households not accessing the services of waste companies in the endemic communities was the high financial charges 55 (46.6%), whiles non-availability of waste companies 304 (74.7%) accounted for that in the non-endemic communities. There were more public waste dump sites in the endemic communities 322 (71.1%) than the non-endemic communities 176 (38.8%). The average time taken for a round trip to the dump waste site and return was 17.24±6.62 in the endemic communities and 11.77±8.51 in the non-endemic communities as shown in table 4.4(b).

Table 4.4b: Solid waste disposal practices among households in cholera endemic and non-endemic communities – (N=906)

Variable	Endemic		Non-Endemic	
	No.	%	No.	%
Disposal of solid waste**				
Collected by a waste company	335	74.0	46	10.2
Burned by household	23	5.1	265	58.5
Public dump site (covered)	2	0.4	3	0.7
Public dump site (opened)	67	14.8	59	13.0
Dump indiscriminately	25	5.5	58	12.8
Buried by household	1	0.2	23	5.1
Waste collection companies available (n=381)				
Alliance waste	0	0	11	23.9
AMA waste company	1	0.3	3	6.5
Private motorized tricycle	290	86.6	0	0
Zoomlion	44	13.1	32	69.6
Duration of waste collection (n=381)				
Daily	78	23.3	0	0
Weekly	74	22.1	46	97.9
Monthly	1	0.3	0	0
As to when the waste bin is full	182	54.3	0	0
Reasons for households refuse not collected by a waste company (n=525) **				
Financial reasons (high service charges)	55	46.6	9	2.2
Waste collection companies are not available	10	8.5	304	74.7
Waste collection companies are available but not reliable	43	36.4	31	7.6
No waste disposal bin	50	42.4	147	36.1
Availability of Public waste dumpsite				
Available	322	71.1	176	38.8
Not available	131	28.9	277	61.2
Distance to public dump site and back in minutes	17.24±6.62		11.77±8.51	

**Multiple responses

The liquid waste disposal practices of the households were surveyed as shown in Table 4.5. A drainage system was available in most households in the endemic communities 342 (75.5%) than in the non-endemic communities 42 (9.3%). Out of the 342 households in the endemic communities that had a drainage system, 323 (94.4%) of them were mostly open gutters. For the 42 households with drainage in the non-endemic communities, the majority of 36 (83.7%) were underground drainage types.

In the endemic communities, about three-quarters, 329 (72.6%) of the liquid waste was from domestic chores and 328 (72.4%) from the bath were disposed directly into the gutters. On the other hand, 235 (51.9%) of the liquid waste from domestic chores and 229 (50.5%) of the bath were disposed onto the compounds in the non-endemic communities.

Table 4. 5: Liquid waste disposal practices among households in cholera endemic and non-endemic communities – (N=906)

Variable	**Endemic**		**Non-Endemic**	
	No.	**%**	**No.**	**%**
Availability of drainage system				
Available	342	75.5	42	9.3
Not available	111	24.5	411	90.7
Type of drainage systems (n=402)				
Open drainage (open gutter)	323	94.4	1	2.4
Covered drainage (covered gutter)	14	4.1	5	11.9
Underground drainage (pipes connection to sewage)	4	1.2	36	83.7
Others	1	0.3	0	0
Liquid waste disposal domestic chores				
Through the sewage system to septic tank	6	1.3	50	11.1
Thrown onto the street/outside	60	13.3	165	36.4
Thrown into gutter	329	72.6	2	0.4
Thrown onto the compound	58	12.8	235	51.9
Others	0	0	1	0.2
Liquid waste disposal from the bath				
Through the sewage system	6	1.3	9	2
Through drainage into a pit	7	1.5	77	17
Thrown onto the street/outside	59	13.0	138	30.5
Thrown into gutter	328	72.4	0	0
Thrown onto the compound	53	11.7	229	50.5

Table 4.6 shows the environmental predisposing factors of cholera in both endemic and non-endemic communities. With regards to water bodies, it was observed that 203 (44.8%) households in the endemic communities had water bodies in their neighbourhoods compared to 183 (40.4%) in the non-endemic communities. The majority of the residents in the endemic communities lived closed to water bodies such as the sea 123 (60.6%), lagoons 59 (29.1%), and rivers/stream 41 (20.2%).

In the non-endemic communities, the households lived in proximity to the lagoons 113 (61.8%), sea 74 (40.4%), and river/streams 31 (16.8%). It was observed that an average time round trip to the water body site and return was 14.70±9.06 in the endemic communities and 11.17±7.27 in the non-endemic communities.

Two hundred (200, 44.1%), of the households, had a market in their neighbourhood, whereas there were no records of a market in the vicinity of the households interviewed in the non-endemic communities. For those communities in the endemic communities with neighbourhood markets, the majority 195 (97.5%) of their households bought foodstuff from those markets. Most of the respondents 160 (80.0%) who bought from the markets indicated that the vendors sometimes screened their smoked/fried fish from flies in the market. The average time taken for a round trip to the market and return was 22.07±9.53 in the endemic communities.

There were more abattoirs or slaughterhouses in the neighbourhoods of households in the endemic communities 39 (8.6%) than in the non-endemic communities 2 (0.4%). In the endemic communities where abattoirs were present, about half 20 (51.3%) of the respondents stated that the butchers never screened their meat, 14 (35.9%) indicated they sometimes screened and 5 (12.8%) stated they often screened. There were only two (2) households in the non-endemic communities who lived near an abattoir in the neighbourhood and one indicated the butchers always protected the meat from flies and dust and the other indicated they did sometimes. The average time taken for a round trip to the abattoir and return was 19.21±4.47 in the endemic communities and 17.5±3.54 in the non-endemic communities.

Table 4. 6: Environmental predisposing factors to cholera in endemic and non-endemic communities – (N=906)

Variable	**Endemic**	**Non-Endemic**

	No.	%	No.	%
Water body in neighbourhood				
Present	203	44.8	183	40.4
Not present	250	55.2	270	59.6
Distance to water body in minutes	14.70±9.06		11.17±7.27	
Type of water body(n=386) **				
River/Stream	41	20.2	31	16.8
Lagoon	59	29.1	113	61.8
Sea	123	60.6	74	40.4
Market in neighbourhood				
Present	200	44.1	0	0
Not present	253	55.9	453	100
Distance to market and back in minutes	22.07±9.53		-	
Food stuff (n=200)				
Food stuff bought from the market	195	97.5	0	0
Food stuff bought from else where	5	2.5	0	0
Vendors screen their smoked/fried fish from flies and dust (n=200)				
Yes, always	2	1	0	0
Yes, often	34	17.0	0	0
Yes, sometimes	160	80.0	0	0
No, never	4	2.0	0	0
Abattoir/slaughter house in neighbourhood				
Present	39	8.6	2	0.4
Not present	414	91.4	451	99.6
Distance from abattoir and back in minutes	19.21±4.47		17.5±3.54	
Butchers screen to protect the meat from flies and dust(n=41)				
Yes, always	0	0	1	50
Yes, often	5	12.8	0	0
Yes, sometimes	14	35.9	1	50
No, never	20	51.3	0	0

**Multiple responses

Table 4.7 shows cholera infection in the households and neighbourhood in the past years. Household respondents in the endemic 453 (100%) and non-endemic 432 (95.4%) communities were aware of the symptoms of cholera. Among the households surveyed, 38 of the households ever reported a case of cholera. Thirty-six (36, 94.7%) of the cases occurred in the endemic communities and 2 (5.3%) in the non-endemic communities.

With regards to cholera cases in the neighbourhood, 189 (41.7%) of the households ever heard of a cholera case in the neighbourhood, 69 (15.2%) never heard of a case, and 195 (43.1%) were not aware of a case in the endemic communities. In the non-endemic communities 20 (4.4%) of the households ever heard of a case of cholera in the neighbourhood, 302 (66.7%) never heard of a case and 131 (28.9%) were not aware of any case in the neighbourhood.

Table 4. 7: Cholera infection in the households and neighbourhood – (N=906)

Variable	**Endemic**		**Non-Endemic**	
	No.	**%**	**No.**	**%**
Cholera symptoms awareness				
Aware symptoms	453	100.0	432	95.4
Not aware of symptoms	0	0.0	21	4.6
Cholera infection in the household				
Ever suffered from cholera	36	7.9	2	0.4
Never suffered from cholera	417	92.0	451	99.6
Cholera infection in the neighbourhood				
Ever reported a case of cholera	189	41.7	20	4.4
Never reported a case of cholera	69	15.2	302	66.7
Don’t Know	195	43.1	131	28.9

4.2 Associations between socioeconomic characteristics of household respondents and cholera in the Greater Accra Region

Tables 4.8 show the association between socioeconomic characteristics of the household respondent and cholera occurrence. A higher proportion of cholera was observed among households whose respondents were in the age groups of 28-37 years 11 (28.9%) and 38-47 years 11 (28.9%) with no recorded case of cholera among those in the age group of 58-67 years. However, the differences in the proportions of cholera in the different age groups were found to be statistically non-significant (p=0.232). With regards to the sex of respondents, female respondents 24 (63.2%) mostly recorded cholera cases in their households than their male 14 (36.8%) counterparts but this was found not to be statistically significant (p=0.555).

More cholera cases were recorded among households whose respondents were cohabitating (14, 36.8%), compared to those who were married (13, 34.2%), with no recorded cholera cases among households whose respondents were either separated or divorced. A significant association was found between the marital status of the respondents and cholera (p=0.009).

The religion of the respondents whose household ever had cholera were mostly Christians 25 (65.8%), followed by Islam 13 (34.2%) with no recorded cases among those with no religion or those who practiced traditional religion. These differences were also found to be statistically significant (p=0.042).

The ethnicity of the respondents whose households had the most cases of cholera was the Ga-Dangmes 14 (36.8%), with the least been the Guans 1 (2.6%). The differences in cholera distribution among the ethnic groups of respondents were found to be statistically significant (p=0.015). With the employment status of the respondents and cholera cases, it was found that 29 (76.3%) in informal employment were the households most affected by cholera but this was not statistically significant (p=0.858) when compared to those who were unemployed 8 (21.0%), retired worker 0 (0.0%), or informal employment 1 (2.6%).

With regard to education, those respondents with JSS/middle school level education 16 (42.1%) had their households mostly affected with the least been among those with tertiary education 2 (5.3%). However, the differences were not statistically significant (p=0.670).

In terms of the wealth status of the respondents, households with medium wealth 19 (50.0%) were more affected by cholera, compared to poor 8 (21.0%) or wealthy 11 (29.0%) households. This was, however, found not to be statistically significant (p=0.176).

With regards to household income or earnings, respondents with low income (<GH¢300) recorded the highest cases of cholera 30 (78.9%) followed by (8, 21.0%) those with a medium-income (>GH¢300 - GH¢1000), and no cholera case was found among those within the higher income brackets (GH¢1000 - >GH¢2000). These differences were found to be statistically significant (p=0.042). Also, those respondents with a family size of 4-6 recorded more cholera cases in the households (20, 52.6%) compared to those with smaller (1-3) and larger family sizes (>7). This was, however, not statistically significant (p=0.385).

Table 4.8a: Associations between socioeconomic characteristics of respondent and cholera in the Greater Accra Region

Variables	Cholera				P-Value
	Yes = 38		No=868		
	No.	%	No.	%	
Age Group					0.232
18-27yrs	9	23.7	136	15.7	
28-37yrs	11	28.9	231	26.6	
38-47yrs	11	28.9	219	25.2	
48-57yrs	5	13.2	136	15.7	
58-67yrs*	0	0.0	88	10.1	
68 yrs and above*	2	5.3	58	6.7	
Sex					0.555
Male	14	36.8	280	32.3	
Female	24	63.2	588	67.7	
Marital Status					**0.009**
Single	10	26.3	130	15.0	
Cohabiting	14	36.8	146	16.8	
Married	13	34.2	509	58.6	
Separated*	0	0.0	20	2.3	
Divorced*	0	0.0	16	1.8	
Widowed*	1	2.6	47	5.4	
Religion					**0.042**
No Religion*	0	0.0	8	0.9	
Christian	25	65.8	679	78.2	
Islam	13	34.2	142	16.4	
Traditional*	0	0.0	39	4.5	
Ethnicity					**0.015**

Akan	8	21.0	116	13.4	
Ewe	5	13.2	83	9.6	
Ga-Dangme	14	36.8	542	62.4	
Guan*	1	2.6	5	0.6	
Mole-Dagbon	10	26.3	119	13.7	
Others*	0	0.0	3	0.3	
Employment					0.858
Unemployed	8	21.0	176	20.3	
Retired Worker*	0	0.0	17	2.0	
Formal Employment*	1	2.6	57	6.5	
Informal Employment	29	76.3	618	71.2	
Educational Level					0.670
No Formal Education	5	13.2	142	16.4	
Primary Education	10	26.3	181	20.8	
JSS/Middle School Education	16	42.1	300	34.6	
Senior High Education	5	13.2	181	20.8	
Tertiary Education*	2	5.3	64	7.4	

P-value is significant if $p < 0.05$, *Fishers Exact Test

Table 4.8b: Associations between socioeconomic characteristics of respondents and cholera in selected households in the Greater Accra Region

Variables	Cholera				P-Value
	Yes = 38		No = 868		
	No.	%	No.	%	
Wealth Status					0.176
Poor	8	21.0	299	34.5	
Medium	19	50.0	324	37.3	
Wealthy	11	29.0	245	28.2	
Income					**0.042**
Low income (<Gh¢300)	30	78.9	486	56.0	
Medium income (>Gh¢300 - Gh¢1000)	8	21.0	322	37.1	

High income (>Gh¢1000 - Gh¢2000)*	0	0.0	50	5.8	
Highest income (>Gh¢2000)*	0	0.0	10	1.1	
Family Size					0.385
1-3	14	36.8	270	31.1	
4-6	20	52.6	431	49.7	
7+*	4	10.5	167	19.2	

P value is significant if p < 0.05, *Fishers Exact Test

A penalized multivariable logistic regression model was used to determine the impact of each socioeconomic predictor variable on cholera risk in the study communities as shown in Table 4.9.

With regards to the marital status, households of cohabiting respondents were significantly protective by 67.0% against cholera compared to households of respondents who were single (OR=0.33, 95% CI: 0.14-0.75, p=0.009). However, after adjusting for other predictor variables found households with respondents who were married to be 64.0% protective against cholera and were statistically significant (AOR=0.36, 95% CI: 0.14-0.90, p=0.028).

Respondents with high income were 65.0% protected against cholera compared to their counterparts who had low income (OR=0.35, 95% CI: 0.16-0.77, p=0.008). This association was not significant after adjusting for other predictor variables (AOR=0.48, 95% CI: 0.20-1.16, p=0.102).

Other predictor variables including; religion, level of education, employment and wealth status of respondents were found not to be significantly associated with cholera as in Table 4.9.

Table 4.9: Assessing the association between socioeconomic factors and cholera using a penalized multivariable logistic regression model

Variable	OR (95% CI)	P-value	AOR (95% CI)	P-value
Marital status				
Single	1		1	
Married	1.23 (0.54-2.82)	0.624	0.36 (0.14-0.90)	**0.028**
Co-habiting	0.33 (0.14-0.75)	**0.009**	1.10 (0.44-2.65)	0.857
Separated	0.3 (0.02-5.37)	0.416	0.35 (0.02-6.76)	0.491
Divorced	0.38 (0.02-6.73)	0.507	0.41 (0.02-7.90)	0.558
Widow	0.39 (0.07-2.24)	0.293	0.46 (0.07-2.89)	0.405
Religion				
No Religion	1		1	
Christian	0.64 (0.04-11.36)	0.760	0.88 (0.04-17.30)	0.935
Islam	1.61 (0.09-29.45)	0.748	1.92 (0.09-39.27)	0.671
Traditional	0.22 (0-11.62)	0.450	0.39 (0.01-22.94)	0.649
Level of Education				
None	1		1	
Primary	1.5 (0.52-4.3)	0.452	1.23 (0.41-3.66)	0.708
JSS/Middle School	1.42 (0.53-3.81)	0.483	0.97 (0.33-2.83)	0.958
SHS	0.79 (0.24-2.61)	0.693	0.57 (0.15-2.16)	0.408
Tertiary	1 (0.22-4.61)	0.996	1.16 (0.16-8.25)	0.883
Employment				
Unemployed	1		1	
Retired worker	0.59 (0.03-10.72)	0.724	1.15 (0.05-24.44)	0.927
Formal Employed	0.54 (0.09-3.15)	0.495	1.27 (0.14-11.73)	0.834
Informal Employed	0.99 (0.45-2.16)	0.981	1.23 (0.52-2.89)	0.637
Income				
Low	1		1	
High	0.35 (0.16-0.77)	**0.008**	0.48 (0.20-1.16)	0.102
Wealth Index				
Poor	1		1	
Medium	2.12 (0.93-4.81)	0.073	1.69 (0.73-3.94)	0.224

Wealthy	1.65 (0.67-4.07)	0.276	1.51 (0.56-4.05)	0.418

OR – Odds Ratio, AOR – Adjusted Odds Ratio, CI – Confidence Interval, Significant ($p<0.05$)

4.3 Associations between behavioural factors and cholera in the Greater Accra Region

4.3.1 Drinking water sources and handling practices among households and cholera

The water sources and handling practices of household members pose a risk to cholera as shown in Table 4.10a. It was observed that households whose main source of drinking water was sachet water recorded the highest cases of cholera 32 (84.2%) compared to those who drank private piped water 3 (7.9%), public pipe 2 (5.3%), or unprotected dug well 1 (2.6%). This was found to be statistically significant (p=0.002). A higher proportion of cholera cases 35 (92.1%) were also found to be associated with households that had their main drinking water sources located in the household dwelling than those in the yard 0 (0.0%) or elsewhere 3 (7.9%) and was found to be statistically significant (p=0.002).

Among the households, more cases of cholera were detected among households that drank untreated water 5 (83.3%) than those that drank treated water 1 (16.7%). However, this was tested statistically and found not to be significant (p=0.516). Drinking from a covered storage vessel recorded more cases of cholera 5 (83.3%) than from uncovered 1 (16.7%) but was not statistically significant (p=0.442).

4.3.2 Food eating habits and cholera

The food eating habits of the households could pose a risk to cholera as shown in Table 4.10a. With the food eating habit, most households ate food cooked from home and outside the home and a recorded majority of the cholera cases 32 (84.2%) than the households that ate only food cooked at home 4 (10.5%) or from outside home 2 (5.3%). The differences were statistically significant (p=0.003).

Most households cook food in the yard, whiles others cooked inside the house or in a separate building. It was observed that those who cooked inside the house recorded most cases of cholera 22 (61.1%). The differences in the distribution of the cholera cases by location were found to be statistically significant (p=0.008).

Most households ate leftover foods, and cholera was found to be common 36 (94.7%) among households that ate left-over foods. Eating leftover foods was however found not to be associated with cholera (p=0.157). The main treatment method of the left-over food before eating was reheating to boil recorded the highest proportion of cholera cases 36 (100.0%) compared to the others, however was found not to be significantly associated with cholera (p=1.000).

With regards to the handwashing before meals, the majority of household members who washed in a bowl of water had cholera 28 (73.7%) compared to those who used the other methods of hand washing. The differences, however, were found not to be statistically significant (p=0.298).

4.3.3 Solid waste disposal practices and cholera

As shown in Table 4.10b, the location of toilet facility in own house dwelling had more cases of cholera 7 (77.8%) than those located in the yard 2 (22.2%) or elsewhere 0 (0.0%). Also, toilet facilities that had a handwashing device, recorded more cases of cholera 31 (93.9%) than those without a handwashing device 2 (6.1%) and was found to be statistically significant (p=0.001).

Various mediums were employed in the storage of refuse as in Table 4.10. Those who stored their refuse in sacks had a higher proportion of cholera cases 29 (76.3%) than the others and was found to be statistically significant (p=0.001).

4.3.4 Liquid waste disposal and cholera

Table 4.10b shows the association between household liquid waste disposal and cholera. Among the several methods employed in liquid waste disposal from the households, the most predominantly used was the disposal into the gutters which recorded the highest cholera cases of 31 (81.6%). When compared to the other methods of disposal was found to be statistically significant ($p<0.001$).

