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ANTIMICROBIAL RESISTANCE OF FOODBORNE PATHOGENS IN STOOL OF DIARRHOEA PATIENTS IN TWO SELECTED HEALTH FACILITIES AND FOOD FROM VENDORS IN SELECTED COMMUNITIES IN GHANA

BY

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DECLARATION

I, Helena Dela declare that the information presented in this thesis is my own and was generated from the original research undertaken by me in the Department of Animal Biology and Conservation Science under the supervision of Prof. Langbong Bimi and Prof. Kennedy Kwasi Addo. I certify that this work, to the best of my knowledge, contains no material previously published or written by another person elsewhere. All references to other works have been duly acknowledged.

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DEDICATION

I dedicate this thesis to the men in my life; my husband, Reverend Gabriel S. K. Dela and my three sons, Daniel, David, and Daaron for the support and being understanding during my busy schedules and frequent travels. Notwithstanding my late father who sacrificed his all to put me through education. You are and will always be my inspiration.

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LIST OF ABBREVIATIONS

AMR	Antimicrobial Resistance
API	Analytical Profile Index
BA	Blood agar
CAUTI	Catheter-associated Urinary Tract Infection
CCDA	Charcoal Cefoperazone Deoxycholate Agar
CDC	Center for Disease Control
CLSI	Clinical and Laboratory Standards Institute
CRAB	Carbapenem-resistant *Acinetobacter baumanii*
DAEC	Diffuse adherent *E. coli*
DALY	Disability Adjusted Life Years
DEC	Diarrhoeagenic *E. coli*
EAggEC	Enteroaggregative *E. coli*
EHEC	Enterohemorrhagic *E. coli*
EIA	Enzyme linked immunosorbent assays
EIEC	Enteroinvasive *E. coli*
EPEC	Enteropathogenic *E. coli*
ESBL	Extended Spectrum Beta Lactamase
ESBL-PE	Extended Spectrum beta-lactam Producing Enterobacteriaceae
ETEC	Enterotoxigenic *E. coli*

FBP	Foodborne Pathogens
GHS	Ghana Health Service
GSA	Ghana Standard Authority
HACCP	Hazard analysis and critical control point
HAV	Hepatitis A Virus
HCT	Horizontal Gene Transfer
HE	Hektoen enteric
HEV	Hepatitis E Virus
HIV/ AIDS	Human Immunodeficiency Virus/ Acquired Immunodeficiency Syndrome
HRV	Human Rotavirus
IDSA	Infectious Diseases Society of America
IRB	Institutional Review Board
Lt	heat Labile gene
MAC	MacConkey agar
MALDI-TOF	Matrix-assisted laser desorption ionization-time of flight mass
MDR	Multidrug resistant
MGE	Mobile Genetic Elements
MRSA	Methicillin-resistant *Staphylococcus aureus*
NAAT	Nucleic Acid Amplification test

NMIMR	Noguchi Memorial Institute for Medical Research
NOV	Noroviruses
OPD	Outpatient Department
PCA	Plate count agar
PCR	Polymerase Chain Reaction
RTE	Ready-to-eat
SMAC	Sorbitol-MacConkey
	spectrometry
SS	Salmonella-Shigella agar
St	heat Stable gene
STEC	Shiga-toxin *E. coli*
TCBS	Thiosulfate citrate bile salts sucrose agar
TCC	Total Coliform count
TPC	Total Plate count
VBNC	Viable but nonculturable
VRBA	Vancomycin resistant *Staphylococcus aureus*
VRE	Vancomycin Resistant *Enterococci*
WASH	Water Sanitation and Hygiene
WHO	World Health Organization
XLD	Xylose Lysine Deoxycholate

ABSTRACT

Foodborne pathogens (FBPs) have been implicated as the cause of so many food poisonings and outbreaks worldwide. Food borne bacteria are the second most common FBP after viruses in causing public health threat due to the gradual emergence of antimicrobial resistance (AMR) in the food chain. Although contaminated food is frequently implicated as the cause of diarrhoeal-related hospitalization, especially during outbreak investigations in Ghana, integrated surveillance has not been conducted to ascertain this fact. This study engaged the various transdisciplinary sectors in human and environmental health to show the holistic approach in understanding the occurrence of AMR of foodborne bacteria in Ghana. Stool of patients attending Maamobi General hospital and Kaneshie polyclinic were collected, and questionnaire administered to patients to understand the risk factors involved in diarrhoea transmission. Ready-to-eat (RTE) food and water were collected from food vendors, palm swabs of food vendors as well as responses on possible risk factors leading to food and water contamination were investigated. Common pathogens isolated in stool were *E. coli*, *E. cloacae*, *K. pneumoniae* in food, while *K. pneumoniae*, *Aeromonas* spp. and *E. cloacae* were isolated from food, water and palm samples from the Maamobi and Kaneshie communities. Among the stool isolates, a total of 69.7% (85/122) Enterobacteriaceae was recovered in the study with an overall ESBL occurrence of 26.5%, predominantly among *E. coli* (13.2%; 10/76), *Klebsiella pneumoniae* (35.7%; 5/14) and *Proteus mirabilis* (57.1%; 4/7). Among the ESBL genes detected, bla_{TEM} (n=14) was common, followed by $bla_{CTX\text{-}M}$ (n=13) and bla_{SHV} (n=4). Thirty-four *E. coli* isolates from the stool samples possessed the heat labile (*Lt*) gene of Enterotoxigenic *E. coli* (ETEC) in the stool samples. All the Enterobacteriaceae isolates were resistant to the penicillins and aminoglycosides apart from *E. coli* which showed reduced susceptibility to both drug classes. The food items showed the common occurrence of *E. cloacae* (16.8%), *Citrobacter* spp (10.1%), *E. faecalis* (7.8%), *Pseudomonas* spp (6.7%) and

K. pneumoniae (4.0%). Resistance genes and Diarrhoeagenic *E. coli* (DEC) showed among two *C. freundii* isolates possessing the bla_{TEM} gene and one *E. coli* possessing the *Lt* gene of ETEC. Over 50% resistance to penicillins and Rifampicin among most of the Enterobacteriaceae (*E. cloacae*, *Citrobacter* spp, *Aeromonas* spp), and non- Enterobacteriaceae (*E. faecalis*) were observed among the isolates recovered. Common pathogens recovered in water and palm were *K. pneumoniae*, *Aeromonas* spp. and *E. cloacae* with one *K. pneumoniae* isolate from the palm of a food vendor possessing the bla_{TEM} gene. Resistance to majority of the antibiotics among the Enterobacteriaceae as well as non-Enterobacteriaceae (*E. faecalis* and *Pseudomonas* spp) was observed. Mean Total Plate Counts (TPC) of 1.4 X 10^5 CFU/g) of unsatisfactory interpretation was observed in stew as well as mean Total Coliform Counts (TCC) of 2.2 X 10^5 CFU/g and 1.7 X 10^5 CFU/g occurring in stew and 'waakye' respectively. Over 50% of all water types examined for mesophilic bacteria by TPC and TCC were considered impure with all palm swabs showing a 100% purity. Environmental samples (food and water) showed the effect of non-adherence to proper hand hygiene on food and water contamination during street food vending and the need for targeted education and enforcement of food safety policies in the country. These findings confirm the existence of AMR FBPs posing a threat to food safety due to the difficult to treat infections in case of transmission. Similar resistance to beta-lactams, Tetracyclines, Azithromycin and Penicillinase antibiotics among the clinical and environmental isolates shows common FBP transmission patterns which warrants the need for more AMR integrated surveillance in the food chain prior to consumption.

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1 CHAPTER ONE: INTRODUCTION

1.1 General Introduction

Foodborne pathogens (FBPs) are organisms or biological agents (viruses, bacteria, parasites) that can cause disease or an illness (Bintsis, 2017). An infection or an illness can occur when the pathogen is ingested, establishes itself and multiplies in a human host. This condition is caused by the intake of contaminated food and water which may occur at any stage in the process of food production to consumption ('farm to fork') (World Health Organisation, 2014). Gastrointestinal disorders are the most common syndromes emerging as a result of these pathogens and result in symptoms such as diarrhoea, vomiting, nausea and abdominal pains (Osei-Tutu & Anto, 2016). Diarrhoea is one of the common reasons why many people seek for medical attention (Larbi et al., 2021). In Africa, 16% of recorded deaths is attributed to diarrhoea whiles Ghana records 25% of pediatric mortality among children under the age of five years (Ameyaw, Acheampong, & Appiagyei, 2017).

Common pathogens that have been noted to cause foodborne diseases include bacteria; *Yersinia enterocolitica, Vibrio spp., Staphylococcus aureus, Shigella spp., Salmonella spp., Listeria monocytogenes, Escherichia coli, Cronobacter sakazakii, Clostridium perfringens, Clostridium botulinum, Campylobacter jejuni* and *Bacillus cereus,* viruses (Hepatitis A and Noroviruses) and parasites (*Toxoplasma gondii, Cyclospora cayetanesis* and *Trichinella spiralis*). The most dominantly reported causative pathogens that cause foodborne infections are bacteria which rank second to Noroviruses (Havelaar et al., 2015). *Salmonella spp.* (21.8%) was reported to be the most common pathogen causing foodborne disease with notable outbreaks in 2015 followed by *Campylobacter* spp. (8.9%) in Europe (EFSA & ECDC, 2016). The adaptive and diverse nature of most bacteria foodborne pathogens enables them with the ability to survive under diverse conditions. Highly resistant spore-forming bacteria *(Bacillus*

cereus, Clostridrium botulinum), heat-resistant toxin producing bacteria (*Staphylococcus aureus, Clostridium botulium*) and low temperature resistant bacteria (*Listeria monocytogenes* and *Yersinia enterocolitica*) are some of the characteristics that enables most bacteria to thrive when faced with harsh conditions (Bintsis, 2017).

FBPs when mismanaged and not kept under control may lead to foodborne outbreaks. Foodborne outbreaks occur when two or more cases of similar illness occur after the intake of a common food (Bintsis, 2017). Food experts attribute foodborne outbreaks to reasons which include the introduction of the contamination; due to inappropriate preparation practices including food vending, proliferation of pathogens as a result of inadequate storage conditions and the survival of pathogens in food during instances when there is a decrease in cooking time leading to increase of pathogens (Kearney, 2018).

There have been several worldwide reported outbreaks of foodborne diseases. *Listeria monocytogenes, Campylobacter* spp*, Salmonella* spp, Shiga-toxin producing *E. coli* (STEC) have been implicated to be the major causes of foodborne outbreaks in the U.S by December 2015 with 82% cases of hospitalizations and deaths respectively (Dewey-Mattia, Manikonda, Hall, Wise, & Crowe, 2018). Cholera has been implicated to be the major FBP that cause most of the outbreaks ever encountered in Ghana over the years. However, there have been other instances where the causative FBPs could not be identified (Ababio & Lovatt, 2015; Saba & Gonzalez-Zorn, 2012). In the U.S. out of the 31 known causes of foodborne diseases, there have been 9.4 million illnesses, 55,961 hospitalizations and 1,351 deaths each year (Kearney, 2018). WHO has recorded Africa to have the highest burden of foodborne diseases with an estimated burden of 91 million illnesses and 135,000 deaths annually (World Health Organization, 2015). In Ghana, according to the Ministry of Food and Agriculture and the World bank, 1 in every 40 Ghanaians suffer from foodborne diseases each year with 65,000 death rate which costs the government of Ghana $69,000,000.00 out of the 420,000 cases that

are reported annually (Ababio & Lovatt, 2015). Also, the loss of productivity due to foodborne diseases in Ghana in 2006 according to the Food and Drugs Authority was approximately 594,279 days (19, 809) months which was a huge loss to the State (Ababio & Lovatt, 2015). There is also a huge direct and indirect cost of US $33 million yearly due to diarrhoea, one of the major symptoms of FBPs in Ghana (Ameyaw et al., 2017).

Foodborne diseases are a major public health concern to all due to the effect it has on morbidity and mortality in both developed and developing countries (Havelaar et al., 2015). Between 1,200 and 1,300 Disability Adjusted Life Years (DALYs) per 10,000 population in two African subregions (including Ghana) have been observed, owing to the occurrence of foodborne pathogens globally by 2010 (Havelaar et al., 2015).

Since the groundbreaking discovery of penicillin in 1928 by Alexander Fleming in curing patients with bacterial infections, antibiotics have proven to be the most reliable antidote to bacterial pathogens. Other microbes (viruses and parasites) have also been managed for many years using various antimicrobials. Recently, due to the increase, abuse, misuse of antimicrobials, there is gradually an emergence of resistance (O'Neill, 2016). Antimicrobial resistance (AMR) can be defined as the situation when microorganisms are able to survive and thrive in the midst of antimicrobial agents (ECDC, 2017). Microbes have gradually mutated to survive in the presence of antimicrobials. In view of this, the discovery of novel antimicrobials is gradually declining because of the increase in AMR (Mendez Arancibia et al., 2009).

AMR infections have been reported to cause at least 50,000 deaths across Europe and U.S annually with 700,000 deaths globally. It has been estimated that there will be 10 million deaths globally by the year 2050 if the AMR problem is not sorted out (O'Neill, 2016). Various examples of resistant bacteria include *E. coli* and *Klebsiella pneumoniae* (production of extended spectrum beta-lactamases (ESBLs), carbapenem and gradually emerging colistin resistant *Pseudomonas aeruginosa*, carbapenem-resistant *Acinetobacter baumannii* (CRAB),

Vancomycin resistant *Enterococci* (VRE) and Methicillin-resistant *Staphylococcus aureus* (MRSA) in Europe and various parts of the world (ECDC, 2017).

Information regarding estimates of AMR burden in Africa and Ghana remains difficult to obtain (Yevutsey et al., 2017). In a study which constituted various bacterial isolates from various clinical specimens collected in Ghana, the presence of some multidrug-resistant strains of *Salmonella* spp, *Staphylococcus aureus* showing high resistance to gentamicin and ciprofloxacin occurred. Resistance to some commonly used antibiotics such as chloramphenicol (75%), ampicillin (76%), tetracycline (82%) and cotrimoxazole (73%) was also reported (Newman, Frimpong, Donkor, Opintan, & Asamoah-Adu, 2011).

Several studies involving stool sample microbiological testing with their antibiotic susceptibility testing (AST) have been performed in Ghana (Akuffo et al., 2017; Newman et al., 2011; Yevutsey et al., 2017). Commonly isolated pathogens showed resistance to ampicillin, tetracycline, chloramphenicol, nalidixic acid and ciprofloxacin. These studies involved patients who presented diarrhoea symptoms to various health facilities in need of treatment.

One of the major reasons leading to diarrhoea in humans is food poisoning caused by various FBPs. Various ready-to-eat (RTE) foods have been implicated to cause food poisoning during patients' visits to health facilities from patients' responses. Symptoms of diarrhoea and complaints involving food consumption have eventually warranted the need for investigations to be carried out. In view of that, several studies in Ghana have been conducted based on the response to foodborne outbreaks due to food poisoning (Ameme et al., 2016; Malm et al., 2015). Outcomes of such studies to some extent give answers for certain foodborne outbreaks and food poisoning incidences but there have been cases where there have been missed opportunities due to the inability to obtain food samples that have been implicated (Malm et al., 2015).

On the other hand, there have been several studies investigating raw and RTE food studies that have been conducted in isolation (Abakari, Cobbina, & Yeleliere, 2018; Adzitey, Ekli, & Aduah, 2020; Feglo & Sakyi, 2012; Marras & Agbendech, 2016; Mensah, Yeboah-Manu, Owusu-Darko, & Ablordey, 2002; Saba & Gonzalez-Zorn, 2012; Yeboah-Manu, Kpeli, Akyeh, & Bimi, 2010). These studies have given information on various types, kinds, locations of food that have been investigated and the different pathogens, coliforms (level of microbial contamination) recovered from them. Food safety and hygiene issues have arisen and alerted the concern for standardized procedures in the food chain.

1.2 Problem statement

Several diarrhoea and food studies have been conducted investigating the presence of FBPs and AMR profiles (Ababio & Lovatt, 2015; Abakari et al., 2018; Akuffo et al., 2017; Amare et al., 2019; Ameyaw et al., 2017; Asamoah, Ameme, Sackey, Nyarko, & Afari, 2016; Feglo & Sakyi, 2012; Mensah et al., 2002; Opintan, Bishar, Newman, & Okeke, 2010; Yeboah-Manu et al., 2010; Yeleliere, Cobbina, & Abubakari, 2017). Although there have been several studies of antimicrobial resistance in human and environment sectors, increasing AMR rates are being reported due to lack of collaboration and integration in Ghana (Abas, Cobbina, & Abakari, 2019; Dsani et al., 2020; Newman & Opintan, 2015; Opintan et al., 2015).

Antimicrobial resistance from a One health standpoint in Ghana remains a problem and has led to difficult to treat enteric diseases (Dela et al., 2022).

1.3 Justification

There is a pool of diverse information regarding the prevalence of FBPs causing diarrhoeal disease which leads to hospitalization and the level of contamination as well as the existence of various bacteria in RTE food sold by vendors in Ghana. Unfortunately, due to the lack of

coordination and an integrated continuous surveillance system between the health and environmental system, there is a gap regarding the relationship between the pathogens recovered. A more in-depth look from a human and environmental perspective (one-health) gives a clear understanding regarding the emergence of FBPs, their mechanisms of spread, treatment, control, management, and their AMR properties to ascertain whether there exists a relationship or not.