Table 4.10a: Associations between household behavioural factors and cholera in the Greater Accra Region

Variables	Cholera				P-Value
	Yes = 38		No=868		
	No.	%	No.	%	
Drinking water sources and handling practices					
Main source of drinking water					**0.002**
Private pipe*	3	7.9	111	12.8	
Public pipe*	2	5.3	220	25.4	
Unprotected dug well*	1	2.6	69	7.9	
Sachet water	32	84.2	468	53.9	
Location of source of drinking water					**0.002**
In own house/dwelling	35	92.1	568	65.4	
In own yard*	0	0.0	11	1.3	
Elsewhere*	3	7.9	289	33.3	
Water treatment(n=406)					0.516
Drinking treated water*	1	16.7	45	11.3	
Drinking untreated water	5	83.3	355	88.7	
Drinking water storage vessel(n=406)					0.442
Covered	5	83.3	379	90.9	
Uncovered*	1	16.7	38	9.1	
Food eating practices					
Food eating habit					**0.003**
Only eat food cooked at home*	4	10.5	195	22.5	
Eat food cooked from home and outside	32	84.2	671	77.3	
Only eat food from outside*	2	5.3	2	0.2	
Location where food is cooked					**0.008**
Inside the house/dwelling	22	61.1	318	36.7	
In a separate building*	2	5.6	163	18.8	
Outdoors (in the yard)	12	33.3	385	44.5	
Leftover foods					0.157
Eaten	36	94.7	853	98.3	

Not eaten*	2	5.3	15	1.7	
Method of treating left over foods before eating					1.000
Eaten cold in the next meal*	0	0.0	22	2.6	
Reheated to boil before eaten	36	100.0	816	95.5	
Warmed in the microwave before eaten*	0	0.0	16	1.9	
Method of washing hands before eating					0.298
Wash hands in a bowl of water	28	73.7	528	60.8	
Wash hands with soap in a bowl of water	9	23.7	223	25.7	
Wash hands under running water*	1	2.6	83	9.6	
Wash hands with soap and water* under running water	0	0.0	34	3.9	

P value is significant if $p < 0.05$, *Fishers Exact Test

Table 4.10b: Associations between household behavioural factors and cholera in the Greater Accra Region

Variables	Cholera				P-Value
	Yes = 38		No=868		
	No.	%	No.	%	
Solid waste disposal factors					
Location of toilet facility					0.103
In own house/dwelling	7	77.8	132	38.6	
In own yard*	2	22.2	194	56.7	
Elsewhere*	0	0.0	16	4.7	
Hand washing facility in the toilet					**0.001**
Available	31	93.9	460	64.8	
Not available*	2	6.1	250	35.2	
Medium of storage of refuse					**0.001**
Plastic bags*	3	7.9	176	20.3	
Cardboard boxes*	0	0.0	20	2.3	
Rubbish bin	5	13.2	229	26.4	
Sacks	29	76.3	332	38.3	
No storage- direct disposal*	1	2.6	98	11.3	
Others*	0	0.0	13	1.5	
Liquid waste disposal factors					
Method of disposal					**<0.001**
Through the sewage system*	0	0.0	15	1.7	
Through drainage into a pit*	1	2.6	83	9.6	
Thrown onto the street/outside*	1	2.6	196	22.6	
Thrown into gutter	31	81.6	297	34.2	
Thrown onto the compound	5	13.2	277	31.9	

P value is significant if $p < 0.05$, *Fishers Exact Test

A penalized multivariable logistic regression model was further used to identify the behavioural risk factors for cholera infection in the study communities as shown in Table 4.11.

With the eating habits of households, the odds of having cholera was 43.44 times more likely among households that only ate food from outside the home compared to those who ate food cooked only at home (OR=43.44, 95% CI:5.96-316.73, p<0.001). This was not significant after adjusting for other predictor variables.

Households that cooked food in the yard and a separate building (detached kitchen) were 54% and 78% less likely to have suffered from cholera respectively compared to those that cooked inside the house dwelling. After adjusting for other predictor variables were found to be significant for cooking outdoors (AOR=0.42, 95% CI: 0.21-0.86, p=0.017) and cooking in a separate building (AOR=0.42, 95% CI: 0.06-0.82, p=0.024).

Predictor variable such as handwashing practice before meals were found not to be associated with cholera.

Table 4.11: Assessing the association between behavioural factors and cholera using penalized multivariable logistic regression model

Variable	OR (95% CI)	P-value	AOR (95% CI)	P-value
Food eating habits				
Only eat food cooked at home	1		1	
Eat food cooked from home and outside	2.1 (0.77-5.71)	0.145	2.09 (0.76-5.81)	0.155
Only eat food from outside home	43.44 (5.96-316.73)	**<0.001**	-	-
Location where cooking is done				
Inside the house/dwelling	1		1	
In a separate building	0.22 (0.06-0.81)	**0.023**	0.22 (0.06-0.82)	**0.024**
Outdoors (in the yard)	0.46 (0.23-0.93)	**0.031**	0.42 (0.21-0.86)	**0.017**
Hand washing practice before eating				
Wash hands in a bowl of water	1		1	

Wash hands with soap in a bowl of water	0.79 (0.37-1.67)	0.534	0.94 (0.42-2.10)	0.876
Wash hands under running water	0.33 (0.06-1.75)	0.194	0.30 (0.06-1.60)	0.160
Wash hands with soap under running water	0.27 (0.02-4.5)	0.361	0.23 (0.01-3.93)	0.312

OR – Odds Ratio, AOR – Adjusted Odds Ratio, CI – Confidence Interval, Significant ($p<0.05$)

4.4 Assessment of the bacteriological quality of drinking water and cholera risk in Greater Accra Region

The household drinking water quality was assessed for the presence of *Vibrio cholerae* strains and indicator (coliform) bacteria (Tables 4.12 and 4.13). High levels of total coliform counts (TCC) were observed in ten communities with a mean count of 7.73 log cfu/100ml and 9.86 log cfu/100ml respectively in the dry and wet seasons as shown in Table 4.12. Feacal coliforms (FCC) were generally present in the private pipe water sources in both seasons except in a few communities: Agege and Dansoman which did not record any counts in both seasons. *Vibrio* counts (VC) were absent in the private pipe water sources except in Mamprobi which recorded 6.91 log cfu/100ml in the wet season. The direct public pipe also had a high mean TCC of 7.82 log cfu/100ml in the dry season and 9.64 log cfu/100ml in the wet season. The mean FCC was also found to be 5.24 log cfu/100ml and 10.06 log cfu/100ml respectively in the dry and wet seasons. No VC was observed in the communities (Agbogbloshie and James Town) during both seasons. When the differences between the mean rank score of the log of TCC (χ^2 =0.182, p=0.669), FCC (χ^2=2.591, p=0.107) and VC (χ^2=0.200, p=0.655) were assessed among the direct water sources there were no statistical differences as shown in Table 4.14.

In the endemic communities' also household drinking water stored in vessels was also analyzed in both seasons. Of the 216 water samples; 110 were private pipes, 23 were public pipes, and 83 were sachet water. The highest mean TCC and FCC in both seasons were found in stored public pipe water compared to stored private pipe water and sachet water in the households (Table 4.12). Suspected *vibrio* counts were present in all household stored drinking water samples (Table 4.12). However, when the differences between the bacterial mean counts were assessed for the stored household drinking water, the mean rank score of the log of TCC (χ^2=7.704, p=0.021), FCC (χ^2 =2.591, p<0.001) and VC (χ^2=12.101, p=0.002) differed significantly (Table 4.14). The bacterial counts were generally higher in the wet season than the dry season during the period for both the water collected directly from the source and that stored in vessels in the households.

In the non-endemic communities also, 24-direct water sources were sampled from private pipe (4), public pipe (16), and unprotected wells (4) in both seasons (Table 4.13). The direct private piped water sources were found in Dawhenya and Kofikope and recorded a mean TCC of 8.57 log cfu/100ml in the dry season and 10.12 log cfu/100ml in the wet season. The mean FCC was 4.50 log cfu/100ml and 8.61 log cfu/ml in the dry and wet seasons respectively, with no recorded VC. The public pipe samples recorded a mean FCC of 8.44 log cfu/ml in the dry season and 9.64 log cfu/ml in the wet season. The mean FCC was 5.24 log cfu/100ml in the dry season and 9.39 log cfu/100ml in the wet season, although six of the communities (Dawa, Dogobom, Adedetsekope, Asigbeykope, Awhiam, Adjumanikope) did not record any feacal coliforms. Anyaman East community, on the other hand, recorded the highest TCC and FCC in both seasons. Except for Awhiam community that had a VC of 6.91 log cfu/100ml, all other communities did not record any counts. The unprotected wells also recorded a mean TCC of 8.61 log cfu/100ml in the dry season and 10.20 log cfu/100ml in the wet season. The mean FCC was 5.53 log cfu/100ml in the dry season and 9.45 log cfu/100ml in the wet season with a mean VC of 10.31 log cfu/100ml. The differences in the mean rank score between the log of FCC among the direct water sources were assessed and found to be statistically significant (χ^2 = 10.212, p=0.006). Whereas, the mean rank score of the log of TCC and VC among the direct water sources did not differ (χ^2=5.171, p=0.075: χ^2=2.029, p=0.363) as shown in Table 4.15.

The two hundred and sixteen (216) household stored drinking water in the non-endemic communities made up of; 34 private piped water, 136 public pipe water, 36 unprotected dug well, 8 dam water, and 2 sachet waters stored in the household. Except VC which was not found in sachet water, TCC, FCC, and VC were generally present in all household water stored in vessels for the period. The bacterial counts in the stored water samples were higher than those collected from the direct water sources. The TCC and FCC were generally observed to be higher during the wet season than the dry season. The differences between the bacterial mean counts were assessed for the household drinking water stored and a statistically significant difference was found in the mean rank score of the log of TCC and FCC (χ^2=14.165, p=0.007: χ^2=33.901, p<0.001), whiles the mean rank scores for log of VC (χ^2=6.051, p=0.195) was not significant (Table 4.15).

Table 4.12: Mean log of bacterial counts in the dry and wet seasons across some selected background characteristics in Endemic communities

Study variable	Characteristics	TCC (log cfu/ 100 ml)		FCC (log cfu /100 ml)		VC (log cfu /100 ml)	
		Dry	**Wet**	**Dry**	**Wet**	**Dry**	**Wet**
Direct water sources	**Community**	**Mean(sd)**	**Mean(sd)**	**Mean(sd)**	**Mean(sd)**	**Mean(sd)**	**Mean(sd)**
Private pipe	Adabraka	8.16	10.04	5.35	9.30	0	0
	Nungua	7.24	12.10	4.50	10.57	0	0
	Agege	8.07	8.85	0	0	0	0
	Chorkor	7.60	8.52	4.60	0	0	0
	Dansoman	7.74	9.39	0	0	0	0
	Kanehsie	8.16	10.93	0	6.91	0	0
	Mamobi	8.04	10.46	4.79	8.29	0	0
	Mamprobi	6.91	9.61	5.13	0	0	6.91
	Nima	7.60	8.52	4.79	8.52	0	0
	Teshie	7.74	10.20	4.50	10.13	0	0
	Total	**7.73(0.41)**	**9.86(0.36)**	**4.81(0.32)**	**8.95(1.34)**	**0**	**6.91**
Public pipe	Agbogbloshie	8.04	10.60	5.13	10.34	0	0
	James town	7.60	10.78	4.79	9.80	0	0
	Total	**7.82(0.31)**	**9.64(1.29)**	**5.24(0.65)**	**10.06(0.38)**	**0**	**0**

Water stored in vessels	Private pipe	8.26(0.38)	10.76(1.13)	5.53(0.45)	10.04(1.09)	2.40 (0.69)	9.91(1.15)
	Public pipe	8.49(0.37)	11.75(0.38)	5.80(0.44)	11.16(0.76)	1.77(0.88)	9.76(1.52)
	Sachet	8.45(0.39)	10.51(1.08)	5.60(0.41)	10.18(0.99)	2.29(0.71)	10.49(0.57)

Note: All bacterial counts have been log transformed, TCC=Total coliform count, FCC=Faecal coliform count, VC=Vibrio count

Table 4.13: Mean log of bacterial counts in the dry and wet seasons across some selected background characteristics in non-endemic communities

Study variable	**Characteristics**	**TCC (log cfu/ 100 ml)**		**FCC (log cfu /100 ml)**		**VC (log cfu /100 ml)**	
		Dry	**Wet**	**Dry**	**Wet**	**Dry**	**Wet**
Direct water sources	**Community**	**Mean(sd)**	**Mean(sd)**	**Mean(sd)**	**Mean(sd)**	**Mean(sd)**	**Mean(sd)**
Private pipe	Dawhenya	8.48	10.09	0	8.70	0	0
	Kofikope	8.66	10.16	4.50	8.52	0	0
	Total	**8.57(0.13)**	**10.12(0.06)**	**4.50**	**8.61(0.13)**	**0**	**0**
Public pipe	Addokope	8.63	9.21	4.79	0	0	0
	Dawa	8.29	0	0	0	0	0
	Dogobom	8.34	8.00	0	0	0	0
	Adedetsekope	8.27	0	0	0	0	0

	Anyaman East	8.81	10.60	5.70	9.39	0	0
	Asigbeykope	8.57	0	0	0	0	0
	Awhiam	8.48	10.76	0	0	0	6.91
	Adjumanikope	8.16	0	0	0	0	0
	Total	**8.44(0.22)**	**9.64(1.29)**	**5.24(0.65)**	**9.39**	**0**	**6.91**
Unprotected Dug well	Angomya-Ada	8.41	11.00	5.67	9.80	0	10.31
	Kopeheum Kasseh	8.81	9.39	5.39	9.10	0	0
	Total	**8.61(0.28)**	**10.20(1.14)**	**5.53(0.19)**	**9.45(0.49)**	**0**	**10.31**
Water stored in vessels	Private pipe	8.92(0.26)	11.10(0.68)	6.16(0.36)	10.27(0.71)	1.84(0.62)	9.92(1.24)
	Public pipe	8.77(0.26)	10.45(1.13)	6.03(0.34)	9.59(1.08)	2.23(0.92)	9.73(1.08)
	Sachet	8.88	12.21	5.74	10.93	0	0
	Unprotected dug well	8.81(0.31)	11.16(0.61)	6.10(0.40)	10.39(0.77)	2.48(0.59)	11.19(0.78)
	Dam	9.05(0.23)	11.83(0.13)	6.44(0.23)	10.60(0.18)	2.91(0.77)	9.24(0.34)

Note: All bacterial counts have been log transformed, TCC=Total coliform count, FCC=Faecal coliform count, VC=Vibrio count

Table 4.14: Assessing the differences in log bacterial count among household drinking water stored in vessels and from direct water sources in endemic communities

	n	Rank Sum	χ^2 with ties	P-Value
Total Coliform count				
Direct source			0.182	0.669
Private Pipe	4	55.50		
Public Pipe	20	244.50		
Water stored in vessels			7.704	0.021
Private Pipe Stored	110	12068.50		
Public Pipe Stored	23	3198.50		
Sachet water	83	8169		
Faecal coliform count				
Direct source			2.591	0.107
Private Pipe	4	70.50		
Public Pipe	20	229.50		
Water stored in vessels			17.156	**<0.001**
Private Pipe Stored	110	12170		
Public Pipe Stored	23	3532.50		
Sachet water	83	7733.50		
Suspected Vibrio count				
Direct source			0.200	0.655
Private Pipe	4	48		
Public Pipe	20	252		
Water stored in vessels			12.101	**0.002**
Private Pipe Stored	110	12094		
Public Pipe Stored	23	3289		
Sachet water	83	8053		

Table 4.15: Assessing the differences in log bacterial count among household drinking water stored in vessels and from direct water sources in non-endemic communities

	n	Rank Sum	χ^2 with ties	P-Value
		Total Coliform count		
Direct source			5.171	0.075
Private pipe	4	67.50		
Public pipe	16	163		
Unprotected dug well	4	69.50		
Water stored in vessels			14.165	**0.007**
Unprotected dug well	36	4423.0		
Dam water	8	1189.5		
Private pipe	34	4357.5		
Public pipe	136	13168.0		
Sachet	2	298.0		
		Faecal Coliform count		
Direct source			10.212	**0.006**
Private pipe	4	63.50		
Public pipe	16	155.50		
Unprotected dug well	4	81		
Water stored in vessels			33.901	**<0.001**
Unprotected dug well	36	4972.0		
Dam	8	1257.5		
Private pipe	34	4743.0		
Public pipe	136	12205.5		

Sachet	2	258.0		
Suspected Vibrio count				
Direct source			2.029	0.363
Private pipe	4	46		
Public pipe	16	195.50		
Unprotected dug well	4	58.50		
Water stored in vessels			6.051	0.195
Unprotected dug well	36	4206.5		
Dam	8	1152.0		
Private pipe	34	3525.5		
Public pipe	135	14226.0		
Sachet	2	110.0		

Table 4.16 & 4.17 shows the different coliform and other bacteria isolated from the household drinking water in the endemic and non-endemic communities. Out of 120 household drinking water samples that were collected, in the endemic communities for each of the seasons, 76 (63.3%) and 82 (68.3%) positive growths were found respectively in the wet and dry seasons. In some of the water samples, multiple growths of the bacteria were found. Among the positive isolates, the predominant organism occurring in both seasons was *Klebsiella pneumoniae* 24 (31.6%) followed by *Escherichia coli* 13 (17.1%). There were no confirmed *Vibrio cholerae* strains in the household drinking water samples in the endemic communities.

Table 4.16: Microorganisms isolated from the household drinking water samples collected in the endemic communities

	Wet Season	
Organisms	**Frequency**	**Percent**
Acinetobacter baumanni/calcoaceticus	1	1.3
Citrobacter freundii	6	7.9

Enterococcus faecalis	1	1.3
Enterobacter sp	9	11.8
Enterococcus sp	3	4.0
Escherichia coli	13	17.1
Klebsiella pneumoniae	24	31.6
Proteus mirabilis	8	10.5
Proteus vulgaris	1	1.3
Pseudomonas aeruginosa	9	11.8
Serratia ficaria	1	1.3
Total	**76**	**100.0**
	Dry Season	
Acinetobacter baumanni/calcoaceticus	3	3.7
Citrobacter freundii	7	8.5
Enterobacter sp	11	13.4
Enterococcus sp	10	12.2
Escherichia coli	16	19.5
Klebsiella pneumoniae	23	28.1
Proteus mirabilis	7	8.5
Pseudomonas aeruginosa	5	6.1
Total	**82**	**100.0**

A hundred and twenty (120) drinking water samples were sampled from the non-endemic communities in each of the seasons (wet and dry). A hundred and four (104, 86.7%) isolates were identified during the wet season (Table 4.17). Of the 11 different bacteria identified in the wet season, *Escherichia coli* 18 (17.3%) was the most common isolate followed by *Klebsiella pneumoniae* 17 (16.4%). In the dry season, 88 (73.3%) had positive growth with *Klebsiella pneumoniae* (22.7%) as the most frequently occurring followed by *Escherichia coli* (18.2%). There were also multiple growths in some of the water samples. No *Vibrio cholerae* strains were isolated in the household drinking water samples.

Table 4.17: Microorganisms isolated from the household drinking water samples collected in the non-endemic communities

	Wet Season	
Organisms	**Frequency**	**Percent**
Acinetobacter baumanni/calcoaceticus	2	1.9
Citrobacter freundii	7	6.7
Enterobacter sp	13	12.5
Enterococcus sp	11	10.6
Escherichia coli	18	17.3
Klebsiella pneumoniae	17	16.4
Proteus mirabilis	15	14.4
Proteus vulgaris	2	1.9
Pseudomonas aeruginosa	16	15.4
Staphylococcus aureus	2	1.9
Yersinia pestis	1	1.0
Total	**104**	**100.0**
	Dry Season	
Aeromonas hydrophilia	2	2.3
Acinetobacter baumanni/calcoaceticus	1	1.1
Citrobacter freundii	7	8.0
Enterobacter sp	14	15.9
Enterococcus sp	10	11.4
Escherichia coli	16	18.2
Klebsiella pneumoniae	20	22.7
Proteus mirabilis	7	8.0
Pseudomonas aeruginosa	11	12.5
Total	**88**	**100.0**

4.5 Environmental assessment for the presence of *Vibrio cholerae* O1/O139 toxigenic strains in the water bodies in the communities

The survey involved the collection of environmental water samples from selected communities where water bodies were present to test for the presence of *V. cholerae* O1/O139 or Non-*V. cholerae* O1/O139 and other *vibrio* species that may pose a risk to cholera transmission in the endemic or non-endemic communities. All 15 water bodies such as lagoons, estuaries, rivers/streams, and dams were identified in the endemic and non-endemic communities (Table 4.18 & 4.19).

A total of 30 environmental water samples were collected from the water bodies during the wet season (June to August 2019) and dry season (January to March 2020). The 15 water samples collected and cultured during the wet season did not yield *V. cholerae* O1/O139 or Non-*V. cholerae* O1/O139. However, 26.7% (4/15) of the water bodies sampled contained *Vibrio fluvialis*. These were isolated from Korle estuarine, Chemunaa estuaries, Sango lagoon, and estuarine in the cholera endemic communities (Table 4.18). OmpW, O1rfb, O139rfb, and ctxA genes for *V. cholerae*, were not detected using the uniplex PCR.

Table 4.18: Detection and Isolation of *V. cholerae* O1, O139, and non-O1/non-O139 from environmental water samples in cholera endemic and non-endemic communities during the wet season

			Culture				**PCR**			
S/N	**Endemic Community**	**Type of Water Body**	**Vibrio cholerae O1**	**Vibrio cholerae O139**	**Vibrio cholerae Non O1/O139**	**Other vibrio**	**OmpW**	**O1rfb**	**O139rfb**	**ctxA**
1	James Town	Korle Lagoon	-	-	-	-	-	-	-	-
2	James Town Beach	Korle Estuarine	-	-	-	Vibrio fluvalis	-	-	-	-
3	Agbogbloshie	Odorna River	-	-	-	-	-	-	-	-
4	Chorkor	Chemunaa Lagoon	-	-	-	-	-	-	-	-
5	Chorkor	Chemunaa Estuarine	-	-	-	Vibrio fluvialis	-	-	-	-
6	Adabraka	Odorna River (Upper stream)	-	-	-	-	-	-	-	-
7	Adabraka	Odorna River (Lower stream)	-	-	-	-	-	-	-	-
8	Teshie South	Sango Lagoon	-	-	-	Vibrio fluvialis	-	-	-	-
9	Teshie South	Sango Estuarine	-	-	-	Vibrio fluivalis	-	-	-	-
	Non-Endemic Community									
10	Dawa	Dam	-	-	-	-	-	-	-	-
11	Dogobom	Dam	-	-	-	-	-	-	-	-
12	Awhiam	Dam	-	-	-	-	-	-	-	-
13	Anyaman East	Dam	-	-	-	-	-	-	-	-
14	Angomya-Ada	River (Upper)	-	-	-	-	-	-	-	-
15	Angomya-Ada	River (Lower)	-	-	-	-		-	-	-

'-'indicates no detection or isolation, '+'indicates detection or isolation.

During the dry season, a total of 15 water samples were collected from the same sites as in the wet season. Of the 15 samples analysed, only 2 (13.3%) were positive for *V. cholerae* O1 and 1 (6.7%) positive for OmpW (Table 4.19). *Vibrio algionolyticus* was also isolated from the Sango lagoon and estuarine.

Uniplex PCR for the amplification of the genes encoding OmpW (310bp), O1rfb (200bp), O139rfb (450bp), and ctxA (310bp) was performed to confirm the presence of these pathogens. In all, 3 (20%) OmpW (310bp) and 2 (13.3%) O1rfb (200bp) showed positive bands for *V. cholerae* (Figures 4.1 – 4.6). *Vibrio cholerae* O1 non-toxigenic strains (O1rfb (200bp), OmpW (310bp) gene-positive, and ctxA (310bp) negative) were detected in two of the water samples in Awhiam and Dawa in the cholera non-endemic community (Table 4.19). Non-*V. cholerae* O1/O139 (OmpW (310bp) positive gene) was also detected in the water sample in Odorna river in the cholera endemic community (Table 4.19).

Table 4.19: Detection and Isolation of *V. cholerae* O1, O139, and non-O1/non-O139 from environmental water samples in cholera endemic and non-endemic communities during the dry season

S/N	Endemic Community	Type of Water Body	Culture: Vibrio cholerae O1	Culture: Vibrio cholerae O139	Culture: Vibrio cholerae Non O1/O139	Culture: Other vibrio	PCR: OmpW	PCR: O1rfb	PCR: O139rfb	PCR: ctxA
1	James Town	Korle Lagoon	-	-	-	-	-	-	-	-
2	James Town	Korle Estuarine	-	-	-	-	-	-	-	-
3	Agbogbloshie	Odorna River	-	-	-	-	-	-	-	-
4	Chorkor	Chemunaa Lagoon	-	-	-	-	-	-	-	-
5	Chorkor	Chemunaa Estuarine	-	-	-	-	-	-	-	-
6	Adabraka	Odorna River (Upper stream)	-	-	-	-	+	-	-	-
7	Adabraka	Odorna River (Lower stream)	-	-	-	-	-	-		-
8	Teshie South	Sango Lagoon	-	-	-	Vibrio algionolyticus	-	-	-	-
9	Teshie South	Sango Estuarine	-	-	-	Vibrio algionolyticus	-	-	-	-
	Non-Endemic Community									
10	Dawa	Dam	+	-	-	-	+	+	-	-
11	Dogobom	Dam	-	-	-	-	-	-	-	-
12	Awhiam	Dam	+	-	-	-	+	+	-	-
13	Anyaman East	Dam	-	-	-	-	-	-	-	-
14	Angomya-Ada	River (Upper)	-	-	-	-	-	-	-	-
15	Angomya-Ada	River (Lower)	-	-	-	-	-	-	-	-

‘-’indicates no detection or isolation, ‘+’indicates detection or isolation

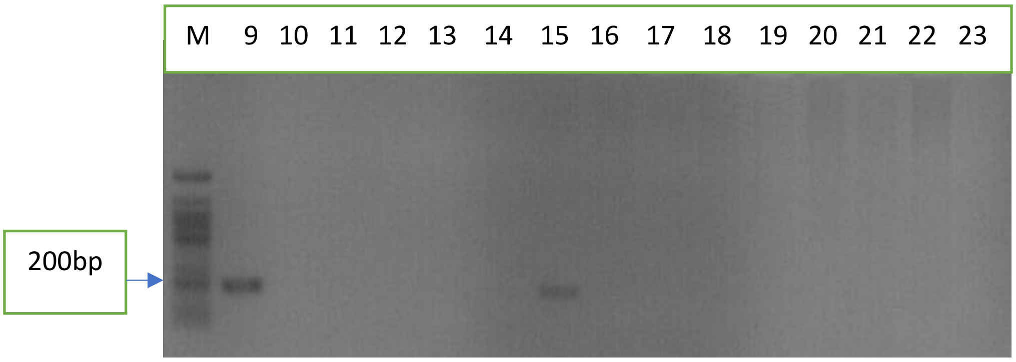

Figure 4.1: Uniplex PCR products of O1rfb [200bp] amplified from environmental water samples and analyzed by 1.5% agarose gel electrophoresis. Lane M, is 50bp molecular base marker. Lane 9 is a positive control. Lane 15 is positive and corresponds to S/N 12 in table 4.18. Lanes 10 to 14 & 16 to 23 are negative. Lane 23 is the negative control.

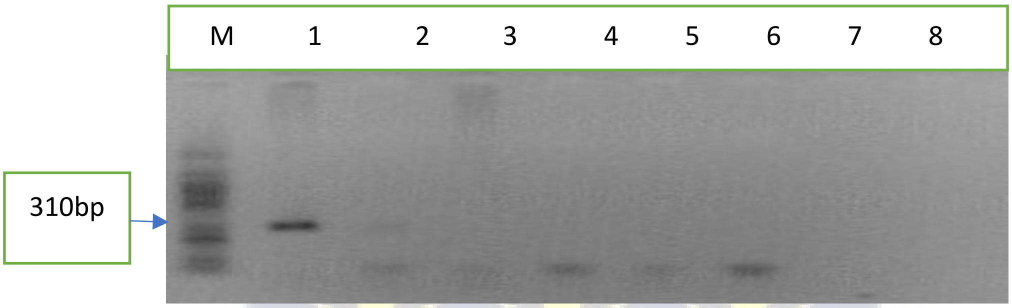

Figure 4.2: Uniplex PCR products of ctxA [310bp] amplified from environmental water samples analyzed by 1.5% agarose gel electrophoresis. Lane M, is 50bp molecular base marker. Lane 1 is a positive control. Lanes 2 to 7 are negatives. Lane 8 is a negative control.

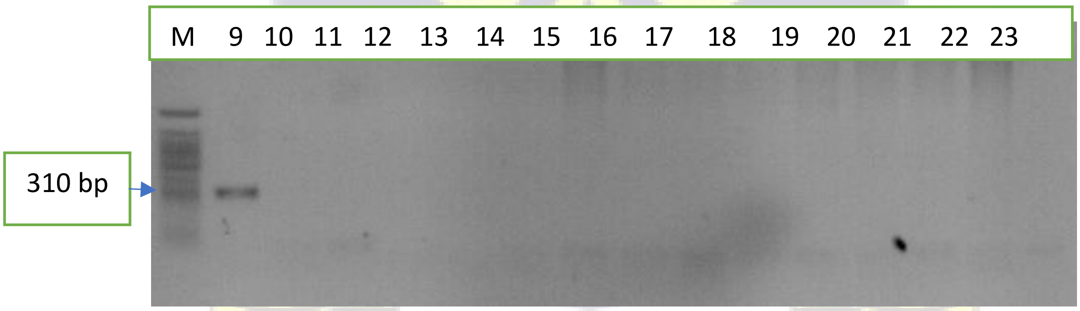

Figure 4.3: Uniplex PCR products of ctxA [310bp] amplified from environmental water samples analyzed by 1.5% agarose gel electrophoresis. Lane M, is 50bp molecular base marker. Lane 9 is a positive control. Lane 10 to 23 are negatives. Lane 23 is a negative control.

4.6 Environmental assessment of the sanitation and cholera risk in the communities

An assessment of the sanitation situation of the households and their neighbourhood was conducted for both endemic and non-endemic communities. Of the ten items which covered responses in the water supply pipelines, refuse, sewage, and excreta waste disposal practices, more than half 300 (66.2%) of the households in the endemic communities recorded poor environmental sanitation, 153 (33.8%) had moderate environmental sanitation and no community had good sanitation as shown in Table 4.20.

All households in Adabraka, Agbogbloshie, Chorkor, Maamobi, and Nima recorded 100% poor environmental sanitation. Agege, Dansoman, James Town, Mamprobi, Nungua recorded less than 50% poor environmental sanitation and above 50% moderate sanitation. Teshie, on the other hand, had a majority of (56, 67.9%) of the households having poor sanitation conditions and 32.1% reported moderate conditions. Kaneshie had 100.0% of the households reporting moderate sanitation.

In most of the reported poor sanitation communities, the water pipeline network connections were laid in open gutters which were choked with refuse. Most of the gutters were choked with garbage, consisting of black polythene bags some containing human excreta, plastic bottles, and sachet water rappers indiscriminately dumped in the drains.

Some waste dumpsites were observed to be overflowing with refuse that attracted houseflies because of the bad odour emanating from the waste. The poor sanitation conditions were common in communities with public toilet facilities. Although some of the toilets were neat and devoid of houseflies' others were unclean with a bad odour that attracted houseflies. Open defecation was observed especially along the undeveloped beaches and waste dumpsites near the bushes around the railway lines in some of these communities. Figures 4.7 to 4.17 show some poor environmental sanitation observed in some of the communities.

In the non-endemic communities, on the other hand, 306 (67.5%) of the households had good sanitation conditions. Among communities where households recorded more than 90% of good sanitation were; Adedetsekope, Angomya-Ada, Asigbeykope, Dogobom, Kopehem-Kasseh, Dawhenya North. One hundred and forty-four households (144, 31.8%) recorded moderate sanitation conditions, with households in Addokope and Awhim recorded 100% moderate conditions. Adjumanikope and Kofikope reported 95.7% and 82.8% good sanitation conditions respectively.

Anyaman East and Dawa communities in the non-endemic communities had over 90% moderate sanitation condition as shown in Table 4.20. Unlike the endemic communities, there were no open gutters, and pipelines were mostly buried in the ground. Their water sources were mostly standing pipes or pipes connected into the household dwelling. The waste in these communities were mostly burnt. Most of the households in the non-endemic communities had toilet facilities. It was however observed that there was open defecation in the bushes in some of the communities.

Table 4.20: Environmental assessment of the sanitation conditions in communities using Principal Components Analysis

	Sanitation condition					
	Poor		Moderate		Good	
	No.	**%**	**No.**	**%**	No.	**%**
Endemic Communities						
Adabraka	30	100.0	0	0.0	0	0.0
Agbogbloshie	32	100.0	0	0.0	0	0.0
Agege	6	46.2	7	53.8	0	0.0
Chorkor	22	100.0	0	0.0	0	0.0
Dansoman	20	43.5	26	56.5	0	0.0
James Town	0	0.0	7	100.0	0	0.0
Kaneshie	0	0.0	25	100.0	0	0.0
Maamobi	50	100.0	0	0.0	0	0.0
Mamprobi	1	4.8	20	95.2	0	0.0
Nima	66	100.0	0	0.0	0	0.0
Nungua	17	28.8	42	71.2	0	0.0
Teshie	55	67.9	26	32.1	0	0.0
Total	**300**	**66.2**	**153**	**33.8**	**0**	**0.0**
Non-endemic Communities						
Addokope	0	0.0	35	100.0	0	0.0
Adedetsekope	0	0.0	0	0.0	36	100.0
Adjumanikope	0	0.0	2	4.3	45	95.7
Angomya-Ada	0	0.0	0	0.0	28	100.0
Anyaman East	2	4.6	42	95.4	0	0.0
Asigbeykope	0	0.0	0	0.0	46	100.0
Awhiam	0	0.0	34	100.0	0	0.0
Dawa	0	0.0	29	93.6	2	6.4
Dawhenya North	0	0.0	0	0.0	44	100.0
Dogbom	0	0.0	0	0.0	25	100.0

Kofikope	1	2.4	2	4.8	39	82.8
Kopehem-Kasseh	0	0.0	0	0.0	41	100.0
Total	**3**	**0.7**	**144**	**31.8**	**306**	**67.5**

* A score of >1 was classified as poor, 0 to 1 moderate and < 1 was classified as good

Figure 4.4: Choked gutters at Adabraka, Accra

Figure 4.5: Choked gutters within households in Agbogbloshie, Accra

Figure 4.6: Waste dump sites near a slum community at Agbogbloshie, Accra

Figure 4.7: Makeshift structures in a slum community along Odorna river in Agbogbloshie, Accra

Figure 4.8: Open defecation along the Odorna river near Agblogbloshie, Accra

Figure 4.9: Waste dumpsite close to the Chemuna Lagoon at Chorkor, Accra

Figure 4.10: Garbage disposed into open drain with water pipelines in Chorkor, Accra

Figure 4.11: Waste dumpsite along a big gutter at Maamobi, Accra

Figure 4.12: Waste overflow at Nungua market dumping site, Accra

Figure 4.13: A public toilet located in the compound of a household in Akpeshika in Nungua, Accra

Figure 4.17: Garbage disposed into open drain with water pipelines in Nima, Accra

4.7 Assessment and identification of environmental risk factors of cholera in the Greater Accra Region

4.7.1 Associations between environmental risk factors and cholera

With regards to environmental factors as in Table 4.21, households with toilet facilities in their household or neighbourhood had more cases of cholera 33 (86.8%) than those without toilet facilities 5 (13.2%), but this was found to be statistically insignificant (p=0.428). Also, those communities with public dumpsites in their neighbourhood recorded more cases of cholera 33 (86.8%) than those communities without 5 (13.2%). The difference was statistically significant (p<0.001).

In terms of the availability of a drainage system for the disposal of household liquid waste, more cholera cases 31 (81.6%) were recorded among households with a drainage system than those without a drainage system 7 (18.4%). This association was found to be statistically significant (p<0.001).

Cholera cases were equally distributed among households in communities with water bodies 19 (50.0%) and those communities without water bodies 19 (50.0%) in the neighbourhood and were found to be insignificantly (p=0.346).

Those neighbourhoods without an abattoir recorded the majority of the cases 35 (92.1%). However, the differences were not statistically significant (p=0.244). The presence of a market in the neighbourhood demonstrated a higher proportion of cholera cases 21 (55.3%) compared to those without a market 17 (44.7%) and was found to be significantly associated with cholera (p=<0.001).

Table 4.21: Associations between environmental risk factors and cholera in Greater Accra Region

Variables	Cholera		P-Value
	Yes = 38	No=868	

	No.	%	No.	%	
		Environmental factors			
Public toilet facility					0.428
Present	33	86.8	710	81.8	
Not present	5	13.2	158	18.2	
Public dumpsite					**<0.001**
Present	33	86.8	465	53.6	
Not present	5	13.2	403	46.4	
Drainage (gutters)					**<0.001**
Present	31	81.6	353	40.7	
Not present	7	18.4	515	59.3	
Water body					0.346
Present	19	50.0	367	42.3	
Not present	19	50.0	501	57.7	
Market					**<0.001**
Present	21	55.3	179	20.6	
Not present	17	44.7	689	79.4	
Abattoir					0.244
Present*	3	7.9	38	4.4	
Not present	35	92.1	830	95.6	

P value is significant if $p < 0.05$, *Fishers Exact Test

A penalized multivariable logistic regression model was further used to determine the impact of the environmental factors on cholera risk in the study communities as shown in Table 4.22. The presence of a toilet facility at home or in the neighbourhood (public toilet) was found to be 72% less likely to have cholera compared to households without toilets or neighbourhood toilets and this was statistically significant after adjusting for other predictor variables (AOR=0.28, 95% CI: 0.08-0.97, p=0.046).

The odds of cholera occurrence were found to be 5.28 times higher among households located in a neighbourhood with a waste dumpsite compared to those with no waste dumpsite in their neighbourhood (OR=5.28, 95% CI: 2.12-13.14, p<0.001). The adjusted model was found to be significant (AOR=2.96, 95% CI: 1.11-7.88, p=0.030).

Households living near a drainage system (open gutters) were 6.12 times more likely to have reported a case of cholera compared to households without a drainage system (OR=6.12, 95% CI: 2.73-13.74, p=<0.001). After adjusting for other predictor variables was found to be significantly associated with cholera occurrence (AOR=5.78, 95% CI: 1.89-17.72, p=0.002).

Households living in a community with a neighbourhood market were 4.72 times more likely to have ever suffered from cholera compared to those living in households without a neighbourhood market (OR=4.72, 95% CI: 2.46-9.06, p<0.001). The adjusted model was however not significant (AOR=1.79, 95% CI: 0.82-3.94, p=0.145).

Table 4.22: Assessment of environmental risk factors of cholera using a penalized multivariable logistic regression model

Variable	OR (95%CI)	P-value	AOR (95% CI)	P-value
Public toilet facility				
Not present	1		1	
Present	1.36 (0.54-3.40)	0.513	0.28 (0.08-0.97)	**0.046**
Public dumpsite				
Not present	1			
Present	5.28 (2.12-13.14)	**<0.001**	2.96 (1.11-7.88)	**0.030**
Drainage (gutters)				
Not present	1		1	
Present	6.12 (2.73-13.74)	**<0.001**	5.78 (1.89-17.72)	**0.002**
Market				
Not present	1		1	

Present	4.72 (2.46-9.06)	**<0.001**	1.79 (0.82-3.94)	0.145

OR – Odds Ratio, AOR – Adjusted Odds Ratio, CI – Confidence Interval, Significant ($p<0.05$)

4.8 Spatial relationship between significant environmental risk factors and the spread of cholera

Cholera cases were spatially distributed in the endemic communities over the period (2012 to 2015) as shown in Figures 4.18 to 4.22. It was observed that the majority of the cholera cases were recorded in 2014 and the least in 2013. Except for Dawhenya North and Kopehem Kasseh in the non-endemic communities that recorded a few cases of cholera during the major outbreak in 2014, no other community recorded a case during the period (Figure 4.20).

In the endemic area, public toilets, public dumpsites, drainage systems, markets, abattoirs, and water bodies (rivers, streams, lagoon, estuarine) were the potential risk factors identified in the communities. All the 12 communities in the endemic area recorded multiple environmental risk factors. In the non-endemic area, water bodies and public toilets were identified in the communities. A penalized multivariate logistic regression model was used to identify public toilets, public dumpsites, and drainage systems (gutters) to be significantly associated with cholera as shown in Figures 4.18 to 4.22.