The cause of diarrhoea among patients who reported to the selected hospital facilities during the period of sample collection was investigated. Patients were provided with questionnaire that gave several information which included the types of food consumed and vending location as well. Responses implicating food as the cause of diarrhoea provided the guide to further investigate the type of foods and vendors involved. Results from stool, food, water samples and palm swabs of vendors provided the following outcomes: common pathogens existing among the samples collected, the similarities and differences among their AMR patterns, phenotypic and genotypic analysis of the foodborne pathogens and the risk factors involved in the transmission of the FBPs recovered. This contributes to the limited information in Ghana regarding an integrated approach towards the investigation of foodborne pathogens incorporating hospitalization as a result and RTE food implicated as the cause. The types of pathogens recovered together with their AMR profiling gives information on case identification, management, prevention, and treatment measures which will serve as an important information in cases of future outbreaks and responses.

Ghana has faced many foodborne outbreaks with cholera being the most recurrent over the past few years (Mireku-Gyimah, Awingura Apanga, & Koku Awoonor-Williams, 2018). Lack of implementation of Water, Sanitation and Hygiene (WASH) have been implicated as some of the reasons for this condition. Multiple diarrhoeal cases stemming from the same/ common

eateries have also been reported (Ameme et al., 2016; Dzotsi et al., 2015; Malm et al., 2015). Food poisoning due to various foodborne pathogens have often been related to such situations.

Several food poisoning cases calls for the need for the investigation of the causes and real time integrated surveillance of FBPs that occur both in humans and the environment. This study however sought to investigate the relationship between the outcome (diarrhoea) and the implicated cause (food). Some of the investigations included finding out whether pathogens recovered in stool samples would be the same or have some similar traits as those found in food and water. Due to the direct food vendor contact with food and water, this study sought to find out whether the contamination there were similar organisms between the food vendors, food and water. Personal hygiene (hand wash), sanitary conditions of food vending sites, method of food preparation were investigated and analyzed in this study to understand the

The One health approach is the incorporation of various transdisciplinary sectors of health from humans, animals, and the environment in addressing a public health concern. A One health view when conducting certain FBPs studies is of uttermost interest to global health. It also gives a clearer understanding of prevalence, mode of spread, similarities, and differences of the FBPs recovered using molecular tools. Their antimicrobial susceptibility patterns also help to inform stakeholders about the antimicrobials that may be losing/ have already lost their potency in the treatment of FBP-related infections.

1.4 Aim of the study

This study sought to identify the trends of antimicrobial resistance among foodborne pathogens isolated, microbial qualities of Ready-to-eat food, water, and food vendors' palms, as well as investigate the risk factors associated with diarrhoea infections of bacteria origin.

1.5 Specific objectives of the study

1. To determine the bacterial FBPs using the Matrix Assisted Laser Desorption Ionization Time of Flight (MALDI-TOF) in stool of patients from Kaneshie Polyclinic, Maamobi General Hospital, as well as food, water, and palm samples from some selected food vendors in two communities where the health facilities are located.
2. To determine the AMR profiles of the isolated bacterial foodborne pathogens in the stool, food and water samples collected.
3. To determine the presence of Extended spectrum beta-lactamase (ESBLs) and diarrhoeagenic *E. coli* (DEC) genes in the isolated foodborne pathogens.
4. To investigate the Total plate count (TPC) and Total Coliform Count (TCC) in food, water, and palm samples.
5. To investigate the risk factors among patients infected with diarrhoea of bacteria foodborne pathogen origin.

These objectives gave an in-depth understanding into the causes of diarrhoea and practices that leading to their transmission. The information derived from this work will help in informing policies on the right treatment regimen in the event of specific FBPs related infections. Outbreaks can also be managed in case they occur.

1.6 Novelty of the study

It is important to note that this study incorporates primarily the One health approach (human, animal, and the environment) in the food chain with the major focus on AMR. Two important techniques; the MALDI-TOF and PCR are the key components of this study which reduces the time and authenticates the results produced.

MALDI-TOF use in this study increased the efficiency and precision of the identification of pathogens. It also reduced the turnaround time in the provision of results as compared to the use of other biochemical methods. This equipment will be useful in the event of an outbreak where urgent results will be needed for interventions to take place. PCR played a key role in understanding the ESBL genes associated with resistance among some of the Enterobacteriaceae pathogens that were recovered. It also helped to investigate the presence of DEC among the *E. coli* isolates from all sample types. This approach is one of first studies in Ghana in the light of investigating FBPs and their AMR properties in stool, food, water, and palm samples.

Based on the food vending aspect in this study, special highlights on food safety issues like WASH, food preparation and other related risk factors will be discussed thoroughly in the chapters ahead.

1.7 Brief methodology

In general, stool samples were collected from diarrhoeal patients who consented in two hospitals: Maamobi General Hospital and Kaneshie polyclinic. Stool samples were collected and transported to NMIMR for testing. Based on responses from the questionnaires that were administered to the patients about instances and locations of suspected eateries that caused infection, study personnel located the food vending sites and paid visits there. Due to difficulty in getting to direction-specific food vendors, a community sampling approach was used to enroll food vendors in both communities. Consenting food vendors were administered questionnaires as well and various samples (food, water for cooking, storage, and rinsing plates, palm swabs of food vendors) collected and sent to NMIMR for further testing. Pathogens were identified by the MALDI-TOF, and ASTs performed to determine their

resistance profiles. PCR was also performed on the Enterobacteriaceae organisms to investigate various ESBL genes that could lead to the resistance to beta lactam antibiotics, including cephalosporins, penicillins and monobactam aztreonam. DEC presence was also investigated among the *E. coli* isolates using PCR. Cleaning and data analysis were performed in SPSS, version 22. Data was analyzed for risk factors associated with disease syndromes.

1.8 Organisation of thesis

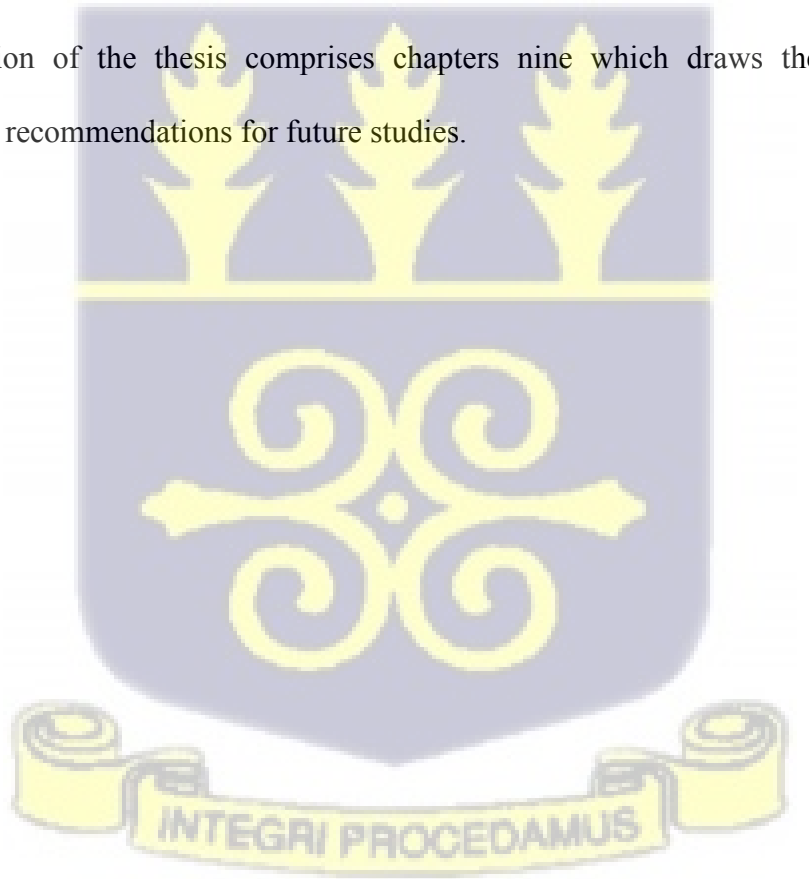
This thesis is structured into three main sections. The first section comprises of three chapters (One, two and three). Chapter one gives the introduction to the study, particularly information on the emergence of AMR in FBPs, giving a synopsis of their effect worldwide from the human, animal, and environment perspective (One health approach). A brief overview of clinical, RTE studies in relation to work done so far on diarrhoea, food, water, and palm in Ghana is outlined. This chapter also provides, the aim and specific objectives of the study and shows the gap this study sought to fill which eventually shows the novelty of this research work.