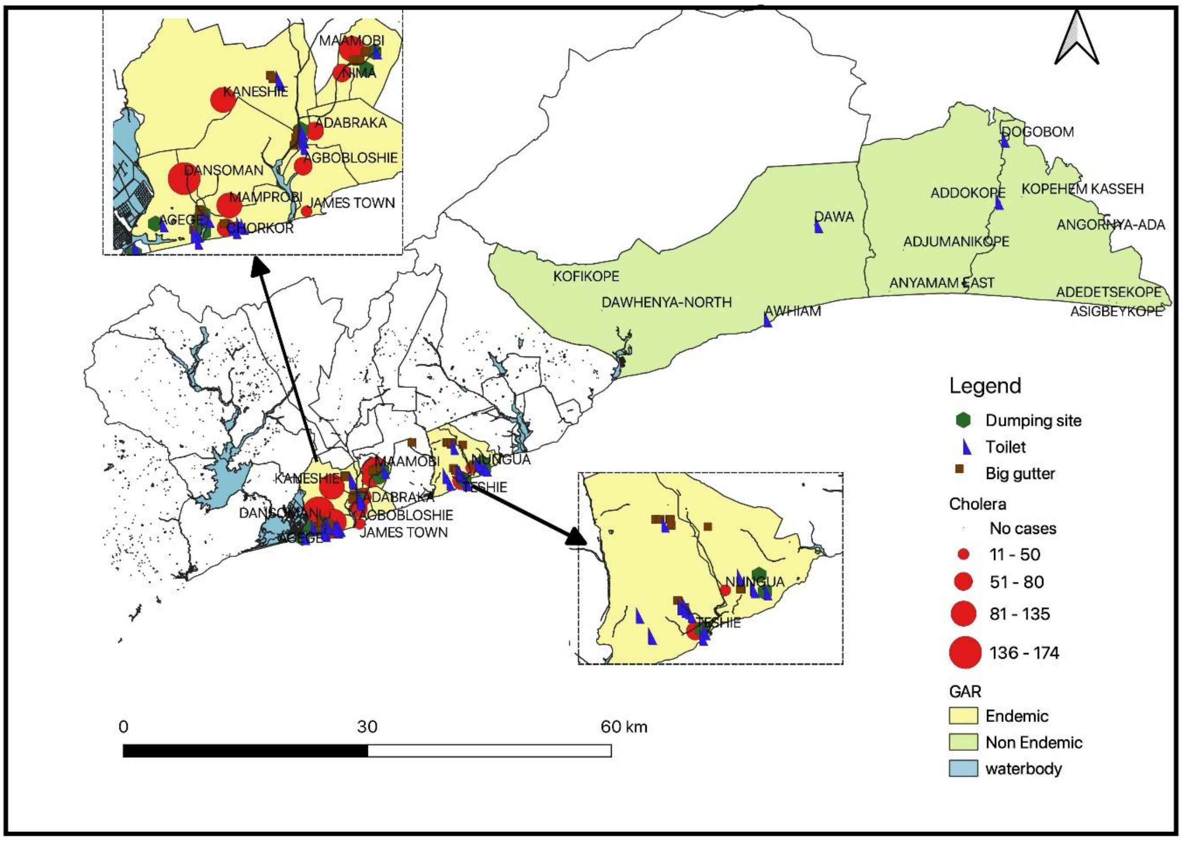

Figure 4.18: Spatial distribution of the significant environmental risk factors and cholera cases in the communities in 2012

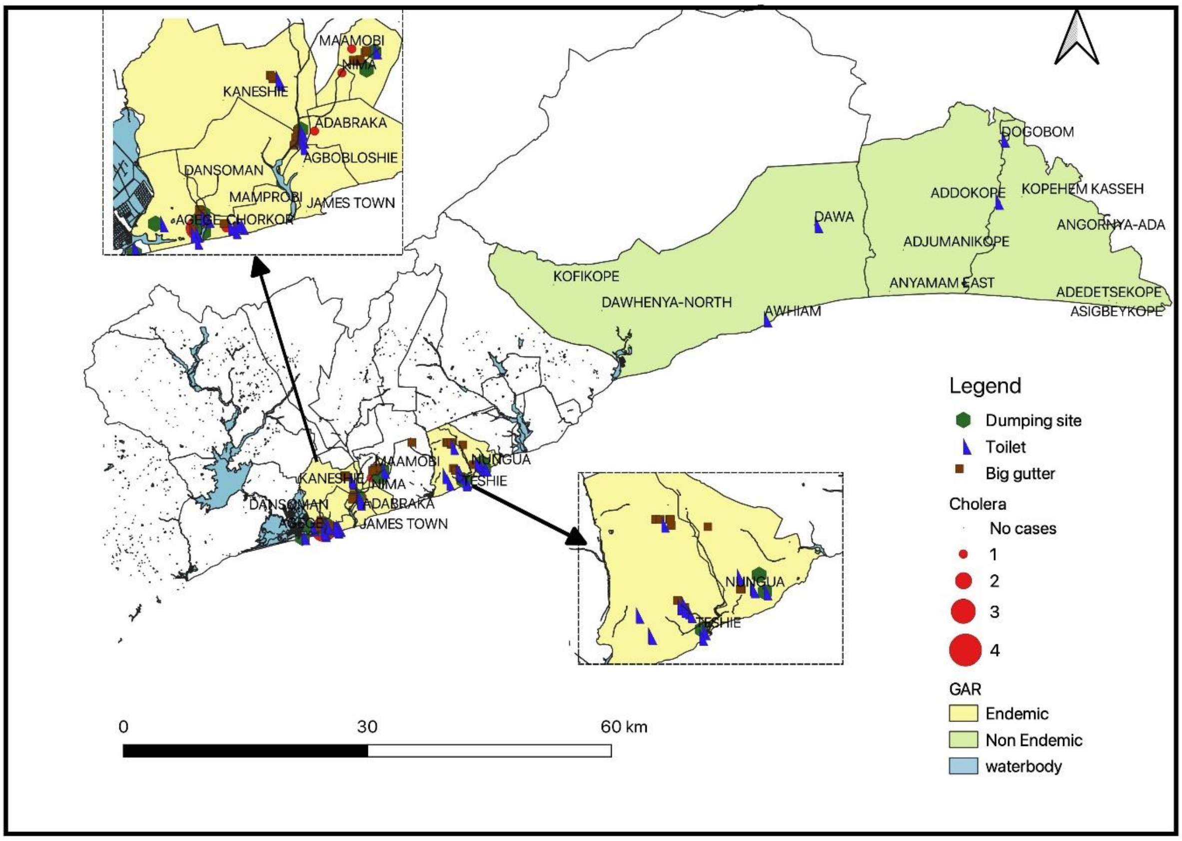

Figure 4.19: Spatial distribution of the significant environmental risk factors and cholera cases in the communities in 2013

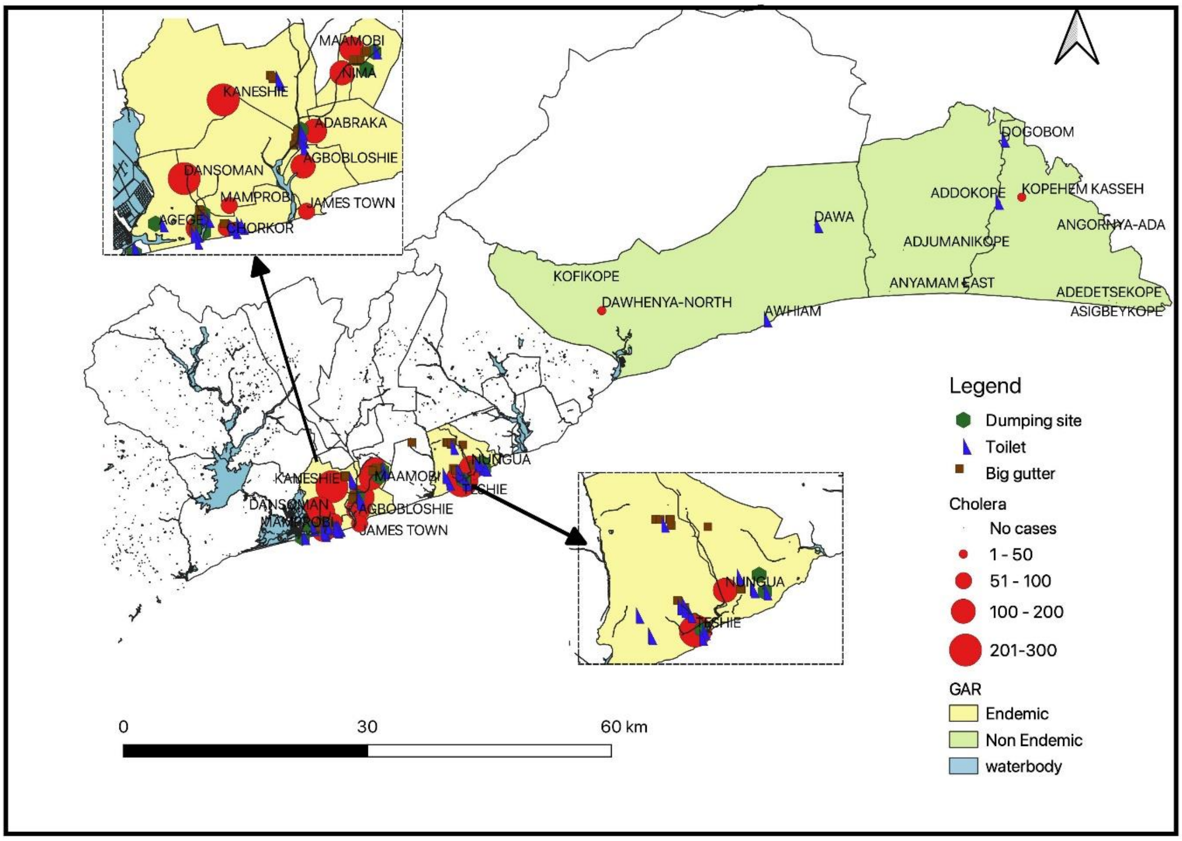

Figure 4.20: Spatial distribution of the significant environmental risk factors and cholera cases in the communities in 2014

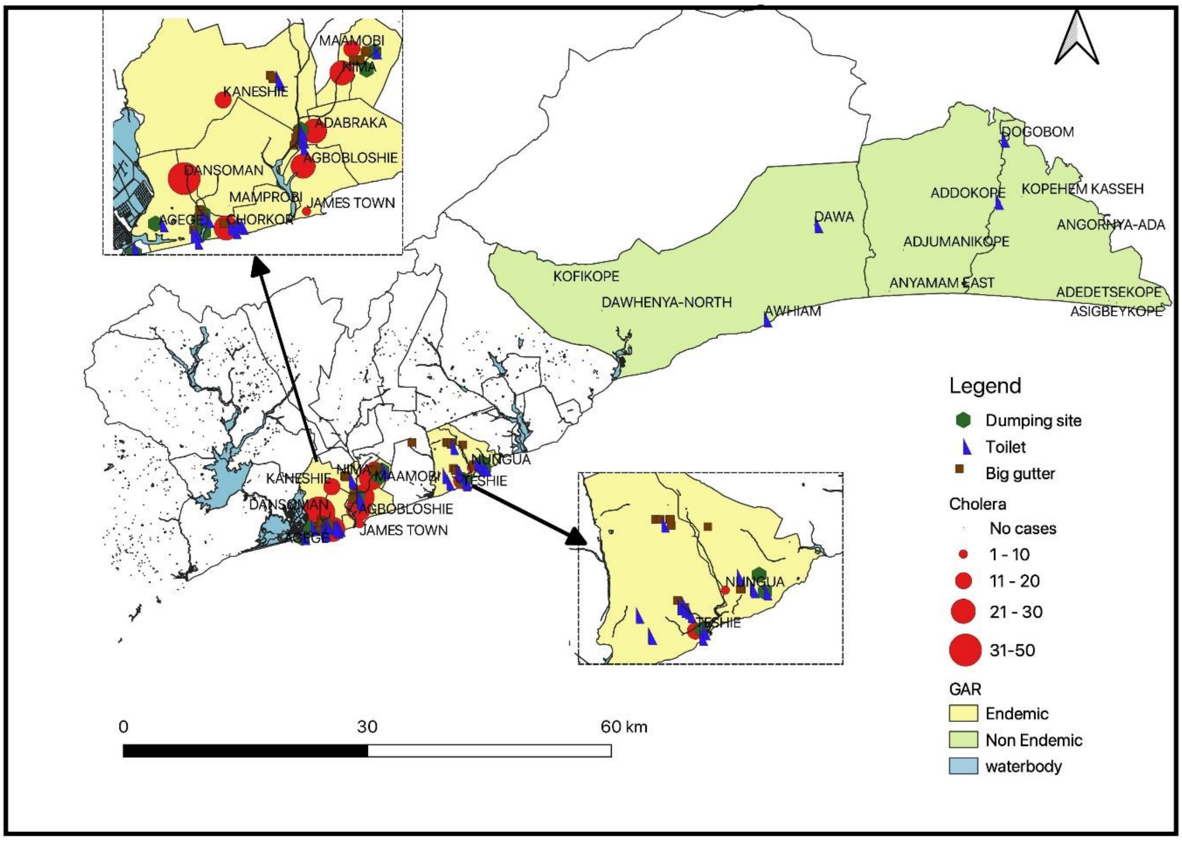

Figure 4.21: Spatial distribution of the significant environmental risk factors and cholera cases in the communities in 2015

Spatial analysis of 500m buffer was created with public toilet facilities as the reference to show the different 'hotspot' communities and the significant environmental risk factors that were associated with the cholera incidence as shown in Figure 4.22.

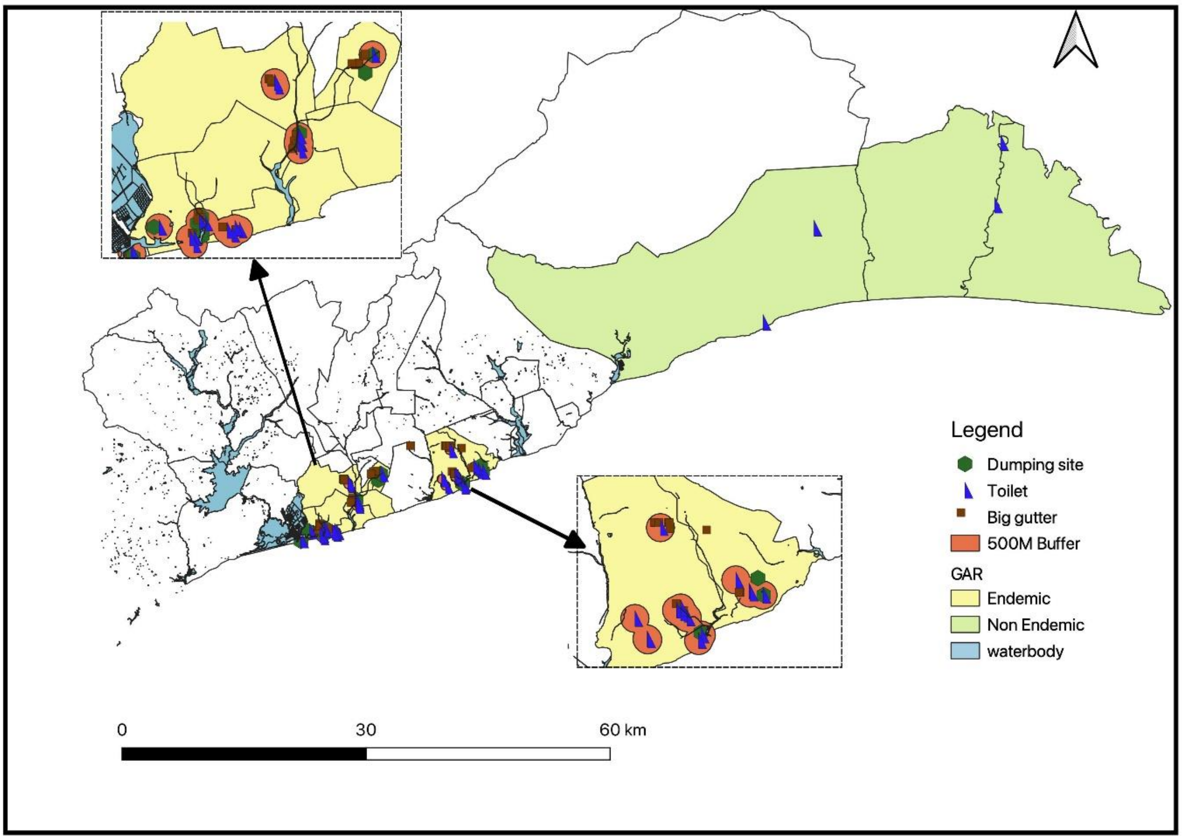

Figure 4.22: Spatial relationship between the significant environmental risk factors and cholera cases within 500m buffer

CHAPTER FIVE

DISCUSSIONS

Summary of Major Findings

The study compared cholera endemic and non-endemic communities to identify the most significant risk factors responsible for the cholera endemicity in the Greater Accra Region. The most important behavioural factor was the unsafe drinking water sources, lack of household toilet facilities. The household cooking location was protective against cholera. The household drinking sources and storage were highly contaminated with total and faecal coliforms.

The study demonstrated the absence of the toxigenic *V. cholerae* O1 and O139 in the environmental water bodies and household drinking water sources or storage. However, revealed the presence of *Vibrio fluvialis* and *Vibrio algionolyticus* in some environmental water bodies.

The environmental assessment showed pipe network contamination, indiscriminate dumping of refuse including human excreta, open defecation was still prevalent in the endemic communities.

The study further identified the significant environmental risk factors such as waste dumpsites, open drainage, and public toilets to be close (500m) to each other and the historical cholera cases.

5.1 Behavioural Factors Associated with Cholera Outbreaks

5.1.1 Water sources and handling factors

The main drinking water sources identified in this study were; private pipe, public pipe, borehole, unprotected well, surface, and sachet water. The study further revealed that most communities in both the endemic (99.8%) and non-endemic communities (84.3%) have access to improved water sources (i.e private pipe, public pipe) and sachet water. The findings suggest an improvement over the national coverage of 44.3% to 79.0% access to improved water as reported by the Ghana Living Standard Survey in 2017 (Ghana Statistical Service, 2018; Government of Ghana, 2019). The improvement may reflect government efforts to focus on water and sanitation issues to achieve the Sustainable Development Goal (SDG) 6 by 2030. The access to improved water by communities, however, does not guarantee safety. This current study found a significant association between the main sources of household drinking water and cholera. An earlier study during the 2014 cholera outbreak in the Osu Klottey sub metropolis in Accra similarly found drinking pipe-borne water to be associated with cholera (Davies-Teye, Vanotoo, Yabani, & Kwakye-Maclean, 2014). The study also observed that the majority of the water sources in the endemic (57.0%) and non-endemic (75.9%) communities were located elsewhere outside their households. A significant association was found between cholera cases and the location of the drinking water sources. This may be attributed to inadequate treatment at the water source or contamination along with the pipe network in the case of pipe-borne water. This is supported by Karikari and Ampofo (2013) of unsuitable levels of chlorine in the Accra water network which may be attributable to incomplete treatment of pipe-borne water.

Despite the challenge with drinking water safety many communities do not treat their water at the point-of-use. This study found low water treatment at the point-of-use in both endemic (9.3%) and non-endemic communities (11.9%). This finding is consistent with other studies which similarly found low treatment of water at the point-of-use (Kaniambady, Vasu, Sandhya, & Kulkarni, 2017; Lartey, 2019). However, this contrasts a community-based study in Mangalore, South India which reported a high (95.0%) household water treatment as a result of the high literacy rate of the participants (Mithra et al., 2010). Although more cholera cases were recorded among households that did not treat their drinking water at the point-of-used it was not significant. Our findings nonetheless give an indication of the low promotion of household water treatment in the communities and the need for water treatment intervention to protect the high-risk population from cholera infection.

The study findings also revealed that the majority of households in the endemic (97.8%) and non-endemic communities (100.0%) experienced pipe-borne water shortages. These shortages are attributed to the high demand exceeding the production as a result of the rapid urbanization of the communities in the region. Amidst water scarcity, households relied on other unimproved water sources such as surface water and dug well as observed in the non-endemic communities, and tanker water supply as found in the endemic communities. This scarcity and rationing of pipe-borne water also make households resort to a longer storage of their water leading to contamination as has been reported in other previous studies (Boateng et al., 2013; Gizachew et al., 2020). There is the need to ensure a regular supply of pipe-borne water to prevent cholera in at-risk populations.