Chapter two gives an extensive literature review of various research activities that have been conducted so far. This is coupled with the gaps that this study intended to address and the additional information it gave based on FBPs and AMR in Ghana. A detailed description of the FBPs in relation to their mode/mechanisms of action and spread is also elaborated in the literature review section. Chapter three comprises of an introduction to the study sites which includes the hospitals and the various food vending sites. This is followed by the sample size calculation and the total number of samples collected. General laboratory processes and data analysis is also outlined.

Section two of the study begins with chapter four and ends with Chapter eight. All the chapters end with a discussion session that reviews all the results and explain how they relate to

literature. Chapter four presents the results of the occurrence of the various foodborne pathogens that were isolated in the study using the MALDI-TOF. Results are presented in a structured way to give a clear understanding about the occurrence of FBPs in stool, food, water, and palm swabs. Chapter five gives the AMR results of the FBPs showing their AST profiles by the respective sites as well. Chapter six shows the occurrence of Extended Spectrum beta-lactamases (ESBLs) and Diarrhoeagenic *E. coli* (DEC) in stool, food, water, and palm isolates using PCR. Chapter seven gives Total plate count (TPC) and total coliform count (TCC) results found in food, water, and palm swabs to understand the microbial quality and safety of food/water samples and hand hygiene of food vendors. Finally, Chapter eight shows the various risk factors that could have been involved in the transmission of FBPs.

The third section of the thesis comprises chapters nine which draws the conclusions, limitations, and recommendations for future studies.



2 CHAPTER TWO: LITERATURE REVIEW

2.1 Overview of foodborne pathogens

Foodborne pathogens are known to cause several mortality and fatality cases worldwide. As the name depicts, they are transmitted through the consumption of food contaminated by pathogens that can survive and thrive in food. Over 200 known diseases are reported to be transmitted through food (Mead et al., 1999). In the United States, there are 48 million cases of mortalities, 128,000 hospitalizations and 3000 deaths annually due to foodborne diseases according to the Center for Disease Control and Prevention (CDC) (Baraketi, Salmieri, & Lacroix, 2018). In Canada, according to Health Canada, an average of 4 million cases of foodborne diseases responsible for causing diseases occur annually.

FBPs are organisms or microbes that cause foodborne illness when ingested by humans. The causes of foodborne illnesses could be viruses, bacteria, parasites, metals, toxins, and prions. In most foodborne infections, when a pathogen is ingested with food, it either establishes itself and mostly multiplies in the human host or attaches itself to the food product and produces a toxin when it is toxigenic. The outcome of this phenomenon is either a foodborne infection or a foodborne intoxication. Reported mortalities mostly due to foodborne infections are complicated due to the difficulty in tracing the source of infection due to the longer incubation period prior to the occurrence of symptoms (Bintsis, 2017).

2.2 Pathogens that cause foodborne diseases

2.2.1 Viruses

Viruses, one of the causative pathogens of FBPs have 10 families that have been identified to be associated with foodborne infections. Their effect ranges from self-limiting diarrhoea

diseases to severe liver disease that can lead to hospitalization (WHO, 2008). There are several examples of viral pathogens that cause foodborne infections. Some of which include Noroviruses (NoV) (one of the most common cause of foodborne gastroenteritis globally through infected food handlers and contaminated produce), Hepatitis A virus (HAV), Hepatitis E virus (HEV) from zoonoses in pigs, Human Rotavirus (HRV)- the leading causative pathogen of viral gastroenteritis in infants and children globally, which causes a severe dehydrating illness and other emerging viruses (Nipah virus, Highly Pathogenic Avian Influenza (HPAI) virus, SARS-causing Coronavirus). In the USA, , two-thirds of all foodborne infections, one-third of hospitalizations and 7% deaths, are attributed to viruses, predominantly of which about 12-47% were caused by NoV(Mead et al., 1999; Scallan et al., 2011). Each year among all diseases that can be attributed to foodborne transmission, ranging from viruses (67%), bacteria (30%) and parasites (3%). Several viral FBPs outbreaks have occurred over the years worldwide. This includes Norovirus outbreak which involved the consumption of oysters in Queensland, Australia in 1996 and several cases of Rotavirus outbreaks through contaminated food causing acute gastroenteritis (Vasickova, Dvorska, Lorencova, & Pavlik, 2005). However, Rotaviruses have been recorded to account for up to 20% mortalities among children in developing countries (Vasickova et al., 2005). Another notable vehicle of Norovirus infection was the Cruise ships which was noted to carry thousands of people from different parts of the world to multiple destinations around the world. In this process, there was access to multiple ports in different countries where passengers had the luxury to sample local foods from various cultures (Qadri, Svennerholm, Faruque, & Sack, 2005; Vasickova et al., 2005) Several outbreaks occurred on a celebrity cruise ship called 'Mercury' in 2010 resulting in more than 10-22% of passengers and 2-4% of crew falling ill due to NoV (Bintsis, 2017).

Each year, two million children under the age of five are hospitalized whereas 527,000 children die due to Rotavirus infection globally (Enweronu-Laryea et al., 2012). In Ghana, acute

gastroenteritis account for 13.1% of hospitalizations among children under five years. Out of this percentage, rotavirus account for 49% and 16.4% of Norovirus infections among childhood diarrhoea in Ghana (Chen, Lartey, Agbemabiese, Armah, & Mahmoud, 2013; Enweronu-Laryea et al., 2012).

2.2.2 Parasites

Parasites rank third among the major cause of foodborne diseases in the world (Bintsis, 2017). Unlike viruses and bacteria that have received a lot of attention, parasites as FBPs have been underrecognized. Due to globalization (travel, transportation and storage of refrigerated food), underlying health conditions of people (ageing , HIV infections, malnutrition) and lifestyle changes (street food dependency, cooking methods), there is the risk of parasitic foodborne pathogens increment (Dorny, Praet, Deckers, & Gabriel, 2009). Examples of parasites that cause foodborne disease include *Cyclospora cayetanesnsis*, Cryptosporidium, Giardia, *Toxoplasma gondii*, *Trypanosoma cruzi*, *Echinococcus* spp., *Taenia* spp., *Trichinella* spp., Trematodes (liver flukes-*C. sinensis* and *Opisthorchis* spp), Cestodes (flatworms-*Diphyllobothrium latum*) among others. Some major parasitic food borne outbreaks that have been recorded includes opisthorchiasis caused by the species, *Opisthorchi felineus* that occurred in Europe between 2007 and 2011 because of the consumption of raw and undercooked fish (Dorny et al., 2009). Cryptosporidiosis (Cryptosporidium) and Trichinellosis (*Trichinella spiralis*) have also caused major foodborne outbreaks in Denmark, France and Serbi respectively (Cacciò, Chalmers, Dorny, & Robertson, 2018).

A study conducted in May 2015 by Danikuu et al., (2015) showed the prevalence of parasites in the stool samples of street food vendors in the Tamale Metropolis, Ghana. *Giardia lambia*

and *Entamoeba histolytica* showed the highest prevalence among the other parasites that were recovered (Danikuu, Azikala, & Baguo, 2015).

2.2.3 Bacteria

Out of the 31 FBPs identified in the U.S, bacteria have been identified to cause most of the hospitalizations and deaths (Law, Mutalib, Chan, & Lee, 2014; Mead et al., 1999; Scallan et al., 2011). Some of the bacterial FBPs include *Salmonella* spp., *Escherichia coli* 0157, *Campylobacte*r spp, *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, *Vibrio* spp., and *Colostridium perfringens*.

Among the causative organisms responsible for foodborne illnesses, non-typhoidal *Salmonella* spp rank second after NoV (11%) and is also the leading cause of death (28%) in the U.S (Scallan et al., 2011). Non-typhoid *Salmonella* spp., is commonly associated with either the consumption of contaminated fruit and vegetables with animal wastes, and infected egg, poultry and dairy products (Bintsis, 2017). The Center for Disease Control (CDC) records an estimate of more than 1 million infections of Salmonella annually with 19,000 hospitalizations and 380 deaths (Scallan et al., 2011). Some outbreaks that have occurred due to *Salmonella* spp includes the outbreak of the strains *Salmonella Bareilly* and *Salmonella Nchanga* (infecting 247 and 11 persons respectively) from 24 states in the US and the district of Columbia from the consumption of frozen raw yellow fin tuna product, called Nakoachi Scrape in 2012 (Bintsis, 2017). Outbreaks involving Salmonella Chester have been reported in several countries in the EU (France, Belgium, the Netherlands, Spain, Denmark, Sweden) between 2014 and 2015 (restaurant outbreak) and in Australia in 2005 which involved tap water consumption (Bintsis, 2017).

E. coli infections can occur due to the consumption of undercooked meat and contaminated products (vegetables, fruits, flour, flour-based mixes and RTEs). Among the six groups of

pathogenic *E. coli* is the Shiga toxin-producing *E. coli* (STEC) which is known to cause episodes of mild to severe diarrhoea with 5-10% of infections developing into Hemolytic Uremic Syndrome (HUS). STEC with the strain 0157: H7 has been estimated to cause 63,000 illnesses, 2, 100 hospitalizations and 20 deaths each year (Scallan et al., 2011). From outbreaks of hamburgers in 1993 in the US to contaminated spinach in Canada and US in 2006, contaminated leafy greens and other vegetables in Germany and flour, flour-based mixes in 2006 also in the US, STEC 0157 has resulted in several hospitalizations and deaths (Bintsis, 2017; Kearney, 2018).

Campylobacter spp. (*C. coli* and *C. jejuni*; the most common species among humans) infections occur through the consumption of undercooked poultry and/ or products that may be contaminated. Listeriosis caused by *Listeria monocytogenes* is a disease that is commonly transmitted by the consumption of contaminated canned and RTE foods even when stored and transported in extreme cold conditions. Pregnant women, newborns, the elderly and immune-compromised individuals are the most infected leading to deaths. In 2011, the US encountered one of the largest and deadliest outbreaks of Listeriosis in many of their states from the contamination of whole cantaloupe melons. This incident caused 146 illnesses, 29 miscarriages and one death due to unsanitary conditions of storage and processing facilities (Bintsis, 2017; Kearney, 2018).

Staphylococcus aureus is also a common cause of foodborne infections through poor personal hygiene, inadequate refrigeration, and cooking of food. It can be found on the skin of mammals and in the case of infected mammalian hosts, found in the blood, mammary glands, intestinal, genitourinary, and upper respiratory tracts. Its non-spore-forming nature makes it survive for a long time and have been isolated in air, dust, sewage and water (Cong, Yang, & Rao, 2020; Pawlak & Johnson, 2012). Common foods prone to *S. aureus* infection include milk, cream pies, ground beef, pork sausage, ground turkey, oysters and shrimps (Kearney, 2018; O'Neill,

2015). The US reports staphylococcal food poisoning of 241,188 illnesses, 1,064 hospitalizations, and 6 deaths annually (Kearney, 2018).

Vibrio cholerae and *Vibrio parahaemolyticus* are the two major implicated *Vibrio* species known to cause foodborne infections. *V. cholerae* is normally associated with the consumption of contaminated seafood that is eaten raw or undercooked and contaminated water sources. Seaweed, crabs, prawns, scallops, oysters and clams are food sources that have been implicated as vehicles for the transmission of *V. parahaemolyticus*. Several *V. parahaemolyticus* outbreaks have occurred both in the US and in certain provinces in China due to the consumption of raw contaminated sea food and other related products. China recorded 933 illnesses and 117 hospitalizations without death, out of 71 outbreaks from 2010 to 2014 from the China National Foodborne Diseases Surveillance Network (Bintsis, 2017). *V. cholerae* is the one of the foodborne illnesses that have caused major outbreaks in Ghana with several reasons including street food vending of contaminated water and food (Mireku-Gyimah et al., 2018; Osei-Tutu & Anto, 2016).

2.3 The importance of antibiotics in the treatment of bacterial infections

Since the discovery of penicillin in 1928 by Alexander Fleming in curing patients with bacterial infections, antibiotics have experienced a high dependency of being used to cure so many infections (O'Neill, 2015). Over the years, several antibiotics emerged with the purpose of tackling different bacterial infections based on the physiological properties (cell wall, cell membrane) of bacteria and genotypic characteristics (protein, nucleic acid synthesis and metabolism) as well. As a result of these characteristics, antibiotics have also been developed based on their mechanisms/ mode of action against the bacteria. These include; a.) interference with cell wall synthesis, b.) inhibition of protein synthesis, c.) interference with nucleic acid synthesis, d.) Inhibition of a metabolic pathway e.) disorganizing the cell membrane (Munita & Arias, 2016; Shaikh, Fatima, Shakil, Rizvi, & Kamal, 2015).

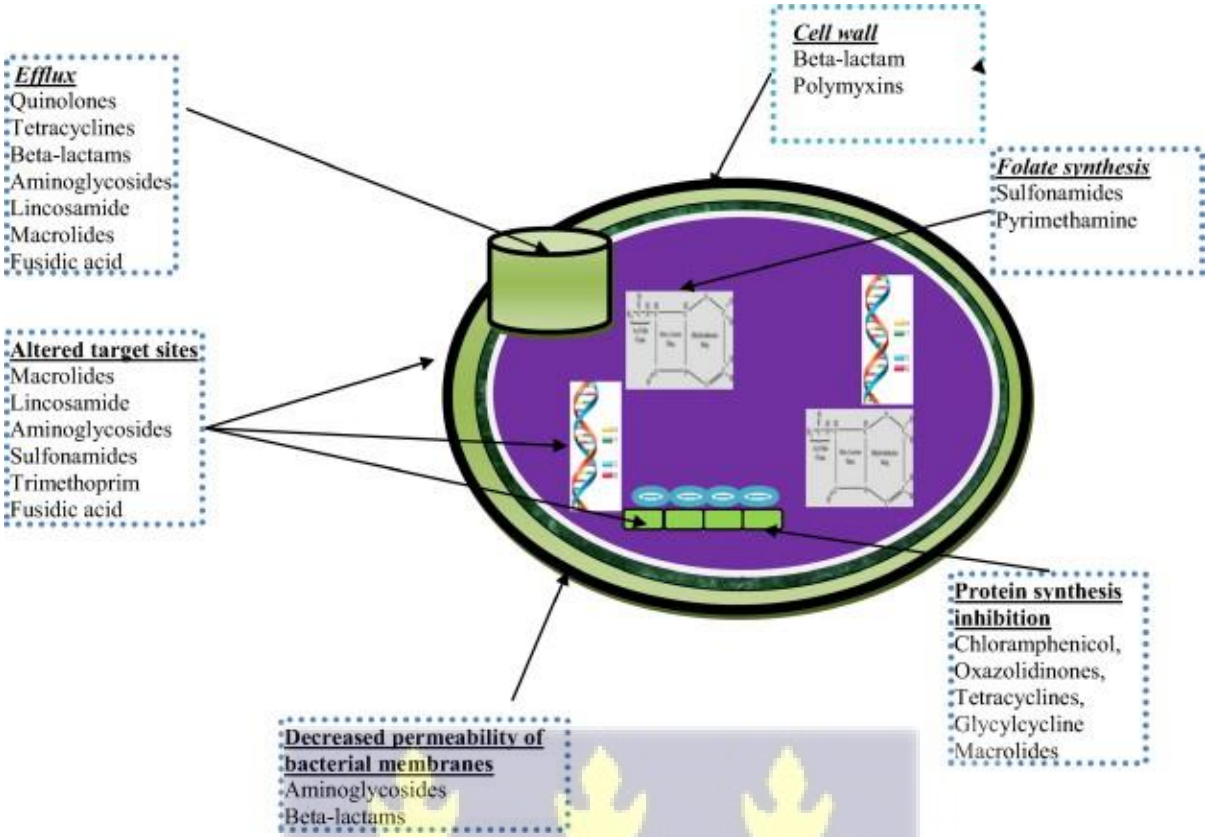


Figure 2.1: Antibiotic resistance vs. antimicrobial activity mechanism

Source: (Shaikh et al., 2015)

As shown in Figure 2.1, various groups of antibiotics that were manufactured over the years had specific targets and properties of the bacteria with the needed mechanisms to tackle. The Beta-lactams and Polymyxins targeted the cell wall in the formation of the peptidoglycan layer, the aminoglycosides and Beta-lactams focused on the disorganization of the cell membrane by decreasing its permeability, while other antibiotics worked against the efflux, folate synthesis, protein synthesis and altered the target sites of the bacteria (Shaikh et al., 2015).

2.4 Antimicrobial Resistance

The WHO has termed AMR as one of the three public health issues of concerns and threats of the 21st century. It has been anticipated that there will be around 300 million premature deaths by 2050 combined with a global economy loss of up to $100 trillion (£64 trillion) due to AMR (Munita & Arias, 2016). Already due to multi-drug resistant infections, there is an estimated economic burden of over $20 billion per year in the US alone, coupled with 23,000 deaths occurring annually (Havelaar et al., 2015).

2.4.1 Mechanisms of antimicrobial resistance

Various mechanisms have been identified to contribute to the AMR in bacteria. Biochemical aspects which involves the antibiotic inactivation either by hydrolysis or redox process, target modification (impotence of antibiotic to bind properly), antibiotic inactivation by group transfer (chemical substitution) and secondly by genetic mechanisms comprised of resistance through mutations and horizontal gene transfer (HGT) (Munita & Arias, 2016; O'Neill, 2016).