5.1.2 Food eating practices

There are many foods related habits that are associated with cholera. This mostly occurs through the unhygienic handling of food which may lead to contamination with the *Vibrio cholerae* bacterium. In this current study, household food eating habits were explored. This study did not find any significant association between households that ate food from outside the home compared to those who ate from only home. This finding is consistent with a study by Moradi et al. (2016) conducted in Iran that did not also find any significant association between cholera and the consumption of food from street vendors. This finding, however, contrasts other studies which attributed cholera infection to the consumption of food sold by street vendors (Nsagha et al., 2015; Ohene-Adjei et al., 2017). They attributed the unhygienic way ready-to-eat foods are handled and sold by vendors to the cholera infection. Although this study did not find any significant relation with food eating habits of the households, street vendor foods are mostly sold by unlicensed vendors whose food safety manners may be compromised and a likely source of cholera transmission (Cummings et al., 2012; Nguyen et al., 2014). Thus, District Assemblies should ensure food vendors are adequately screened and licensed to operate to protect the population from cholera.

The study also assessed the location where food was cooked in the household. It was observed that the majority of the households in both endemic (51.9%) and non-endemic communities (37.3%) cook in the yard compared to those who cooked inside the house or in a separate building. This study demonstrated that households that cooked in a separate building or the yard were protected from cholera infection compared to households that cooked in household dwelling. Despite this finding, the protection from cholera is dependent on the hygienic conditions of the cooking area.

This study also investigated the household's treatment of their vegetables before consumption. The study revealed that vegetables were treated with; only water, water and heat steam, water and salt, salt solution and heat steam, vinegar and heat steam. The study observed that most communities in the endemic (67.1%) and non-endemic (80.4%) treated their vegetables by washing with only water. The good practice is to wash with salt and heat steam or washed in vinegar and heat steam which only a few did. This finding however was not significantly associated with cholera. Notwithstanding, it is worrisome that most households are treated only by washing with water as these vegetables could be contaminated on the farm with sewage or manure of human origin and brought onto the market for sale. This was the case in Zambia, where the outbreak was linked to the consumption of raw vegetables (DuBois et al., 2006). A similar study in Ethiopia was also linked to the consumption of raw vegetables (Dinede et al., 2020). There is a need for education on the adequate treatment of vegetables before consumption to avoid contracting cholera.

The eating of leftover foods is common in most countries, however, the risk of cholera transmission becomes important when the food is treated before eating (Tang et al., 2013). In this study, there was no significant association between cholera and the households' habit of eating leftover foods. The study findings revealed that the majority of the households treated their leftover foods by heating them before eating. Only 4.9% of households in the non-endemic communities claimed they did not heat their leftover foods before eating and were at risk of cholera. Nonetheless, no significant association with the methods of household treatment of leftover foods and cholera was observed. This contrast a study carried out in Sierra Leone which found eating hot food to be protective (Nguyen et al., 2014). *V. cholerae* can survive in food that is refrigerated or stored at room temperature and heating the food is the remedy to destroy the bacterium in the food (Tang et al., 2013; Waturangi et al., 2015).

Handwashing before eating is a good practice to prevent many diseases including cholera. This study assessed the handwashing habit of households before meals and found a majority of households in both endemic (59.2%) and non-endemic (63.6%) communities wash their hands with only water before meals. On the other handwashing techniques used were; washing with soap in a bowl of water, washing hands in a running pipe-borne water, and washing with soap and running pipe-borne or 'Veronica bucket' (i.e a bucket with a tap connected). The washing of hands in a bowl of water is inappropriate because the germs that are washed off are picked up again. Only a few households practiced proper handwashing with soap under running tap water. The results of the findings however did not reveal any significant association between hand washing before meals and cholera infection. This study, however, contrasts other studies that reported handwashing with soap under running water to be protective against cholera (Dan-Nwafor et al., 2019; Richterman, Sainvilien, Eberly, & Ivers, 2018; Wolfe, 2018).

The proper washing of fruits with soap under running pipe-borne water was also not practiced in the studied communities. The majority of the households washed their fruits including mangoes, oranges, apples, watermelons, etc in a bowl of water. This practice has the disadvantage of recontamination of the fruits in the same water during the washing process. This study did not find any significant association between cholera and the washing of fruits before eating. This study's findings contrast that of Moradi et al. (2016) conducted in Iran which associated the eating of fruits to cholera outbreaks which they speculated spread cholera in the province. This often occurs when the fruits are inadequately washed before eating.

5.1.3 Solid waste disposal factors

The faecal oral route transmission of diarrheal diseases can be prevented through the proper disposal of human excreta (Chikwe et al., 2020). In this study, it was observed that 70.9% of households in the endemic and 51.7% in the non-endemic communities did not have toilet facilities in their homes and relied on public toilets or practice open defecation. This is consistent with other previous studies conducted in Prampram and Dodowa in the Greater Accra Region which revealed 85.0% and 85.9% of households respectively lacking access to toilet facilities in their homes (Akpakli, Manyeh, Akpakli, Kukula, & Gyapong, 2018; Obeng et al., 2015). The lack of funds and inadequate space to construct toilets especially in the poverty-driven communities may explain the low access to toilet facilities in the households in the endemic communities. The much-improved access to household toilet facilities in the non-endemic communities on the other hand may be due to compliance of household heads to the district by-laws to include toilet facilities in the building plans. This study however did not find any association between cholera and the availability of household toilet facilities. This notwithstanding, the lack of household toilets may encourage the practice of open defecation especially at night if household members cannot visit the public toilet facilities. The average round trip time to go and use a public toilet facility was also examined in this study. It was observed that it takes an average round trip of 12.17 minutes and 9.50 minutes for households in the endemic and non-endemic communities respectively to go and use a public toilet facility. Although it appears closer to the households, access could be a challenge at night. As such household's ownership of toilets should be encouraged and public toilet facilities limited to public places such as markets, shopping malls, or lorry stations. This will help to prevent people from defecating into chamber pots and plastic bags and throwing them into gutters at night when it becomes impossible to visit the public toilets.

With regards to the sharing of household toilets, the study revealed that 71.3% of households in the endemic and 86.8% in non-endemic communities shared their toilets with other households or the public. This study corroborates with the finding from the study by Akpakli et al. (2018) which found 85.9% of households in Dodowa sharing toilet facilities. Toilets that are shared by more than one household are considered unimproved according to WHO because of the likelihood of contact with human excreta and the high risk of cholera infection (UNICEF and WHO, 2019). It was observed that shared toilet facilities exceed the national coverage of 56.7% in this study, demonstrating the challenge of the use of unimproved sanitation facilities in Ghana (Appiah-Effah et al., 2019). This contrasts with the study by Kaniambady et al. (2017) in Karnataka, India where all households used improved sanitation facilities. The high use of shared toilet facilities in our study area maybe because most of our participants lived in compound houses where toilet facilities are shared.

This study supports Just et al. (2018), that demonstrated the increasing incidence of diarrhoeal diseases with increasing coverage of sharing toilets using mathematical modelling. This they attributed to the challenge of maintaining good hygienic practices in shared toilets. A survey of 570 households in a slum community by Heijnen, Routray, Torondel, and Clasen (2015) also suggested that shared toilets were more likely to be unclean with faeces and flies. This study also found some households in the endemic communities to be using bucket toilets which have a high risk of cholera transmission because of the possibility of contact with faeces and prohibited by the District Assembles. Although shared toilets are mostly used by households in urban communities the hygienic conditions of such facilities must be maintained to avoid spreading cholera and other diarrhoeal diseases.

Handwashing after defecation is important in preventing faecal oral route transmission of diseases (Chikwe et al., 2020). The availability of handwashing devices near the toilet facilities encourages people to wash their hands after defecation. In this current study, 82.4% of households in the endemic communities had handwashing devices (running tap water and soap) near the toilet facilities for immediate use after defecation compared to 41.8% in non-endemic communities. A study in Kersa Woreda reported only 8.3% of the households have hand washing facilities near the toilet (Mengistie & Baraki, 2010). The availability of handwashing devices at the toilet facilities was significantly associated with cholera ($p=0.001$). This suggests that having a handwashing device does not necessarily guarantee effective handwashing. In the non-endemic communities where toilet ownership was common, they lacked handwashing devices. There is the need for the provision of handwashing devices at all toilet facilities (home or public) and community members should be educated on effective handwashing after defecation to prevent the transmission of cholera.

In urban communities, large tonnes of waste is produced as a result of the rapid urbanization and high population density (Anaman & Nyadzi, 2015; Ohene-Adjei et al., 2017). Unfortunately, the waste companies are not able to manage the large quantities of this waste produced in the urban communities and these are often left to overflow and, in some cases, disposed of indiscriminately into gutters and on the street. In this current study, it was found that most households temporally stored their waste in sacks and rubbish bins before disposal. Households' temporal storage of waste was significantly associated with cholera. This may be explained by the odour generated from the waste that might attract flies or other rodents which could act as vectors in the transmission of cholera especially if the waste is kept for long periods before final disposal.

In this study majority (86.6%) of the waste was collected by the motorized tricycles also known locally as 'Aboboyaa' in the endemic communities whereas Zoomlion Company mostly (69.6%) collected the waste in the non-endemic communities. The engagement of these motorized tricycles waste collectors to do the door-to-door collection of solid waste has significantly improved waste collection in most homes as they do not have to wait for the waste companies' vehicles which are not regular (Issahaku, Nyame, & Brimah, 2014). This has helped to manage the tonnes of waste to substantial levels, although more needs to be done. For household's solid waste that is not collected by any waste company, they were disposed of through burning, burying, indiscriminate dumping, or at public dumpsites. This attitude of disposal could be attributed to a lack of education on the harmful effects of such practices as similarly observed by Kanhai, Agyei-Mensah, and

Mudu (2019) in Accra. The burning of waste was the most common means of disposing of waste in the non-endemic communities because of its rural setting and the lack of waste bins and vehicles to convey the waste. Therefore, motorized tricycles should be encouraged to augment the services of the other waste companies that exist in such communities. Households that were not patronizing the services of the waste companies in both communities gave reasons as the high charges and the lack of waste bins.

5.1.4 Liquid waste disposal factors

Liquid waste disposal from households involves wastewater from the bath and other domestic chores. In this study the drainage systems identified for disposal of liquid waste were; open gutters and those with pipe connections underground into a septic tank. In the study also, the liquid waste from the domestic waste which includes that from kitchen and washings were either disposed through the sewage system into a septic tank or thrown onto the streets, gutters, or compounds. Our findings reveal 94.4% of the drainage in the endemic communities to be open gutters whiles 83.7% of the drainage in the non-endemic communities was the underground drainage type. Irrespective of the type of drainage there was no significant association with cholera. The liquid waste from the bath was discarded in a similar manner depending on whether the bath was connected to a septic tank or into a gutter or sock away. The methods of household liquid waste disposal were significantly associated with cholera. This may be attributed to the sanitary conditions of the drains which were predominantly open gutters.

5.2 Bacteriological quality of household drinking water

The bacteriological quality of water can be affected by the natural presence of microorganisms or from contamination by human activities which may pose a risk to cholera and other diarrhoeal diseases (WHO, 2017c). The total coliform and faecal coliform counts are known to indicate faecal contamination of the drinking water and high risk to potentially harmful pathogens. This current assessment of the bacteriological quality of water was carried out to determine the risk of household drinking water and cholera infection. The presence of *V. cholerae* or bacterial indicators of water quality in direct water sources and the household drinking water storage were assessed. In the endemic communities, the water source collected for drinking was from a private pipe or public pipe. In the non-endemic communities on the other hand the sources were from private, public and unprotected wells. This study demonstrated that the direct pipe, public pipe, unprotected well water sources collected from most of the communities during the dry and wet season were contaminated with total and or faecal coliform counts above the WHO and Ghana standards of zero (0) cfu/100 ml for drinking water both in the endemic and non-endemic communities, making them unwholesome for consumption (Ghana Standard Authority, 2009; WHO, 1993). These findings are consistent with other studies that recorded high total and faecal coliform counts in directly collected piped and well water (Oyedum, Adabara, & Kuta, 2016; Tekpor, Akrong, Asmah, Banu, & Ansa, 2017). The high bacterial counts in the piped water sources may be attributed to the practice of laying pipelines in gutters and the lack of maintenance of the pipelines. Inadequate chlorination may also account for these high bacterial counts as reported by Karikari and Ampofo (2013) in an earlier study that revealed unsuitable chlorine levels and the presence of faecal coliform in the Accra water network. The differences in bacteria counts between the direct pipe and public taps in the endemic communities did not show any statistical significance, indicating that their contamination levels were similar.

The same was the bacteria counts in the non-endemic communities except faecal coliform counts that showed significant differences in private, public, and unprotected well water. The private and public piped water are supplied by Ghana Water Company and may just be a reflection of their service delivery that needs improvement to reduce the risk of cholera and diarrheal disease transmission. The unprotected wells may have run-off water contamination of the wells especially during the rainy season that might account for the high counts from the direct well water. This notwithstanding, the direct main public tap water in Dawa, Aedetsekope, Asigbeykope, and Adjumanikope did not record any coliform counts and was considered wholesome by the standards in the wet season. These communities have a community public standpipe located at a central point in the community and either connected to a borehole or directly to a high-level elevated tank, thus have few pipeline connections, unlike in the endemic communities where the public standpipes were connected to the general pipeline distribution and subject to more contamination. With regards to seasonality, higher total and faecal coliform counts were generally observed in the wet season than in the dry season in both the endemic and non-endemic communities. This finding is in agreement with other studies that similarly found higher total and faecal coliform counts in the wet season than the dry season (Dongzagla, Jewitt, & O'Hara, 2021; Odonkor & Mahami, 2020). This may occur as a result of the poor environmental conditions around some of these water sources in the communities.

In this study, the water stored for drinking by the households was also assessed for the level of contamination. The source water was collected and stored mostly in plastic containers and a few in earthenware/pot. The study results revealed that irrespective of the water source (a private pipe, public pipe, sachet water, well, or dam) the contamination levels for total and faecal coliforms were higher than that directly collected from the water source. In the endemic communities, our study found a significant difference between the bacterial contamination levels and the water sources stored in the household. This was similarly observed in the non-endemic communities, except the *Vibrio* count which did not show any significant differences with the water sources stored. This study is consistent with the findings of Meierhofer, Wietlisbach, and Matiko (2019) in Kenya which found higher contamination levels in the storage containers. The reports of Boateng et al. (2013) and Agensi et al. (2019) in Tamale, Ghana, and Kisoro, Uganda respectively, are similarly in line with this study's findings. The cleanliness of the storage vessels, methods of drawing water from the storage container, and the length of the storage were implicated for the high bacterial counts (Asfaw, Reta, & Yimer, 2016; Lartey, 2019). This study findings also revealed that sachet water described as 'pure water' and increasingly relied on by most residents in the endemic communities recorded similarly high levels of contamination with total and faecal coliforms as the private pipe and public tap water. Earlier studies in Ghana and Nigeria have variously indicated sachet water to be contaminated with faecal coliform and other pathogenic bacteria and not wholesome (Aslan et al., 2020; Mosi, Adadey, Sowah, & Yeboah, 2019; Oluwafemi & Oluwole, 2012). Semey et al. (2020) explained in their study that the contamination might be due to the unhygienic handling by factory workers during production. It may also occur if the source water used in the packaging is already contaminated without any further treatment. Dzodzomenyo et al. (2018) have further indicated that some of the sachet water companies are not formally registered by the Food and Drug Authority and might be violating the guidelines of the standard for quality water production. It is therefore not surprising that Aslan et al. (2020) study that examined the sachet bag observed some bags not to have a batch number, date of manufacture, and no information on treatment. This is worrisome as the shift to drinking sachet water might not necessarily be protective against cholera.

Further analysis was conducted to confirm the suspected *Vibrio* isolates that were mostly detected in the household drinking water storage in both seasons. The most frequently isolated organisms in both seasons were; *Escherichia coli* and *Klebsiella pneumoniae*. The presence of these organisms indicates the possibility of the presence of potentially harmful bacteria. The suspected *Vibrio* counts observed were confirmed to be negative for *Vibrio* *cholerae*, instead were identified as *Pseudomonas aeruginosa*, *Proteus sp.* and *Aeromonas hydrophilia*. Our findings did not detect the presence of *V. cholerae* and contrast that of Yirenya-Tawiah et al. (2018) that found *Vibrio cholerae* O1 strains in household drinking water storage in some communities in Accra just before the major cholera outbreak in 2014. The absence of *V. cholerae* in the current study explains the reason for the absence of a cholera outbreak during the study period. The findings further strengthens the importance of regular environmental surveillance to predict cholera outbreaks.

5.3 Assessment of environmental water bodies for the presence of *V. cholerae* O1/O139

The water bodies in the selected communities for the study were assessed for the presence of toxigenic *V. cholerae* O1/O139, a potential risk for cholera infection. This study revealed the presence of non-toxigenic *V. cholerae* O1 strains in dam water in Awhiam and Dawa in the non-endemic communities. *V. cholerae* non O1/non O139 was also found in the Odorna River in the endemic communities. These findings are consistent with that by Bwire et al. (2018) that examined major surface water bodies in Uganda but did not find any toxigenic *V. cholerae* O1/O139 strains. Similar studies in Cameroon and Burkina Faso did not also identify toxigenic strains in surface water (Akoachere & Mbuntcha, 2014; Kaboré et al., 2018). The findings however contrast an earlier study by Abana et al. (2019) that identified toxigenic strains in environmental water samples in some cholera endemic communities in Accra.

Other studies in Haiti also identified toxigenic *V. cholerae* O1 from some surface water (M. T. Alam et al., 2015; M. T. Alam et al., 2014). Toxigenic *V. cholerae* is believed to be transiently present in surface water during outbreaks. The Abana et al. (2019) study was conducted after the major 2014 cholera outbreak and the spillover of the cases into 2015 may explain the presence of the toxigenic strains. The absence of the toxigenic strains of *V. cholerae* O1 and O139 may explain the reason for the absence of an outbreak of cholera in the region. It also indicates that environmental surveillance of *V. cholerae* may be able to predict future outbreaks. This notwithstanding, non-toxigenic *V. cholerae* strains exist naturally in coastal waters, including areas where clinical cases did not exist (Almagro-Moreno & Taylor, 2014). These non-toxigenic strains however have been recognized to be associated with localized outbreaks of diarrhoea and gastroenteritis and are of public health concern (Aydanian et al., 2015; Kaboré et al., 2018; Marin et al., 2013). It has also been reported that a few non-toxigenic *V. cholerae* strains could evolve to become pathogenic strains under selection pressure and their presence in the aquatic environment in the communities is important (Islam et al., 2020). This is however yet to be established in the Ghanaian water bodies as other researchers have disputed the existence of the possibility of an indigenous source (aquatic source or human carriers) and have attributed cholera outbreaks to mobile populations within the sub-region (Moore et al., 2018).

This study did not detect *V. cholerae* O139 strains in the water bodies and the findings are consistent with similar studies in Burkina Faso and Cameroon but contrast the findings of Bwire et al. (2018) in Uganda which observed the presence of non-toxigenic *V. cholerae* O139. Hitherto, *V. cholerae* O139 strains were limited to India, Bangladesh, and some East Asian countries (Chowdhury et al., 2015; Mahapatra et al., 2014). This suggests the possibility of a spread to areas that were not originally indigenous for *V. cholerae* O139 strains. All the *V. cholerae* strains identified in this study were identified during the dry season and contrast other studies in Cameroon and Uganda where *V. cholerae* were frequently identified during the rainy season (Akoachere & Mbuntcha, 2014; Bwire et al., 2018). Although our detection occurred during the dry season there were intermittent rains during the period and the warmer temperatures could have supported their proliferation as reported by Emch et al. (2008) in an earlier study. Nevertheless, cholera transmission can occur during any season but usually more commonly spread with the flood waters in the rainy season.