Various genetic explanations have been outlined to explain the evolution and the continuous spread of AMR. Two main ways include, a.) mutations in the genes in the bacterium that would have responded to the mechanism/ mode of action of the compound in the antibiotic or, b.) the receipt of foreign DNA coding responsible for resistance determinants from neighboring bacteria which is termed horizontal gene transfer (HGT) (Munita & Arias, 2016; Shaikh et al., 2015; WHO/Europe, 2011). Horizontal gene transfer is facilitated using three different mechanisms: conjugation, transformation, or transduction. Conjugation which is normally referred to as bacterial 'sex' uses various Mobile Genetic Elements (MGEs) as means of sharing important genetic information after cell-to-cell contact. Examples of MGEs include plasmids and transposons (O'Neill, 2016; Shaikh et al., 2015; World Health Organization, 2017b).

2.5 Acute diarrhoeal infections/ Gastroenteritis (ADI/ GE)

Symptoms of foodborne infections (bacteria, viruses, parasites, etc.) vary from mild gastroenteritis resulting in acute diarrhoeal infection to neurologic, hepatic and renal syndromes which can cause other complicated diseases in humans (Mead et al., 1999). Gastroenteritis is also referred to as acute diarrhoeal infection. Most acute gastroenteritis cases (70%) is as a result of foodborne diseases and is a major cause of morbidity and mortality worldwide (Humphries & Linscott, 2015; World Health Organization, 2017a). It is recorded that 31 major pathogens acquired in the US caused 9.4 million episodes of diarrhoeal illness, 55,961 hospitalizations and 1,351 deaths annually (Humphries & Linscott, 2015). Other conditions such as nausea, vomiting, abdominal and stomach cramping, fever, headache, dehydration, myalgia, arthralgias are some of the other symptoms that result from foodborne infections. Out of the numerous symptoms of possible foodborne infections, diarrhoea is one of the symptoms that presents itself in several of suspected causative FBPs.

2.5.1 Diarrhoea

Diarrhoea can be described as the passage of three or more loose or liquid stools per day (mostly with symptoms lasting for less than 14 days); defined by the Infectious Diseases Society of America (IDSA) (Humphries & Linscott, 2015). Diarrhoea also gives the opportunity to examine, test and diagnose (based on other factors) to investigate the pathogen that may be causing the foodborne infection. Stool samples can be examined based on their characteristics such as bloody (*Salmonella*, *Shigella*, or enterohaemorrhagic *E. coli*), foul smelling (*C. difficile* or *Giardia* infection) or watery (viral cause or *Giardia* infection).

Examination in the absence of testing using symptoms only is also not specific, factors like history (medical, travel, employment), diet, antibiotic use are factors to be considered before diagnosis and treatment (Switaj & Christensen, 2015). Stool cultures are performed for patients

with diarrhoea occurring less than a day, fever or temperature of >38.5˚C, dehydration, systemic illness, bloody stools, or a clinical history of a likelihood of a bacterial infection based on the Infectious Diseases Society of America (IDSA) guidelines (Humphries & Linscott, 2015).

Other traditional methods such as bacteria microbiology, microscopy with and without selective stains, immunofluorescence and antigen testing can be used to investigate the cause of various diarrhoea. Advanced testing such as the use of the MALDI-TOF, PCR and gut microbiome technique can also be used in testing (Jagadeesan et al., 2019).

2.5.2 Pathophysiology of bacterial diarrhoea

The small intestine is responsible for absorbing fluids in the gut. Therefore, when a disorder sets in, the fluids are not absorbed well. This propels different toxins to be released which causes the excretion of fluid from the intestinal lining, resulting in relatively loose or watery stools. Adherence, mucosal invasion and toxin production have been identified as some of the mechanisms through which diarrhoea is caused by gut bacteria (Sattar & Singh, 2018).

Adherence of the mucosal lining of the gut by some bacteria triggers various adhesins and other cell surface proteins that helps in their attachment to other cells. Bacteria such as *V. cholerae* uses this virulence factor by adhering to the brush border of the erythrocytes in the small intestines through specific surface adhesins, toxin-coregulated pilus and other accessory colonization factors. Enterotoxigenic *E. coli* (ETEC) uses similar adherence virulence properties by producing adherence proteins called colonization factor antigen. When it colonizes the upper part of the small intestines, it then produces an enterotoxin, either heat labile (*Lt*) or heat stable (*St*) which causes toxigenic secretory diarrhoea portrayed by an enormous intestinal fluid secretion (Akhtar & Sutjita, 2006; Humphries & Linscott, 2015).

Mucosal invasion is a typical phenomenon that occurs among *Shigella* and Enteroinvasive *E. coli* (EIEC) infections which involve the invasion of the mucosal and epithelial cells, multiplication in the epithelial cells and finally spreads to other neighbouring or adjacent cells. The final disease manifests as dysentery which involves cytotoxin production, bacterial invasion and a final destruction of the intestinal mucosal cells (Qadri et al., 2005).

Finally, the final manifestation of toxin production (enterotoxins) which mostly causes watery diarrhoea, affects the secretory mechanisms in the intestinal lining and cytotoxins that destroy the mucosal cells and causes other diarrhoea of similar nature. Aside these virulence mechanisms, the inoculum size of different types of bacteria during invasion, determines an infection or the extent to which an infection can occur (Bintsis, 2017). Example is Shigella or enterohemorrhagic *E. coli* (EHEC) which causes infection with a minimum of 10-100 bacteria unlike *V. cholerae* which will involve one hundred thousand or one million bacteria in order to cause an infection (Sattar & Singh, 2018).

2.5.3 Laboratory methods for the identification of bacterial diarrhoea

In preserving the integrity of stool samples for laboratory testing, Cary-Blair transport medium is normally used for transportation and anticipation of delay of more than 2 hours before culture (Humphries & Linscott, 2015). One of the primary tests that are performed is the examination of fecal specimen for the presence of fecal leukocytes using Loeffler's methylene blue or Gram stain. Loeffler's methylene blue method involves the mixing of equal amounts of methylene blue and fecal suspension prepared on a slide and viewed on 400X magnification using a light microscope. Positives are established when 3 or more leukocytes are observed in a high power filed, also in four or more microscopic fields. A positive outcome when observed does not mean an infection but an inflammation of the colon which is normally associated with salmonellosis, campylobacteriosis, shigellosis, EIEC, EHEC, Crohn's diseases. Gram stain

methods on the other hand are not reliable due to smear thickness, staining and sensitivity issues (Akhtar & Sutjita, 2006).

Various differential media have been used to culture different bacterial organisms. For example, among Shiga-like toxin producing *E. coli*, 80% of the serotype belonging to this group do not ferment D-sorbitol and therefore made Sorbitol-MacConkey (SMAC) agar a medium of chic for its isolation. *Vibrio* species on the other hand is tested using Thiosulfate citrate bile salts sucrose agar (TCBS) which possesses sulfur in combination with ferric acid to detect hydrogen sulfide production, fermentable carbohydrate and alkaline PH for their detection and enrichment. *Aeromonads* spp and *Plesiomonas* spp, are recovered on sheep blood agar (BA) and MacConkey (MAC) agar while Shigella species and Salmonella species grow on differential media such as Xylose Lysine Deoxycholate (XLD), Salmonella-Shigella agar (SSA), Hektoen enteric (HE), deoxycholate-citrate, and MAC agars (Humphries & Linscott, 2015). Latex agglutination tests are used to confirm the species of *Campylobacter* and the rapid detection of *E. coli* serogroup 0157 in primary cultures. To identify the different serogroups among Vibrio species, serotyping using strain specific antisera (Ogawa, Inaba). Various biochemical tests aside serological tests are also used in identifying different bacteria that cause diarrhoea (Akhtar & Sutjita, 2006).

Further identification of bacteria is carried out using either manual identification system such as API 20E and other automated equipment such as Phoenix, Vitek2, MicroScan and MALDI-TOF MS. Unfortunately, most of these systems are unable to classify the bacteria organisms that are recovered up to the genus level; examples include *Salmonella, Shigella, Aeromonas*, *Shigella* and *Yersinia.* The MALDI-TOF MS is able to detect both *Salmonella* and *Campylobacter* to the genus level aside its ability to detect organisms directly off the primary plate of isolation; examples HE, XLD and SSA (Humphries & Linscott, 2015). Other non-

culture methods of testing for enteric diarrhoea pathogens include the use of Enzyme linked immunosorbent assays (EIAs) and nuclei acid amplification tests (NAATs).