Other *Vibrio's* with similar phenotypic resemblance to *V. cholera*e were further analysed and revealed to be *Vibrio fluvialis* and *Vibrio algionolyticus*. These pathogens can be easily isolated with routine cultural techniques, but are difficult to differentiate between the species (Ramamurthy, Chowdhury, Pazhani, & Shinoda, 2014). This study used the mini Antigenic Profile Index (API E20) to identify them for the first time in the Ghanaian water bodies. *Vibrio fluvialis* was detected in the lagoon and estuaries in the endemic communities during the rainy season. The *V. algionolyticus* on the other hand was identified in the Sango lagoon and estuarine in Teshie during the dry season. Like the other *Vibrios*, these have also been found to exist naturally in coastal water bodies with poor sanitation conditions. *V. fluvialis* is a fast emerging pathogen that presents with a cholera-like diarrhoea, gastroenteritis, and septicaemia in immunocompromised patients (Liu, Chiu, Chao, Hou, & Lai, 2011; Usó, Tirado, Moreno, & Campos, 2010). The *V. fluvialis* has haemolysin and metallprotease toxin genes which increases their virulence and are associated with foodborne outbreaks especially among people who eat inadequately cooked seafoods (Chowdhury et al., 2012; Liang, Cui, Du, Kan, & Liang, 2013). A suspected case of cholera that was reported from a fisher folk at the time of this study, was confirmed to be caused by *V. fluvialis* and is an important organism to study in the future to elucidate its public health importance for inclusion in the national disease surveillance programme. The *V. algionolyticus* is also known to cause chronic diarrhoea, otitis, wound infection, and septicaemia when exposed to contaminated seawater (Citil, Derin, Sankur, Sahan, & Citil, 2015).

5.4 Assessment of Environmental Sanitation Condition of the Communities

The assessment of the environmental sanitation condition revealed that most of the communities in the endemic communities had poor sanitation with a few categorized to be moderate. The poor environmental sanitation was associated with contamination of the water supply pipe network laid in open gutters, indiscriminate dumping of refuse including human excreta as well as the presence of open defecation. The findings reflect a systematic review on cholera outbreaks in Ghana by Donkor and Namaitijiang (2019)that described the sanitation situation to be unsatisfactory. The poor environmental sanitation in Accra has similarly been reported by Ohene-Adjei et al. (2017) during the major cholera outbreak in 2014. In their study, they indicated that the index cases for the 2014 cholera outbreak were found in locations in Accra where unhygienic and poor environmental sanitation existed. Dzotsi et al. (2016) in an isolated case-control study in Accra during the cholera outbreak in 2014, also attributed contamination of water supply pipelines, crude dumping of refuse, poor drainage, choked gutters, and open defecation to the outbreak. In that study, they observed all the cholera cases resided in communities that had generally poor sanitation conditions. Although governments in the past and present have attempted to address the sanitation situation through the creation of a ministry for sanitation and water resource, 'national sanitation day' and the 'one house, one toilet' initiative by the Accra Metropolitan Assembly, sanitation issues remain a challenge as revealed by our current study. This notwithstanding most communities in the non-endemic area had good or moderate sanitation because they had their pipelines buried in the ground with no open gutters even though open defecation was observed in bushes in some of the communities, they were not washed to contaminate the water pipelines. On the whole, the non-endemic communities had a lesser risk of cholera transmission than the endemic communities. This indicates that we are not out of the cholera menace yet and need to further enforce and implement the Assembly by-laws on sanitation to prevent these diseases.

5.5 Assessment of Environmental related factors associated with cholera

5.5.1 Environmental factors and cholera

This study assessed the associations between cholera and other potential environmental factors such as the presence of public toilets, waste dumpsites, open gutters, water bodies, and abattoirs in the neighbourhood of the households.

This study's findings indicate that 68.9% of endemic communities had public toilets compared to 17.7% in the non-endemic communities. This reflects the lack of household toilet facilities in the endemic communities. The findings however indicated that households with neighbourhood public toilets were 72% protected from cholera compared to those households without any neighbourhood toilets and this was significant. The availability of public toilets in communities without household toilets could encourage their usage and prevent open defecation which is associated with cholera risk. This however was not the case in this study as some household members were engaged in open defecation despite the presence of public toilets. A minimal amount ranging from fifty Ghana pesewas to one Ghana Cedi was charged at the time of the study to use the public toilet facilities. Some households with large family sizes claimed they could not afford the daily use of the public toilets because of the cost. Others gave reasons for the poor hygienic conditions of the toilet facilities that made them resort to open defecations. There may be other cultural or belief systems as suggested by Osumanu, Kosoe, and Ategeeng (2019) in their study in the Wa Municipality that could also influence the practice of open defecation and thus present the risk to cholera. The Community-Led Total Sanitation (CLTS) approach and the enforcement of the District Assembly by-laws on sanitation could help prevent open defecation in these communities.

This study findings revealed more waste dumpsites in the endemic (71.1%) communities than the non-endemic communities (38.8%). It further found that households with neighbourhood waste dumpsites were more likely to contract cholera than those without waste dumpsites in their neighbourhood and this was significant. These households were estimated to be located at the average round distance of 17.24 minutes in the endemic communities and 11.77 minutes in the non-endemic communities. Other studies have similarly suggested proximity to the waste dumpsite as a risk factor to cholera. For instance, Suleman, Darko, and Agyemang-Duah (2015) indicated in a related study in Sawaba in the Kumasi Metropolis that waste dumpsites located at a distance of fewer than 5 minutes to the households reported more cases of cholera. Other geospatial analyses by Olanrewaju and Adepoju (2017) in Ife, Nigeria, and Osei and Duker (2008b) in Kumasi also suggested proximity to waste dumpsite as a risk factor for cholera infection. The houseflies and other flies hovering around the waste dumpsites are vectors of transmitting disease pathogens and could aid in the contamination of nearby exposed foods in the households.

This study's findings showed that there were more open gutters in the endemic communities (94.4%) than in the non-endemic communities (2.4%). The presence of open gutters in the neighbourhood was more likely to suffer from cholera compared to those households without neighbourhood open gutters and this was significant. The drainage system in most households is open gutters and liquid waste together with solid waste is disposed. There is no proper drainage system in most households in the disposal of liquid waste. As such most households dispose of the house liquid and solid waste into the gutters. These often result in the choking of gutters and serve as a reservoir for many pathogens including *Vibrio cholerae*.

This study assessed the proximity of the water bodies in selected communities and cholera infection. The study identified water bodies including lagoons, rivers, streams, and sea to be present and located at an average of 14.70 minutes round trip distance to endemic and 11.17 minutes in the non-endemic communities. This study's findings however did not reveal any significant association between household's location to the water bodies and cholera infection. This contrasts with the study by Stoltzfus et al. (2014) that showed the proximity of 5 km from the population to water bodies to have an increased risk of cholera in Kenya. Other studies support the findings that proximity to water bodies increased the risk of cholera infection (Bompangue et al., 2008; Kiiru et al., 2013; Nkoko et al., 2011; Olanrewaju & Adepoju, 2017). Although this study did not find any significant relation, it is known that water bodies that contain phytoplankton and zooplankton serve as a reservoir for *V. cholerae* and may explain the reason for the increased risk of cholera (Colwell et al., 1996; de Magny et al., 2011). This is especially important during the rainy season when floodwater from the water bodies contaminates the drinking water sources or if water is directly used for domestic chores. Other studies have also speculated that swimming or bathing in contaminated water bodies poses a risk of cholera infection (Rebaudet, Sudre, Faucher, & Piarroux, 2013). It is obvious households who are fisher folks or live near water bodies such as lakes, rivers, and sea may swim in them not knowing the risk it poses.

Concerning the proximity of households to markets, this study found markets only in the endemic areas and they were within 11.04 minutes average walking distance away. The findings revealed that households with neighbourhood's markets were more likely to suffer from cholera compared to those households without markets in their neighbourhoods which was significant. However, in a penalized logistic regression model, this was found not to be significant. This contrasts the Olanrewaju and Adepoju (2017) study which found 7 out of 18 markets to be in close proximity to historical cholera cases in Ife, Nigeria. Other studies also found cholera hotspots to be located close to market places (Griffiths et al., 2021; Luquero et al., 2011; Zerbo, Delgado, & González, 2020). They attributed their findings to the waste that is dumped near marketplaces to be the possible reason. In our current study, similar heaps of waste were found in most of the marketplaces. Managing and disposing of waste at the marketplaces could help to contain the spread of cholera in such neighbourhoods with markets.

This study identified a few unapproved abattoirs or slaughterhouses in the endemic communities (8.6%) and non-endemic communities (0.4%). They were located at an average distance of 9.61 minutes and 8.75 minutes in the endemic and non-endemic communities respectively. The study did not find any significant association between the presence of neighbourhood abattoir or slaughterhouses and cholera. The Olanrewaju and Adepoju (2017) study in Nigeria indicated 2 out of 36 abattoirs to be associated with historical cholera cases. In unapproved abattoirs that are not equipped with facilities to protect against houseflies, the meat attracts flies which could aid transmission by contaminating the meat or nearby foods in the neighbourhood.

5.6 Spatial relationship between significant environmental risk factors and the spread of cholera

Geographical Information System (GIS) was used to map the location of the significant environmental risk factors of cholera and to determine the spread in the communities. In this study, the Spatio-temporal effect observed from 2012 to 2015 demonstrates a possible environmental influence of cholera in the Greater Accra Region. In this study, public toilets, waste dumpsites, and open drainage were the significant environmental risk factors found to be associated with cholera. The proximity of the location of cholera cases to these environmental risk factors also shows the relationship to the transmission and endemicity of cholera in the communities.

This study revealed that waste dumpsites, open drainage, and public toilets risks factors were within 500m proximity in the cholera endemic communities. The multiple presences of these environmental factors may explain the reason for the transmission and the endemicity of cholera in the endemic communities compared to the non-endemic communities. This is consistent with the study of Olanrewaju and Adepoju (2017), which also found cholera to occur close to waste dumpsites near markets, rivers/streams, and abattoirs. The effect of living near environmental risk factors was similarly demonstrated by Osei and Duker (2008b) in a study in Kumasi. They expressed the spread to the effect of distance on spatial interaction with the risk. Thus, suggesting the risk of contracting cholera to be minimal as the risk was further away. Public toilets, waste dumpsites, and open drainage may constitute a major breeding site for vectors such as houseflies and rodents which are capable of spreading diseases including cholera. Fotedar (2001) in a study in India demonstrated the potential for houseflies (*Musca domestica*) or flies to act as mechanical vectors for the spread of *V. cholerae*. Public toilet facilities that are unclean and smell bad odour attract houseflies and may aid in the spread of the cholera bacterium to households in close proximity. Waste dumpsites may serve as breeding sites for disease-causing flies and rodents depending on the state of waste and the length of storage.

The findings from this study agree with an earlier study by Osei and Duker (2008b) which demonstrated the role of proximity and density of open refuse dumps to cholera transmission in Kumasi. They indicated that open refuse dumps serve as niches for cholera infection and individual households living within a 500m radius were more at risk of cholera infection. The study of Olanrewaju and Adepoju (2017) also identified waste dumpsite as a major risk for cholera. With the current use of motorized tricycles in the Accra Metropolis, waste collection has improved except for the very deprived slum communities where indiscriminate dumping of waste is still rampant. This study also found proximity to open drainage to play a role in the spread of cholera. The practice of dumping biodegradable waste including human excreta in plastic bags remains a challenge in most of the endemic communities and most often choke the drains. These choked drains may serve as breeding sites for flies which may aid the spread of cholera in nearby households in the communities. Except for a few public toilets in the non-endemic communities, waste dumpsites and open drainages were uncommon and do not have a high risk of cholera spread. It is therefore not surprising that the non-endemic communities did not report a case of cholera over the years despite the ongoing surveillance. It was only in 2014 when the country experienced a major cholera outbreak that Dawhenya North and Kopehem Kasseh communities in the non-endemic area reported a few imported cases from the Accra Metropolis. This was however, not wide spread and was contained. In order to reduce the risk of cholera spread the siting of public toilets, waste dumpsites should be sited at beyond 500m radius in the communities. Open drains should also be covered.

5.7 Limitations of the Study

The findings from this study are subject to some limitations. The most important limitation lies in the fact that our study was cross-sectional in design and concluding on causality, however inferences about associations between the potential risk factors and cholera occurrence were made.

The respondents to the questionnaire were the head of households, nonetheless, difficulties with the recall of household information on the reported household cholera infection was possible. This might have accounted for the low number of household cholera cases reported in the endemic area even though the endemic communities were purposefully selected.

The self-reporting of the cholera cases in the households and social desirability biases, unmeasured confounders and possibility of residual confounding was also a limitation of the study.

The line list of historical cholera cases obtained from the study did not have information on the GPS location of the cases. As such the maps were drawn based on the aggregated data of cases in each particular community, instead of the case-by-case mapping to demonstrate how the cases clustered within the communities.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study assessed the behavioural risk factors in both endemic and non-endemic communities. The findings showed that household drinking water was contaminated from the source and unsafe for drinking. However, most households did not treat them at the point-of-used prior to consumption, which may have contributed to the cholera outbreaks in the communities. Additionally, it showed that households who cooked outside/yard or in separate building had a lower chance of contracting cholera and this practice should be promoted. The study further demonstrated that households lacking toilet facilities shared with other households or relied on public toilets or engaged in open defecation. Through this behaviour, others run the risk of coming into contact with human excreta in the environment, which increases their risk of contracting cholera or other diarrheal diseases.

The bacteriological assessment of the household drinking water quality revealed that total and faecal coliform counts exceeded the Ghana and WHO recommendation in the endemic and non-endemic communities. Thus, suggesting the contamination to likely be from source, length of storage or from the container and unfit for human for consumption. The sachet water which was the most preferred drinking water source in the endemic communities was also highly contaminated with total and faecal coliforms and increased the risk of cholera spread. The bacterial counts followed a seasonal pattern peaking during the wet season compared to the dry season possibly resulting from contamination of water sources from run-off flood waters. *Escherichia coli* and *Klebsiella pneumoniae* were the most common coliforms recovered with no *V. cholerae strains* isolated from both the direct or stored household drinking water.

Toxigenic strains of *V. cholerae* O1 and O139 were absent in the environmental water bodies in the study communities suggesting a reduced infection rates in both endemic and non-endemic communities. However, other *Vibrio* strains such as *Vibrio fluvialis* and *Vibrio algionolyticus* which presents with cholera-like diarrhoea were isolated in the lagoon and estuarine waters in the endemic communities.

When compared to non-endemic communities, the endemic communities had less satisfactory (poor) sanitation standards, as evidenced by the contamination of pipe networks, indiscriminate disposal of waste, including human excreta, and the prevalence of open defecation.

The three main environmental risk factors identified were public toilets, waste dumpsite, and open drainage. The multiple presences of these environmental risk factors within a 500 m radius to each other provided evidence of the interaction of the risk and the relationship to cholera endemicity in the endemic communities compared to the non-endemic communities.

6.2 Recommendations

The following recommendations were made from the study findings:

1. The household drinking water from direct and stored vessels were contaminated with coliforms, an indication for the possible presence of pathogenic organisms. However, the water was consumed without treatment by most households. It is therefore recommended as a short-term measure, to the Municipal and District Health Directorates in the Greater Accra Region to;

1. Educate the community leaders (chiefs, assembly men, community volunteers) to support their respective communities in promoting household drinking water

treatment at the point-of-used to reduce the risk of transmission of cholera and other diarrheal diseases.

2. Sachet water which is highly patronized in the endemic communities was also highly contaminated with coliforms and a high risk to cholera infection. As a short-term measure, Ghana Standard Authority/Food and Drug Authority should;

1. Regularly monitor the bacteriological quality of sachet water sold on the market and sanction sachet water manufacturing companies not conforming to the quality standard.

3. Most households in the endemic and non-endemic communities that lacked toilet facilities shared toilets with other households or used public toilets or were engaged in open defecation. To avoid the spread of diarrheal diseases including cholera it is important for every household to have a toilet. It is therefore recommended as a short to medium-term measure, to the Municipal or District Assemblies in the Greater Accra Region to strengthen and enforce laws on;

1. ‘One-house-one-toilet’ policy in the endemic communities and adopt the ‘community-led total sanitation’ approach in the non-endemic communities to improve on household access to toilets and prevent open defecation.

4. The environmental sanitation conditions assessed revealed the endemic communities to have unsatisfactory (poor) sanitation. This was due to the indiscriminate dumping of solid waste mostly resulting from the absence of bins and the unavailability of waste collection companies to pick up the waste in some communities. It is therefore recommended as a short to medium-term measure, to the Municipal or District Assemblies to improve on waste collection and disposal in the communities by;

1. The provision of waste bins to households and to also place bins at vantage points in the communities

2. The use of motorized tricycles on door-to-door waste collection complement the effort of the waste collection companies to rid the communities of solid waste.

3. Community participation in the monthly clean-up campaigns to improve the sanitation conditions of the communities.

4. Considering siting public waste dumpsites at a distance of 500 m from the households to reduce the risk of cholera transmission in the communities.

5. Future research is needed to:

1. Further, determine the importance of environmental surveillance of the cholera aetiological agents in the environmental water bodies or household drinking water in predicting cholera outbreaks.

2. To determine the emerging pathogenic strains of *V. fluvialis* and *V. algionolyticus* isolated from the environmental water bodies to establish their public health importance and possible inclusion in the national surveillance system.

6.3 Contribution to Knowledge

1. This research provides evidence that *V. cholerae* O1 and O139 is not permanently present in the Ghanaian water bodies.

2. To the best of my knowledge *V. fluvialis* and *V. algionolyticus* were isolated for the first time in the Ghanaian environmental water bodies. These *Vibrios* are fast emerging pathogens that present with cholera-like diarrhoea, gastroenteritis, and septicaemia in immunocompromised patients and if their public health importance is established could be included in the national surveillance system.

3. This research demonstrated that the mini Antigenic Profile Index for Enterobacteriaceae (API 20E) could be used routinely to identify other *Vibrios* such as *V. fluvialis* and *V. algionolyticus* in patients presenting with cholera-like diarrhoea for effective management.

4. This research work also demonstrated that multiple presences of environmental risk factors close to each other could account for cholera endemicity or persistence in a community.

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APPENDICES

Appendix A: Bacteriological Media Preparations

Buffered Peptone Water (BPW) (Oxoid, UK)

BPW is a non-selective pre-enrichment medium used for recovering bacteria from foods or clinical specimen.

It was prepared by weighing an amount of 20g of BPW dehydrated agar and dissolved in 1litre of distilled water. It was dispensed into bottles and sterilized in an autoclave at 121^{0}C for 15minutes at 15lbs pressure.

Eosin Methylene Blue Agar (EMB) (Microbes Laboratories)

EMB agar is a selective and differential medium used to isolate faecal coliforms.

It was prepared by weighing an amount of 35.96g of dehydrated EMB and dissolved in 1 litre of distilled water. The medium was heated to boil to dissolve the media completely. The medium was distributed into bottles and sterilized by autoclaving at 121^{0}C for 15minutes at 15lbs pressure. The medium was allowed to cool to about 50^{0}C and dispensed into 15ml sterile petri dishes.

Violet Red Bile Agar (VRD) (Oxoid, UK)

VRB gar is a selective medium used to detect and enumerate lactose fermenting colonies.

It was prepared by weighing 38.5g of VRB and dissolved in 1 litre of distilled water. It was heated to boil and cool to 50^{0}C before dispensing into 15ml sterile petri dishes.

Plate Count Agar (PC) (Neogen Company, UK)

PC is used to enumerate bacteria in water, wastewater, food and dairy products in the laboratory.

It was prepared by weighing an amount of 23.5g of the dehydrated PC agar and dissolved in 1 litre of distilled water. It was heated to boil and dissolve completely. The media was autoclaved at 121^{0}C for 15minutes at 15lbs pressure. The medium was allowed to cool to 45 to 50^{0}C and dispensed into 15ml sterile petri dishes.

Thiosulfate-Citrate-Bile-Sucrose Agar (TCBS) (Park Scientific Ltd)

TCBS is a selective medium used to isolate Vibrio species.

It was prepared by weighing 88g of TCBS dehydrated powder and dissolved in 1 litre of distilled water. It was allowed to soak for 10 minutes and swirled to mix completely. It was then brought to boil and cooled to 47^{0}C and poured into 15ml sterile petri dishes.

Tryptone Soy Agar (TSA) (Neogen Company, UK)

TSA is a general purpose, non-selective media that allows the growth of most bacteria.

It was prepared by weighing 39g of TSA powder and dissolved in 1 litre of distilled water. It was mixed and boiled to dissolve completely. It was then autoclaved at 121^{0}C for

15minutes at 15lbs pressure and distributed into 15ml sterile petri dishes after cooling to 50^0C.