2.6 Antimicrobial resistance of diarrhoea pathogens/ Bacterial transfer of antimicrobial resistance in the gut and current trends

Commensals in the gut play key roles in protecting the gut against pathogenic bacteria that may want to colonize the gut by reducing pH near the wall of the gut and forming a protective barrier. Sometimes, they produce antibiotic-like substances that are antifungal or antiviral (Kariuki & Hart, 2001). Unfortunately, in the course of time, some of these commensals have been reported to cause disease by serving as reservoirs due to their acquisition, accumulation, and transfer of antimicrobial determinants of resistance and virulence in the gut. Examples include Carbapenem-resistant *Acinetobacter baumannii*, vancomycin-resistant *Lactobacillus* and other resistance types reported among *Staphylococcus* spp, *Klebsiella* spp, *Streptococcus* spp and *Escherichia* spp (Hartantyo et al., 2020; Rolain, 2013). Some bacteria can spread the AMR determinants/ genes to different bacteria which accounts for the rapid evolution and spread of AMR. A typical example is *Klebsiella* spp, aside the potential of causing diarrhoeal disease among severely ill patients including HIV/ AIDS patients have the ability to also transfer AMR in the gut (Taitt et al., 2017).

Diarrhoea that is caused by bacterial food pathogens account for one of the reasons why people seek medical attention on a daily basis worldwide (Li, 2013). In Ghana, there have been various food poisonings that have been reported (Ababio & Lovatt, 2015; Ameme et al., 2016; Malm et al., 2015). The underlying cause of some of these diarrhoea cases have been attributed to contaminated food and water. Antimicrobials ideally have not been recommended to be used for treating diarrhoea cases, since some of the bacteria are self-resolved or treated by normal infusions when contracted (Humphries & Linscott, 2015). Various food borne organisms such as *Shigella sp.* and *Salmonella sp*. have been isolated and identified in stool samples in Ghana

with Tetracycline and Ciprofloxacin showing resistance in most diarrhoeal isolates (Akuffo et al., 2017; Duedu, Offei, Codjoe, & Donkor, 2017; Opintan et al., 2015) with multidrug resistant bacteria identified among some of them (Opintan et al., 2015).

Multidrug Resistance (MDR) can be described as the case when an organism experiences resistance to at least one drug from three different antibacterial classes (Fisher & Mobashery, 2010; Shaikh et al., 2015). There is gradual emergence of various MDR occurrence in diarrhoeal pathogens, e.g. Extended spectrum beta-lactamase-Producing Enterobacteriaceae (ESBL-PE) in *E. coli*, *K. pneumoniae*, and *P. aeruginosa*, methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *S. pneumoniae* and Vancomycin-resistant *Enterococcus* (VRE) found in either community infections or hospital-acquired infections (Fisher & Mobashery, 2010).

2.6.1 Extended spectrum beta-lactamase Enterobacteriaceae (ESBLs)

Currently, AMR has rendered ineffective some of the common drugs which used to be effective. One of such examples are the Beta-lactam antimicrobial agents which are the most frequently used group of antibiotics used in the treatment of bacterial infections among Gram-negative bacteria globally. These bacteria have gradually mutated in their β-lactamases to the extent of conferring resistance to even newly developed β-lactam antibiotics (Shaikh et al., 2015). Extended-spectrum β-lactamases (ESBLs) are the enzymes that are known to cause this phenomenon. Their activity causes difficulty in the therapeutic treatment of infections due to difficulty in detection and inconsistency in reporting. This causes an increase in the spread and hospitalization (Duedu et al., 2017; Opintan et al., 2015; Shaikh et al., 2015).

2.6.2 Mechanism of ESBLs

ESBLs are enzymes that are known to break down antibiotics belonging to the cephalosporin and penicillin groups. Their possession of β-lactamases enables them to be transmitted and encoded by genes which can be transferred from one bacterium to another. There are four classes of β-lactamases according to the Bush–Jacoby–Medeiros functional scheme. This classification is based on the functional enzymatic properties of their substrate and inhibitor profiles (Bush, Jacoby, & Medeiros, 1995; Fisher & Mobashery, 2010). Several genes have also been identified to cause resistance against various broad-spectrum beta-lactam antibiotics. Their classifications are based on the types of antibiotics on which they confer resistance (Bush et al., 1995; Bush & Jacoby, 2010; Fisher & Mobashery, 2010; Shaikh et al., 2015; Tham et al., 2010).

ESBL types include the *SHV* family of B-lactamases that confer resistance to broad-spectrum penicillins which include tigecycline, ampicillin and piperacillin. However, the oxyimino substituted cephalosporins are not affected by the *SHV* family of β-lactamases. Up to about 20% of plasmid-mediated ampicillin resistance in *K. pneumoniae* species can be attributed to this family. *TEM*, the next β-lactamase family is known for the hydrolysis of penicillin and first generation cephalosporins but is also unable to attack the oxyimino cephalosporin (cefuroxime, cefotaxime, ceftriaxone, ceftazidime and cefepime) like the *SHV* family (Bush & Jacoby, 2010; Cantón et al., 2008; Margulieux et al., 2018; Rolain, 2013; Rossolini, D'Andrea, & Mugnaioli, 2008). The *CTX-M* family, the most common example of ESBL genetic variant possess the ability to hydrolyze cefotaxime but are not able to attack the β-lactamase inhibitor tazobactam. Sulbactam and clavulanate drugs are also to some extent effective in *Enterobacteriaceae* species that possess this gene unlike tazobactam which is highly effective. Molecular mechanisms of acquiring the aforementioned genes is by amino acid substitutions of their parent enzymes in *SHV* and *TEM* ESBLs, while *CTX-M* ESBLs are acquired via

horizontal gene transfer by the help of genetic devices like conjugative plasmid or transposons (Bush et al., 1995; Shaikh et al., 2015). The *OXA* family of ESBL is effective for the hydrolysis for the oxacillin and cloxacillin group of antibiotics. Other ESBLs include the *PER*-type (hydrolyses penicillins and cephalosporins but susceptible to clavulanic acid), *GES*-1 type, *VEB*-1, *BES*-1 and *SFO*-1 (Shaikh et al., 2015).

Phenotypic indicators of ESBL presence is very important in genomic resource constrained laboratory settings (Kariuki & Hart, 2001; Luzzaro et al., 2001; Oduro-Mensah et al., 2016; Ouchar Mahamat et al., 2019; Tham et al., 2010). Due to the rapid emergence of MDRs worldwide, ESBLs to be specific, it is very important to inform empirical treatment to some extent using basic microbiological techniques like the Kirby Bauer method for AST. Initial resistance to Ceftriaxone and Ciprofloxacin are normally used as a criteria in selecting potential ESBLs among *E. coli*, *K. pneumoniae* and other *Enterobacteriaceae spp* isolates (Dsani et al., 2020; Oduro-Mensah et al., 2016; Opintan et al., 2015). In this method since the ESBLs can confer resistance to penicillin and cephalosporin antibiotics by destroying the beta-lactam antibiotic, clavulanic acid is incorporated in the test to inhibit the beta-lactamase enzyme. In this test, clavulanic acid inhibits the beta-lactamase for Cephalosporin (Ceftazidime/Cefotaxime) to become active on the bacteria, thus creating a zone of inhibition. Therefore, the more active the cephalosporin, the wider zone of inhibition and vice versa (Oduro-Mensah et al., 2016; Opintan et al., 2015; Ouchar Mahamat et al., 2019).

2.6.3 Emergence of ESBLS in Europe, occurrence in Ghana, spread and possible risk factors

Since the first discovery of ESBLs detected in Germany in 1983, recovered from different clinical *E. coli* isolates, there has been an increase of detection, spread, virulence and types across the globe (Bush et al., 1995; Cantón et al., 2008; Kariuki & Hart, 2001). From the initial

discovery of *SHV, TEM* ESBLs to the discovery of *CTX* ESBLs, they have gradually evolved from being enumerated from only nosocomial infections to being community acquired (Cantón et al., 2008). They have been associated with various Enterobacteriaceae species, mostly, *E. coli*, *K. pneumoniae*, *Enterobacter* spp., *Proteus* spp. *Citrobacter* spp. and recently recovered from *Pseudomonas aeruginosa* and *Acinetobacter baumanii* (Cantón et al., 2008; Luzzaro et al., 2001; Shaikh et al., 2015) from different clinical and community isolates.

Reports of risk factors responsible for possible ESBL transmission/ acquisition among patients have included prior fluroquinolone use and possible influx of community associations (Ben-Ami et al., 2006; Cantón et al., 2008; Shaikh et al., 2015). An example of environmental bacteria that could confer ESBL resistance from the community to the hospital is *Kluyvera species* which has been found out to contain the *CTX-M* gene (Ben-Ami et al., 2006). There is an indication of possible ESBL spread to the community via plasmids from hospital-acquired infections from *Enterobacteriaceae* spp. (Ben-Ami et al., 2006). Plasmid-mediated transfer from the community due to the possession of $bla_{CTX\text{-}M}$ genes from *E.coli* isolates have formerly been associated with various epidemics in Europe (Cantón et al., 2008).