MacConkey Agar (MAC) (Difco Laboratories)

MAC is a selective and differential medium designed to isolate non-fastidious gram-negative bacteria.

It was prepared by weighing 51.53g of the MAC powder and dissolved in 1 litre of distilled water. It was heated to boil with gentle agitation to dissolve completely. It was then autoclaved at 121^0C for 15minutes at 15lbs pressure and distributed into 15ml sterile petri dishes after cooling to 50^0C.

Simmons Citrate Agar (Neogen Company, UK)

Simmons Citrate is used to differentiate the bacteria ability to utilized citrate.

An amount of 24g of the dehydrated powder was dissolved in 1 litre of distilled water, swirled to mixed and brought to boil. It was then dispensed into tubes and sterilized by autoclaving for 15mins at 1210C at 15lbs pressure. The tubes were slanted and allowed to cool and solidified.

Motility Indole Ornithine (MIO) (Becton, Dickinson and Company, France)

MIO is used to differentiate Enterobacteriaceae based on motility, ornithine decarboxylase activity and indole production.

It was prepared by weighing 31g of the powder and dissolved in 1 litre of distilled water by heating with frequent agitation to boil for 1minute. It was then dispensed into tubes and autoclaved at 1210C for 15minutes at 15lbs pressure.

Triple Sugar Iron Agar (TSI) (Rapid Labs, UK)

TSI is a microbiological media with the ability to test bacteria fermentation of sugars (1% lactose, 1% sucrose, 0.1% glucose) with the production of hydrogen sulphide.

It was prepared by weighing 65g of the dehydrated powder of TSI and dissolved in 1 litre of distilled water. It was mixed and heated to boil to completely dissolve the agar and dispensed into tubes and sterilized at 121^0C for 15minutes at 15lbs pressure. The tubes were then slanted and allowed to solidify before used.

Luvia Broth

A litre of Luvia broth was prepared by weighing;
Tryptone -10g
Yeast extract – 5g
Sodium chloride – 10g
NaOH – 0.2g
And dissolved in 1 litre of distilled water and dispensed in bottles. This was sterilized at 121^0C for 15minutes at 15lbs pressure and cooled to room temperature before used.

Appendix B: Biochemical Test and Staining Techniques

Biochemical Test

Oxidase Test

The test use Kovac's oxidase reagent (tetramethyl-p-phenylenediamine dihydrochloride) that changes colour when it becomes oxidized by cytochrome C oxidase.

Purpose

This test is helpful in identifying members of Neisseria, Enterobacteriaceae, Pseudomonadaceae, to aid in differentiating Gram negative non-enterics from Enterobacteriaceae, and in identifying a few others.

Requirements

1. Oxidase reagent droppers 0.5ml (1% tetramethyl-p-phenylene-diamine dihydrochloride) (Becton Dickson Company, USA)
2. Positive control bacteria- *Pseudomonas aeruginosa* ATCC 27853
3. Negative control bacteria-*Escherichia coli* ATCC 25922

Procedure

1. A test bacterium grown within 18 to 24 hours on TSA was used.
2. Two to three drops of the oxidase reagent was dispensed on some of the growth.
3. This was examined within 20 seconds for a colour change.

Results

1. The organism was considered positive for the presence of cytochrome C oxidase if a dark-purple colour develops within 20 seconds and negative if there was no colour.

Catalase Test

Hydrogen peroxide, an end product of aerobic carbohydrate metabolism, is extremely toxic to bacteria. Bacteria containing the enzyme catalase break down hydrogen peroxide into oxygen and water. Bacteria that possess catalase demonstrate the reaction in 3% hydrogen peroxide by the rapid appearance of gas bubbles.

Purpose

The catalase test facilitates the detection of the enzyme catalase in bacteria and used to differentiate between bacteria that contains the enzyme and those that does not.

Requirements

2. Catalase Reagent Droppers contain: 3% Hydrogen Peroxide and 0.05% or less of stabilizer (Becton Dickson Company, USA)
3. Platinum inoculating loop
4. Glass slides
5. Bunsen burner
6. Positive control bacteria- *Staphylococcus aureus* ATCC 33592
7. Negative control bacteria-*Enterococcus faecalis* ATCC 29212

Procedure

1. A drop or two of the catalase reagents was taken onto a clean glass slide.
2. An inoculating needle was used to pick a well-isolated colony grown within 18 to 24 hours, without touching the media and placed on the catalase reagent.
3. This was examined for the rapid production of gas bubbles.

Results

1. Positive catalase reactions were denoted by the production of bubbles.

Triple Sugar Iron Test

The Triple Sugar Iron (TSI) agar test is designed to differentiate among the different groups or genera of the Enterobacteriaceae, which are all gram-negative bacilli capable of fermenting glucose with the production of acid, and to distinguish them from other gram-negative intestinal bacilli. The differentiation is based on fermentation of glucose and lactose or sucrose and hydrogen sulphide (H_2S) production. TSI medium contains 10 parts of lactose: 10 parts of sucrose: 1 part of glucose and peptone. Phenol red and ferrous sulphate serve as indicators of acidification and H_2S formation, respectively. The acid base indicator phenol red incorporated for detecting carbohydrate fermentation is indicated by the change in colour of the carbohydrate medium from orange red to yellow in the presence of acids. In case of oxidative decarboxylation of peptone, alkaline products are built and the pH rises. This is indicated by the change in colour of the medium from orange red to deep red. Sodium thiosulfate and ferrous ammonium sulphate present in the medium detects the production of hydrogen sulphide and is indicated by the black colour in the butt of the tube.

Purpose

The TSI is designed to differentiate among bacteria based on the differences in carbohydrates fermentation patterns and hydrogen sulphide production.

Requirements

2. TSI medium prepared
3. Straight inoculating needle
4. Bunsen burner
5. Control organisms e.g *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922

Procedure

1. The top of a well-isolated colony was touched using a sterile straight inoculation needle
2. The inoculation was done by first stabbing through the centre of the medium to the bottom of the tube and then streaking on the surface of the agar slant.
3. The Inoculated TSI tube was loosely closed and incubated at 35°C in ambient air for 18 to 24 hours.

4. The TSI tube was examined for colour change in the slant and butt, blackening and cracks in the medium.

Results interpretation

1. If lactose (or sucrose) was fermented a large amount of acid was produced and turned the phenol red indicator yellow in the butt and slant.
2. If lactose was not fermented but the small amount of glucose was, the oxygen-deficient butt was yellow but the slant indicating the acid produced was yellow.
3. If neither lactose/sucrose nor glucose was fermented, both the butt and the slant were red.
4. If organisms generated gases was indicated by bubbles/cracks on the medium.
5. If H_2S was produced resulted in the blackening of the medium.

Motility Indole Ornithine Medium (MIO)

The casein and gelatine peptones, yeast extract and dextrose provide nitrogenous and carbonaceous substances, vitamins and minerals essential for bacterial metabolism. When ornithine decarboxylase is present, the ornithine is decarboxylated to putrescine which causes a rise in the pH and corresponding colour change of bromocresol purple from yellow to purple.

Purpose

MIO medium is used to demonstrate motility, indole production and ornithine decarboxylase activity for the differentiation of Enterobacteriaceae.

Requirements

6. MIO medium prepared (Becton, Dickinson and Company, France)
7. Kovac's reagent
8. Straight inoculating needle
9. Bunsen burner
10. Positive control bacteria- *Escherichia coli* ATCC 25922
11. Negative control bacteria- *Klebsiella pneumoniae* ATCC 13883

Procedure

12. The top of a well-isolated colony grown between 18 to 24 hours was touched using a sterile straight inoculation needle.
13. The inoculation was done by a single stab through the centre of the medium to within ¼ inch of the bottom of the medium.
14. The Inoculated MIO tube was incubated at 35 ±2°C for 18 to 24 hours.
15. The MIO tube was examined for growth, presence of motility, ornithine decarboxylase and indole reactions. If the reaction was negative was incubated for a further 24 hours.

Results

1. Motility was indicated by growth extending from the line of inoculation. The nonmotile organisms grew only alone the line of inoculation.
2. Decarboxylation of ornithine was indicated by the development of a turbid purple to faded yellow purple colour. A negative reaction was indicted by a yellow colour.

3. Indole production was indicated by the formation of a pink to red colour after the addition of three or four drops of kovac's reagent to the surface of the medium and gentle shaking. A negative reaction was indicated by the development of a yellow colour.

Control Organisms

No.	Control Organisms used	Results after 18 – 48 hrs
1	*Escherichia coli*	Motility – Positive
	ATCC 25922	Ornithine – Positive
		Indole - Positive
2	*Klebsiella pneumoniae*	Motility – Negative
	ATCC 13883	Ornithine – Negative
		Indole - Negative

Gram Staining Technique

Gram staining technique is a differential stain used to classify bacteria into Gram-positive and Gram-negative bacteria.

Requirements

1. Becton Dickinson (BD) Gram Stain (2g crystal violet, 20ml 95% ethyl alcohol, 0.8g ammonium oxalate and 100ml distilled water)
2. BD Grams iodine (2g potassium iodide, 1g iodine crystal, 100ml distilled water)
3. BD Decolourizer (50ml acetone and 50 ml ethanol)
4. BD Safranin (4g safranin, 200ml 95% ethanol, 800ml distilled water)
5. Glass slide
6. Bunsen burner
7. Platinum inoculating loop
8. Water
9. Staining rack
10. Slide rack
11. Gram Positive bacteria – *Staphylococcus aureus* ATCC 33592
12. Gram Negative bacteria - *Escherichia coli* ATCC 25922

Procedure

1. One or two pure colonies were touched and a smear prepared on a clean slide by mixing and spreading it out. The slide was labelled and placed on the staining rack to air dry.
2. The smear was then heat fixed by passing the slide two to three times in a Bunsen burner flame with smear uppermost.
3. The smear was covered completely with the BD Gram stain and left on it for 60 seconds and washed off with water.

4. The smear was covered completely with Gram's iodine solution and allowed to stand for 30 seconds and washed off with water.
5. The smear was decolourized with BD decolourizer for 10 seconds and washed with water.
6. The smear was completely covered with BD safranin for 60 seconds to counterstain and washed with water.
7. The back of the slide was wiped of the excess water and air dried on a slide rack
8. The slide was examined with the microscope using the X100 objective and oil immersion on it.

Results

1. GRAM positive bacteria are violet in colour
2. GRAM negative bacteria are red/pink

Appendix C: Antigenic Profile Index 20 Enterobacteriaceae Biochemical Testing

Oxidase Test

The Oxidase Test was first performed on the colonies to be tested according to the manufacturer's instructions (Becton Dickson Company, USA).

Preparation of the API Strip

An incubation box consisting of a tray and lid was prepared and about 5ml of distilled water distributed into the honey-combed wells of the tray to create a humid atmosphere.

The strip was removed from the packaging and placed in the incubation box.

Preparation of the inoculum

An ampule of the API NaCI 0.85% medium (5ml) was inoculated with the test organism using a sterile pipette used to remove the colony from the culture plate. (Young colonies grown within 18 to 24 hours were used).

This was carefully emulsified to achieve a homogenous bacterial suspension.

Inoculation of the Strip

Using the same pipette, the bacterial suspension was distributed into the tubes of the strip without creating bubbles in sequence as in the table.

Table: API 20E Biochemical Tests and their Abbreviations

Test sequence	Biochemical Test	Test Abbreviation
1	O-Nitrophenl-B-D-galactosidase	ONPG
2	Arginine dihydrolase	ADH
3	Lysine decarboxylase	LDC
4	Ornithine decarboxylase	ODC
5	Citrate Utilization	CIT
6	Hydrogen Sulfide production	H_2S
7	Urease production	URE
8	Tryptophane deaminase production	TDA
9	Indole production	IND
10	Acetoin production	VP
11	Gelatinase production	GEL

12	Glucose fermentation	GLU
13	Mannitol fermentation	MAN
14	Inositol fermentation	INO
15	Sorbitol fermentation	SOR
16	Rhamnose fermentation	RHA
17	Sucrose fermentation	SAC
18	Melibiose fermentation	MEL
19	Amygdaline fermentation	AMY
20	Arabinose fermentation	ARA

For the CIT, VP and GEL tests both the tubes and the cupule of the test was filled with the bacterial suspension.

For the other tests only, the tubes were filled.

For ADH, LDC, ODC, H_2S and URE an anaerobic condition was created by overlaying with mineral oil.

The tubes and the cupules were incubated at $36^0C \pm 2^0C$ for 18 to 24 hours.

Reading of results
After the incubation period the Strip was read.

If three or more tests are positive all the spontaneous reactions are recorded on the result sheet.

For tests such as TDA, IND and VP that required addition of reagents that was performed as follows;

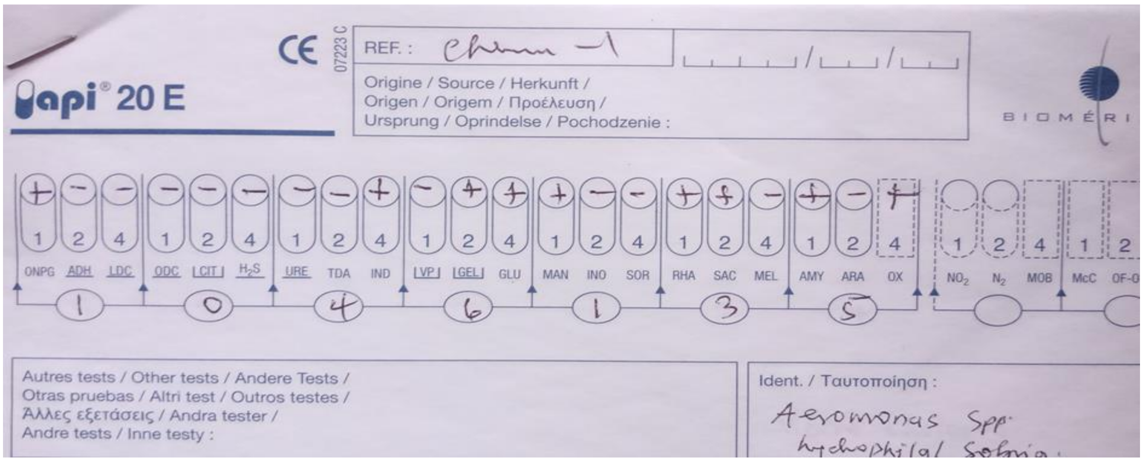
TDA – A drop of TDA reagent was added. A reddish-brown colour indicated a positive reaction

IND – A drop of JAMES reagent was added. The development of a pink colour indicated a positive reaction.

VP – A drop of VP 1 and VP 2 reagents were added. The development of a pink or red colour within 10 minutes indicated a positive reaction.

Interpretation
On the result sheet the tests are separated into three groups and a value of 1, 2, or 4 indicated for each.

By adding together, the values corresponding to positive reactions within group, a 7-digit profile number was obtained for the 20 tests of the API 20E Strip. The oxidase test which was performed first constitutes the 21st test and has a value of 4 if it was positive.



Identification

The identification was performed by keying in the 7-digit numerical profile in the API 20 E database (bioMérieux, France).

Appendix D: Polymerase Chain Reaction work

Zymo Research DNA Extraction Kit (USA)

Kit Content (ready for use)

Quick DNA mini preparation	D3024
Kit size	50 preparations
Genomic lysis buffer	50ml
DNA Pre-wash buffer	15ml
g-DNA wash buffer	50ml
DNA elution buffer	10ml
Zymo-Spin Columns	50
Collection tubes	100

Preparation of PCR reagents

Primers reconstitutions (Inqaba Biotec West Africa Ltd)

1. *ctx*AF – sequence:CTCAGACGGGATTTGTTAGGCACG – 44.92nmoles. 100µM stock was prepared by adding 449.19µl of Tris EDTA buffer.
2. *ctx*AR – sequence:TCTATCTCTGTAGCCCCTATTACG – 35.27nmoles. 100µM stock was prepared by adding 352.69µl of Tris EDTA buffer.
3. O139*rfb*F – sequence: AGCCTCTTTATTACGGGTGG – 79.87nmoles. 100µM stock was prepared by adding 798.66µl of Tris EDTA buffer.
4. O139*rfb*R – sequence: GTCAAACCCGATCGTAAAGG – 31.17nmoles. 100µM stock was prepared by adding 311.69µl of Tris EDTA buffer.

5. *omp*WF - sequence: CACCAAGAAGGTGACTTTATTGTG – 45.03nmoles. 100µM stock was prepared by adding 450.3 µl of Tris EDTA buffer.
6. *omp*WR - sequence: GGTTTGTCGAATTAGCTTCACC – 39.11nmoles. 100µM stock was prepared by adding 391.05µl of Tris EDTA buffer.
7. O1*rfb*F - sequence: GTTTCACTGAACAGATGGG – 42.14nmoles. 100µM stock was prepared by adding 421.4 µl of Tris EDTA buffer.
8. O1*rfb*R - sequence: GGTCATCTGTAAGTACAAC – 64.87nmoles. 100µM stock was prepared by adding 648.7µl of Tris EDTA buffer.

Preparation of working primer solution from the stock

To prepare 10µM from the working stock solution, 10µl of the reconstituted stock was taken and 90µl of nuclease-free water added.

All stock and the working primer solutions were stored at -20^{0}C.

Preparation of 1.5% agarose gel for PCR Amplification

1. 1.5% (1.5g) of agarose was weighted and dissolve in 100ml 1% Tris acetate EDTA buffer.
2. It was heated to melt completely and allowed to cool to about 45^{0}C.
3. 3µl of ethidium bromide was added to the 100ml.
4. The gel casting tray was arranged together with the comb.
5. The molten agarose gel was poured into the casting tray and allowed to set.

Preparation of Master Mix for PCR

1.

	Components	Volume for 1 tube	Volume for 38 tubes
1	1 Taq 2X Master Mix buffer	6.25µl	237.5µl
2	10µM *ctx*AF primer	0.25µl	9.5µl
3	10µM *ctx*AR primer	0.25µl	9.5µl
4	Template DNA	1µl	-
5	Nuclease-free water	4.75µl	179.5µl
6	Total vol.	12.5µl	436µl

2.

	Components	Volume for 1 tube	Volume for 38 tubes
1	1 Taq 2X Master Mix buffer	6.25µl	237.5µl

2	10µM *omp*WF primer	0.25µl	9.5µl
3	10µM *omp*WR primer	0.25µl	9.5µl
4	Template DNA	1µl	-
5	Nuclease-free water	4.75µl	179.5µl
6	Total vol.	12.5µl	436µl

3.

	Components	Volume for 1 tube	Volume for 38 tubes
1	1 Taq 2X Master Mix buffer	6.25µl	237.5µl
2	10µM O1*rfb*F primer	0.25µl	9.5µl
3	10µM O1*rfb*R primer	0.25µl	9.5µl
4	Template DNA	1µl	-
5	Nuclease-free water	4.75µl	179.5µl
6	Total vol.	12.5µl	436µl

4.

	Components	Volume for 1 tube	Volume for 38 tubes
1	1 Taq 2X Master Mix buffer	6.25µl	237.5µl
2	10µM O139*rfb*F primer	0.25µl	9.5µl
3	10µM O139*rfb*R primer	0.25µl	9.5µl
4	Template DNA	1µl	-
5	Nuclease-free water	4.75µl	179.5µl
6	Total vol.	12.5µl	436µl

Appendix E: Outputs for Multicollinearity, goodness of fit and reliability tests

Multicollinearity Test

Socioeconomic Characteristics

Variable	**VIF**	**SQRT VIF**	**Tolerance**	**R-Squared**
Religion	1.02	1.01	0.9797	0.0203
Marital Status	1.13	1.06	0.8819	0.1181
Employment status	1.13	1.06	0.8887	0.1113
Educational level	1.48	1.22	0.6751	0.3249

Wealth index	1.23	1.11	0.8144	0.1856
Income	1.32	1.15	0.7568	0.2432
Mean VIF	**1.22**			

Behavioural

Variable	VIF	SQRT VIF	Tolerance	R-Squared
q59eat	1.01	1	0.9913	0.0087
q61cook	1.01	1.01	0.9897	0.0103
q66hawas	1.02	1.01	0.985	0.0150
Mean VIF	**1.01**			

Environmental Risk Factors

Variable	VIF	SQRT VIF	Tolerance	R-Squared
Landfill site	1.18	1.08	0.8498	0.1502
Toilet facility	1.18	1.09	0.8491	0.1509
Drainage system	1.48	1.22	0.6766	0.3234
Market	1.46	1.21	0.684	0.316
Mean VIF	**1.32**			

Goodness of Fit Test

	Socio-demographics	Behavioural	Environmental
Log-likelihood			
Intercept only	147.835	-145.264	-152.259
Full model	132.719	-137.288	-132.412
Information criteria			
AIC	315.439	294.575	282.825

BIC	435.665	342.621	326.106
R^2			
Cox-Snell/ML	0.033	0.018	0.043
Cragg-Uhler/Nagelkerke	0.118	0.064	0.15
Efron	0.041	0.025	0.042
McFadden	**0.102**	**0.055**	**0.130**
McFadden(adj)	-0.067	-0.014	0.071
McKelvey & Zavoina	0.175	0.149	0.307
Tjur's D	0.047	0.023	0.047

Reliability test for sanitation index score

Test Scale	Mean (unstandardized items)
Average interitem covariance	0.0286011
Number items in the scale	13
Scale reliability coefficient	0.7591

Appendix F: Household Questionnaire

Form Number

House Number/landmark

Head of household or responder's contact/ phone number

Name of location/zone

Name of Community

Name of Sub Metropolis

Name of Metropolis/Municipality/District

Indicate whether community is Endemic or Non endemic for cholera

NAME OF RESEARCH ASSISTANT

NAME: ……………………………………… DATE………………………………………

DATA KEYED IN BY

NAME: ……………………………………… DATE: ……………………………………

RESULT CODES

☐☐

01 Completed

02 Not completed

Record Start Time (GMT) ☐☐☐☐

Record Finish Time (GMT) ☐☐☐☐

Household GPS Coordinates:
Lat:____

Long:____

INTRODUCTION AND INFORMED CONSENT

NOTE: DO NOT BEGIN INTERVIEW WITHOUT GOING THROUGH THE CONSENT PROCESS. REFER TO INFORMED CONSENT FORM ATTACHED. The head of household or senior member (male or female) who can provide the household information is to be interviewed.

INSTRCTIONS: Circle the appropriate response codes or specify your response by writing in the space provided

Q.no.	Questions	Codes/Answers	Skips	FIELDS
1.	ID	☐☐☐☐☐☐		ID
2.	Date of Interview			DOI
	Socio-demographic Factors			
3.	How old are you?(age in completed years)			AGE

4.	What is your sex?	1. Male 2.Female		SEX
5.	What is your marital status?	1. Single 2. Cohabitating 3. Married 4. Separated 5. Divorced 6. Widowed		MAR
6.	What religion do you belong to?	1.No religion 2.Christian 3.Islam 4.Traditional 5.Others (specify) ----------------------------------		RELG
7.	What is your nationality?	1.Ghanaian 2.Non Ghanaian (specify) ---------------------------------------		NAT
8.	To which ethnic group do you belong?	-------------------------------		ETHN
9.	What is your current employment status?	1.Unemployed 2.Retired worker	→ Skip to11	EMPL

		3.Formal employed 4.Informal employed	
10.	What is your occupation?	--	OCCP
11.	What is your educational level?	1.No formal Education 2.Primary Education 3.JSS/Middle school Education 4.Senior High Education 5.Tertiary Education	EDUC
12.	What is your income level? (salary, business, remittance, pensions etc) (Average per month)	1.Low income (<GhȻ300) 2.Medium income (>GhȻ300 - GhȻ1000) 3.High income (>GhȻ1000 – GhȻ2000) 4.Highest income (>GhȻ2000)	INCOM
13.	Does your household have electricity supply?	1.Yes 2.No → Skip to15	ELECT
14.	What is the source of the electricity?	1.ECG 2.Own generator 3.Solar cells 4.Other (specify) --	SOELE
15.	Does your household have a Television set?	1.Yes 2.No	TV

16.	Does your household have a refrigerator?	1.Yes 2.No	REFRI
17.	What type of fuel does your household use for cooking? (Multiple response possible)	1.Electricity 2.LPG 3.Natural gas 4.Biogas 5.Kerosene 6.Coal 7.Charcoal 8.Wood 9.Straw/grass 10.Animal dung 11.Other (specify) …………………………………….. 12. No food cooked in the household	FUEL
18.	In what type of building does the household live?	1.Detached house 2.Semi-detached 3.Flat/apartment 4.Compound house (rooms) 5.Tent 6.Improvised house (kiosk/container) 7.Living quarters attached to office/shop 8.Uncompleted building 9.Other (Specify) --------------------------------	DWELL

19.	What kind of material was used for the building?	1.Cemented block house 2.Brick house 3.Stone house 3.Mud house 4.Wooden/plywood house 5.Other (Specify) -------------------------------		MATER
20.	How long have you been living in this community? (in years)			LENGT
21.	How many people live in your household including you?			NUMB
	Water related factors			
22.	What is the <u>main</u> source of drinking water for members of your household? (Choose only one)	1.Piped into house 2.Piped to yard/plot 3.Public tap/standpipe 4.Tube well or borehole 5.Protected dug well 6.Unprotected dug well 7.Protected spring	Skip to 27 Skip to 27	WASO

		8.Unprotected spring 9.Rain water 10.Tanker truck 11. Cart with small tank 12.Surface water (river/dam/lake/pond/stream/canal/irrigation channel) 13.Bottled water → 14.Sachet water → 15.Others (specify) -----------------------------------	Skip to 27 Skip to 27	
23.	Where is that drinking water, source located?	1.In own house/dwelling → 2.In own yard → 3.Elsewhere	Skip to 25 Skip to 25	WASOL
24.	How long does it take to go there, get your drinking water, and come back? (in minutes)			TIMWS
25.	Do you do anything to the water to make it safer to drink?	1.Yes 2.No → 3.Don't know →	Skip to 28 Skip to 28	
26.	What do you usually do to make the water safer to drink?	1.Boil 2.Add bleach/chlorine/alum 3.Strain through a cloth/ strainer 4.Use water filter (e.g ceramic, sand,		WTREAT

	(Multiple response possible)	composite etc) 5.Solar disinfection 6.Let it stand and settle 7.Camphor/naphthalene 8.Purification tablet 9.Others (specify) ---	
27.	If your main source of drinking water is bottled or sachet water, what is the brand that your household mostly drink?	---	
28.	How does your household store drinking water? (Multiple response possible)	1.Plastic container (buckets/barrels/jerry cans) 2.Pot/earthenware vessels 3.Metal container 4.Elevated polytank 5.Bottled/sachet → Skip to 30 6.Others (specify) ---------------------------------------	WASTOR
29.	Is the storage vessel for the stored drinking water always covered or closed?	1.Yes 2.No 3.Don't know	STCOV

30.	Apart from the drinking water sources, what other water sources do you use for domestic purposes such as bathing, cooking and washing of utensils (plates, spoons, bowls etc) (Multiple response possible)	1.Piped into house 2.Piped to yard/plot 3.Public tap/standpipe 4.Tube well or borehole → 5.Protected dug well → 6.Unprotected dug well → 7.Protected spring → 8.Unprotected spring → 9.Rain water → 10.Tanker truck → 11. Cart with small tank → 12.Surface water (river/dam/lake/pond/stream/canal/irrigation channel) → 13.Others (specify) → ------------------------------------	 Skip to 32 Skip to 32 Skip to 32 Skip to 32 Skip to 32 Skip to 32 Skip to 32 Skip to 32 Skip to 32 Skip to 32	WADOME
31.	If your household uses piped borne water, do you experience any shortages? (Choose only one)	1.Always 2.Often 3.Sometimes 4.Never 5.Don't know		WASUL
	Solid waste disposal factors			
32.	What kind of toilet facility is used by members of your household at home? (choose only one)	1.Flush to piped sewer system 2.Flush to septic tank 3.Flush to pit latrine 4.Flush to somewhere else 5.Flush don't know where it goes		KTOI

		6.Ventilated improved pit latrine (KVIP)		
		7.Pit latrine		
		8.Pit latrine with slab		
		9.Pit latrine without slab/open pit		
		10.Bucket toilet		
		11.No facility/bush/field →		
		12.No facility/use public toilet →	Skip to 38	
		13.Others (specify)	Skip to 36	

33.	If there is a toilet facility at home, do you share this with other households?	1.Yes, with other households only		TOISHAR
		2.Yes, with public →	Skip to 35	
		3.No →	Skip to 35	
34.	How many households use or share this toilet facility?			NUMTOI
35.	Where is this household toilet facility located?	1.In own house/dwelling →	Skip to 37	TOILOC
		2.In own yard →	Skip to 37	
36.	How long does it take to go to the public toilet, use it, and come back? (minutes)			
37.	Is a hand washing facility/device available for use immediately after defecation at the toilet?	1.Yes		HWDH
		2.No		

38.	Where do you usually store your household refuse (rubbish) before disposal?	1.Plastic bags 2.Cardboard boxes 3.Rubbish bin 4.Sacks 5. No storage-direct disposal 6.Others (specify) ------------------------------		REFSTOR
39.	How does the household dispose of refuse (rubbish)? (Multiple response possible)	1.Collected by a waste company 2.Burned by household 3.Public dump site (covered) 4.Public dump site (opened) 5.Dumped indiscriminately 6.Buried by household 7.Others (specify) -----------------------------	 → Skip to 42 → Skip to 42 → Skip to 42 → Skip to 42 → Skip to 42 → Skip to 42	REFDIS
40.	Which waste collection company collects your household waste?	--		WASCOM
41.	How often is refuse (rubbish) collected by the waste collection company?	1.Daily 2.Weekly 3.Monthly 4.As to when the waste bin is full 5.Never collected	→ Skip to 43 → Skip to 43 → Skip to 43 → Skip to 43 → Skip to 43	TWASC
42.	Why is your household refuse not collected by a waste collection company?	1.Financial reasons (charges are high) 2.Waste collection companies are not available 3. Waste collection companies are		WNC

		available but not reliable		
	(multiple responses possible)	4.No waste disposal bin		
		5. others (specify)		
		--		
43.	Do you have a public refuse (rubbish) dump site or landfill site near your neigbourhood?	1.Yes		PUBREF
		2.No →	Skip to 45	
		3.Don't know →	Skip to 45	
44.	How long does it take you to walk from home to the refuse disposal site or landfill site? (in minutes)			TPUBREF
	Liquid waste disposal factors			
45.	Is there a drainage system to take away the household liquid waste?	1.Yes		BATHF
		2.No →	Skip to 47	
		3.Don't know →	Skip to 47	
46.	What kind of drainage system is it?	1.Open drainage (open gutter)		KDRAIN
		2.Covered drainage (covered gutters)		
		3.Underground drainage (pipes connection to sewage)		
		4. Others (specify)		

47.	How does your household dispose-off liquid waste from kitchen and washings?	1.Through the sewage system to septic tank 2.Thrown onto the street/outside 3.Thrown into gutter 4.Thrown onto the compound 5.Others (specify) -------------------------------------	DISLIQK
48.	How does your household dispose of the liquid waste from the bath?	1.Through the sewage system 2.Through drainage into a pit (e.g soak away) 3.Thrown onto the street/outside 4.Thrown into gutter 5.Thrown onto the compound 6.Others (specify) -------------------------------------	DISLIQB
	Environmental related factors		
49.	Is there any water body (sea, river, lagoon, stream, pond,lake etc) located near your neighbourhood?	1.Yes 2. No → Skip to 52	AQR
50.	What type of water body is located near your neighbourhood? (Multiple response	1.Stream 2.Lagoon 3.Pond 4.Lake	TYAQR

	possible)	5.River 6.Sea 7.Others (specify) ---		
51.	How long does it take you to walk from home to the water body site and back? (in minutes)			TAQR
52.	Do you have a market near your neighbourhood?	1.Yes 2.No → If yes, what is the name of the market? ______________________________	 Skip to 56	MARKET
53.	How long does it take you to walk from home to the market and back? (in minutes)			TMARKET
54.	Do you buy your food stuff from that market?	1.Yes 2.No →	 Skip to 56	BFSTUFF

55.	Do vendors screen their smoked or fried fish from flies and dust in that market?	1.Yes, always 2.Yes, often 3. Yes, sometimes 4. No, never	VENSFISH
56.	Do you have an abattoir/slaughter house near your neighbourhood?	1.Yes 2.No → Skip to 59	ABATT
57.	How long does it take you to walk from home to the abattoir/slaughter house and back? (in minutes)		TABATT
58.	Do butchers screen or protect the meat from flies and dust?	1.Yes, always 2.Yes, often 3. Yes, sometimes 4. No, never	BUTCS

Behavioural Factors

Food safety

59.	What is the food eating habit of your household? (choose only one)	1.Only eat food cooked at home 2.Eat food cooked from home and outside home 3.Only eat food from outside home → Skip to 62	EATHAB

60.	Does your household usually cook food at home?	1.Yes, always 2.Yes, often 3. Yes, sometimes 4. No, never → Skip to 63	COOK
61.	Where is the cooking done in your household?	1.Inside the house/dwelling 2.In a separate building 3.Outdoors (in the yard) 4.Others (specify) ---------------------------------	WCOOK
62.	How does your household mostly treat raw vegetables such as cabbage, lettuce, cucumber, carrot, spring onions etc before eating it at home? (choose only one)	1.washed in water only 2.washed in water and heat steam 3.washed in salt water 4.washed in salt water and heat steam 5.heat steam or parboiled only 6.washed in vinegar 7.washed in vinegar and heat steam 8.other (specify) --------------------------------------- 9.Does nothing to it	RAWVEG
63.	Does your household eat left over foods?	1.Yes 2.No → Skip to 66	LEFTOV

64.	How does your household <u>always</u> store the left-over foods? (choose only one)	1.left uncovered in the room/kitchen 2.Covered and left in the room/kitchen 3.Put in the refrigerator/fridge 4.Put in a freezer 5. Others (specify) ---	STOLEFT
65.	How is left over foods usually treated before eating in your household? (choose only one)	1.Eaten cold in the next meal 2.Reheated to boil before eating 3.Warmed in the microwave before eaten 4.Others (specify) ---	TRELEFT
66.	How does your household member wash their hands before meals at home? (choose one common hand washing procedure often used at home)	1.Wash hands in a bowl of water 2.Wash hands with soap in a bowl of water 3.Wash hands under running water (from tap or poured) 4. Wash hands with soap under running water (from tap or poured) 5.Wash hands with water and ashes 6.Other (specify)	HAWASH

		--	
67.	What does your household mostly do before eating fruits e.g mangoes, oranges, water melon, apple etc in your home? (choose only one)	1.They are washed with soap under safe running water 2.They are washed with safe running water 3.They are washed with water and soap in a bowl 4.They are washed with water in a bowl 5.They are not washed 6.Other (specify) ---	FRUIT
	Cholera infection in the households and neighbourhood in the past		
68.	Have you heard about cholera (profuse painless diarrhoea with or without vomiting?	1.Yes 2.No	
69.	Has any member of your household suffered from cholera before?	1.Yes 2.No → Skip to 74	CHOL

70.	What treatment did you immediately give to the household member to stop the diarrhoea?	1.Nothing 2.Traditional herbs 3.Home prepared salt solution 4.ORS 3.Others (specify) --	CHOLTRE
71.	Where was the household member taken to for health care?	1.Hospital/Polyclinic 2.CHPs 3.Pharmacies/Drug chemical shop 4.Traditional healer 5.Stayed at home 6.Others (specify) --	HELSEEK
72.	How long did it take you to decide to send the house member to clinic/hospital/CHPs when he/she started showing signs of cholera? (report in days)	1.≤ 2 days 2.>2 days	TSEEHEL
73.	How long did it take you to reach the health facility (hospital/clinic)? (report in hours)	1.<5 hours 2.≥5 hours	TTHF

74.	Has any person suffered from cholera in your neigbourhood before?	1.Yes 2.No 3.Don't know	END	HISCHOL

Appendix G: Environmental Health Inspection Guide

FORM NUMBER

ID	
Community location/zone	
Name of community	
Name of Sub Metro	
Name of Metro/Municipal/District	
Date of Environmental Assessment	

No.	Ask and Observe in households and community	Sanitation
	Water Supply Pipelines	
1.	Are there exposed water pipelines?	1. Yes 2. No
2.	Are pipelines laid through open drains or gutters?	1. Yes 2. No
3.	Are there any broken or leaking pipelines?	1. Yes 2. No
4.	Does water accumulate around the pipelines?	1. Yes 2. No
	Take photographs of these exposed or broken pipelines and geopoints of these.	
	Refuse Disposal	
5.	Is there a refuse (rubbish) dumping site or landfill site in the neighbourhood?	1. Yes 2. No
6.	Is the refuse dump containers overflowing?	1. Yes 2. No
7.	Are there flies around the refuse dumpsite or landfill site?	1. Yes

		2.	No
8.	Is the refuse dumpsite or landfill site near a water source?	1. 2.	Yes No
	Take photographs of these and geopoints of refuse disposal site		
	Sewage Disposal		
9.	Are the gutters or drains choked with waste/refuse/human excreta and impedes free flow of storm and waste water?	1. 2.	Yes No
10.	Are there over growth of weeds providing cover for indiscriminate disposal of sewage?	1. 2.	Yes No
11.	Are there evidence of liquid waste or sewage (effluent) discharge into a water body (e.g sea, river etc) in the community?	1. 2.	Yes No
	Take photographs of these and geopoints of public toilets		
	Excreta Disposal		
12.	Are there public toilets in the community?	1. 2.	Yes No
13.	Does the public toilet facility attract house flies?	1. 2.	Yes No
14.	Is there any evidence of open defecation in the community?	1. 2.	Yes No
	Take photographs of these and geopoints of public toilets		

Appendix H: Consenting Process

Volunteer Agreement

I have read the foregoing information or it has been read to me on **'Identification and mapping of risk factors associated with cholera in endemic communities in the Greater Accra Region'**. I had the opportunity to ask questions and was told my confidentiality will be assured by not linking my name to any data, participation was voluntary and I could stop at any stage of the study, there was no direct benefit to me and no unforeseen risk associated. I was also told there was no compensation for participating in the study and that I will be given a copy of the consent form. My questions were answered to my satisfaction and I agree to participate as a volunteer.

____________________________ __________________________________

Date Signature or thump print of volunteer

If a volunteer does not understand the English language, a translator will translate in the volunteer native dialect and sign.

____________________________ __________________________________

Date Signature or thump print of translator

If a volunteer cannot read the form themselves, a witness must sign here:

I was present while the benefits, risks and procedures were read to the volunteer. All questions were answered and the volunteer has agreed to take part in the research.

______________________ ______________________________

Date Signature of Witness

I certify that the nature and purpose, the potential benefits, and possible risks associated with participating in this research have been explained to the above individual.

_____________________ ______________________________

Date signature of Person who obtained consent

Appendix I: Ethical Clearance

GHANA HEALTH SERVICE ETHICS REVIEW COMMITTEE

In case of reply the number and date of this Letter should be quoted.

Research & Development Division
Ghana Health Service
P. O. Box MB 190
Accra
Tel: +233-302-681109
Fax + 233-302-685424
Email: ghserc@gmail.com
30th January, 2019

MyRef. GHS/RDD/ERC/Admin/App 19/036
Your Ref. No.

Anthony Dongdem
University of Ghana
School of Public Health
P.O. Box LG 13
Legon- Accra

The Ghana Health Service Ethics Review Committee has reviewed and given approval for the implementation of your Study Protocol.

GHS-ERC Number	**GHS-ERC006/01/19**
Project Title	Identification and Mapping of Risk Factors Associated with Cholera in Endemic Communities in the Greater Accra Region, Ghana
Approval Date	30th January, 2019
Expiry Date	29th January, 2020
GHS-ERC Decision	Approved

This approval requires the following from the Principal Investigator

- Submission of yearly progress report of the study to the Ethics Review Committee (ERC)
- Renewal of ethical approval if the study lasts for more than 12 months,
- Reporting of all serious adverse events related to this study to the ERC within three days verbally and seven days in writing.
- Submission of a final report after completion of the study
- Informing ERC if study cannot be implemented or is discontinued and reasons why
- Informing the ERC and your sponsor (where applicable) before any publication of the research findings.
- Please note that any modification of the study without ERC approval of the amendment is invalid.

The ERC may observe or cause to be observed procedures and records of the study during and after implementation.

Kindly quote the protocol identification number in all future correspondence in relation to this approved protocol

SIGNED...
DR. CYNTHIA BANNERMAN
(GHS-ERC CHAIRPERSON)

Cc: The Director, Research & Development Division, Ghana Health Service, Accra