There have been several reported cases of ESBL presence in various isolates across many African countries (Ouchar Mahamat et al., 2019; Tansarli, Poulikakos, Kapaskelis, & Falagas, 2014). In a laboratory-based nationwide surveillance conducted in 2014 to monitor AMR and its spread in Ghana, a high percentage of enterobacteria (*E. coli* and *Klebsiella* spp) showed a 74% ESBL presence showing resistance to ampicillin, third-generation cephalosporins, and fluoroquinolones among stool, urine, blood and sputum samples which were collected nationwide (Opintan et al., 2015). Since then, several AMR studies in Ghana have recorded the presence of ESBLs among diverse samples; ranging from clinical, animal, to environmental, that have been tested (Dsani et al., 2020; Egyir, Nkrumah-Obeng, et al., 2020; Falgenhauer et al., 2019; Liu et al., 2017; Oduro-Mensah et al., 2016).

Owing to travel destination, antibiotic use and travelers' diarrhoea have been identified as some of the risk factors for the transmission of extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-PE) among travelers in some parts of the world (Tham et al., 2010). Some studies have shown possible transfer of ESBL presence in stool due to the close relatedness/ interaction between humans and animal/ animal products in Ghana (Dsani et al., 2020; Falgenhauer et al., 2019).

2.6.4 ESBLs in stool

The spread of ESBL-related infections has been observed globally occurring in different types of clinical samples like blood, urine, surgical site infections, respiratory secretions, money, food and also in stool samples of diarrhoea patients (Gedik, Voss, & Voss, 2013; Harwalkar et al., 2013; Shaikh et al., 2015; Tham et al., 2010). They exist in different foodborne pathogens such as *Pseudomonas aeruginosa, Vibrio cholerae, E. coli, Salmonella spp, Klebsiella pneumoniae*, and other ESBL Producing *Enterobacteriaceae* (PE) (Shaikh et al., 2015; Tham et al., 2010). These organisms portray the various ESBL types and exhibit them in the various phenotypic AMR profiling.

Various Enterobacteriaceae pathogens are commensal bacteria in the intestinal tract of humans and various animals which could propagate the spread of various types of resistance (Kariuki & Hart, 2001; Ouchar Mahamat et al., 2019). Commensal bacterial species could play the role of harboring resistant genes which can be further transmitted to other pathogenic genes which may be present in the gut by genetic transfer. Several gastrointestinal commensal bacteria have previously been discovered to exhibit resistance to various antibiotics which could serve as a precursor for further resistance on other non-pathogenic and pathogenic bacteria. Examples include *E. coli* and *P. mirabilis* (Kariuki & Hart, 2001; Luzzaro et al., 2001). Inpatients possessing fecal carriage of ESBL-producing organisms are normally at risk of subsequent

infectious episodes and may lead to other MDR conditions. This can cause several treatment failures which can lead to increased mortality cases (Ben-Ami et al., 2006).

In Africa, generally, the prevalence of ESBLs in stool isolates is the highest among all other samples and has also shown increasing trends over time (Storberg, 2014). *CTX-M*-15 is the most common ESBL gene amidst the other ESBL genes (*SHV, TEM* and *CTX-M*) in most parts of Africa. This was a noticeable trend among *K. pneumoniae* ESBL prevalence in Tunisia, Algeria, Egypt and Guinea-Bissau (Storberg, 2014). In a study conducted to determine the molecular epidemiology of ESBL-producing *E. coli* from the intestinal tract of children admitted at the Agogo children's hospital against poultry in Ghana, ESBL *E. coli* was 29% with *CTX-M*-15 showing the highest occurrence, followed by *SHV* and *CTX-M*-14 genes (Falgenhauer et al., 2019).

Advanced analysis aside the phenotypic testing of ESBL presence in Ghana has given detailed understanding of the various ESBL genes that are present, giving a better understanding of resistance showing among various classes of antibiotics (Falgenhauer et al., 2019; Obeng-Nkrumah, Twum-Danso, Krogfelt, & Newman, 2013; Oduro-Mensah et al., 2016; Storberg, 2014). The presence of ESBLs in stool reduces the efficacy of Beta-lactam antibiotics when used to treat diarrhoeal infections causing difficulty in treatment and prolonged hospitalizations (Ben-Ami et al., 2006).

2.7 Diarrhoeagenic *E. coli* (DEC)

E. coli has its natural habitat in the gastrointestinal tract of humans and other warm-blooded animals. In view of this, their presence in water and food poses suspicions of possible direct or indirect fecal contamination of enteric pathogens (Krumperman, 1983). Several foodborne illnesses because of contaminated food and water have been occurring; with *E. coli* being one

of the causative organisms. This has mainly been because of the lack of adherence to personal hygiene and sanitation in the food chain. It is one of the most common FBPs that have been recovered in raw/ uncooked food, prior to purchase by users, in utensils/ surfaces, palm of vendors and finally in the cooked food; showing their ability to be introduced or persist in food if the requisite measures are not taken in handling them (Abas et al., 2019; Amare et al., 2019; Dsani et al., 2020; Falgenhauer et al., 2019; Hassan et al., 2018; Lima et al., 2017; Mensah et al., 2002; Rane, 2011; Yeboah-Manu et al., 2010).

Shiga-toxin *E. coli* (STEC) is one of the common strains of *E. coli* that is known to cause a lot more deaths and hospitalizations all over the world and food is one of the known vehicles of pathogen infection (Dewey-Mattia et al., 2018; Rane, 2011).

It has been reported that vendors can play a very important role in the transmission of foodborne pathogens such as *E. coli*, *salmonella* spp., *campylobacter* spp. in the mode being carriers of the disease of transfer of the organisms from their fingertips as the bacteria are able to stay on surfaces for long when the appropriate measures are not taken (Hassan et al., 2018; Mensah et al., 2002; Rane, 2011).

Most *E. coli* strains of bacteria are commensal organisms in the gut, but also with severely adaptive clones that have the potential to cause disease in humans. There are six strains of *E. coli* that have been identified to cause enteric infections and therefore termed diarrhoeagenic *E. coli* (DEC) with different virulent factors and of great public health concern (Vidal et al., 2005). Known to affect people of all age groups, DEC affects travelers, children under five years old and adults as well. Enterotoxigenic *E. coli* (ETEC), the best documented which is known to be the predominant cause of traveler's diarrhoea worldwide and transmissible among children under five has been recorded as one of the common types of DEC in some parts of Chile and Africa as well (World Health Organization, 2017a). It produces two types of enterotoxins which are either heat labile (*Lt*), known to be the most common type of ETEC,

widely transmitted among all age groups or heat stable (*St*), with a structural and functional resemblance of the cholera toxin, more commonly associated with childhood diarrhoea. Although self-resolving within a few days especially among adults, due to the growing issue of AMR, reports of resistance among tetracyclines, trimethoprim-suphamethoxazole, ampicillin and currently, quinolones have been reported (Mendez Arancibia et al., 2009; Vila et al., 1999). Transmission of ETEC have also been attributed to the consumption of contaminated food and water (Rane, 2011).

Also known to cause watery, bloody and persistent diarrhoea among travelers, children under five and HIV patients, Enteroaggregative *E. coli* (EAggEC) similarly to ETEC is of public health concern (Gassama-Sow et al., 2004). The *aaFII* gene signals for the detection of this strain and possess fimbrial structures which is related to adhesion to Hep-2 cells and human erythrocytes helps the EAggEC to adhere strongly to the intestinal surface and therefore causing infection (Vidal et al., 2005). Enteropathogenic *E.coli* (EPEC) is common and serious among infants of less than a year old but gradually becoming non-existent among 0-6 month old babies due to the exclusive breastfeeding advocacy by UNICEF/WHO (Okeke, 2009). EPEC possess genes (*eae* and *bfp*) which are responsible for adherence factor plasmids and bundle forming pili which are typical of this strain of *E.coli* (Vidal et al., 2005).

Enteroinvasive *E. coli* (EIEC) is closely related to *Shigella* spp due to virulent properties such as the possession of a large invasion plasmid that encodes the Mxi-Spa type III secretion system and the invasion plasmid antigen (Ipa) effectors that gives them the advantage of invading eukaryotic cells. With the virulence genes; *ipaH* and *VirF*, EIEC is known to cause mucoid and bloody stool among diarrhoeal patients and have also been investigated to be transmitted through contaminated food and water based on their ability to survive in low-pH environments (Bintsis, 2017; Hu et al., 2014; Humphries & Linscott, 2015). Several foodborne shigellosis outbreak have occurred in the past in several parts of Africa and are able to also

show some kind of seasonality in these outbreaks (Okeke, 2009). However, Shigella has shown no association with HIV-positive diarrhoeal patients based on studies conducted in some African countries like Senegal (Gassama-Sow et al., 2004).

Enterohemorrhagic *E. coli* (EHEC) which is closely related to Shiga-toxin producing *E. coli* (STEC) due to the presence or absence of the LEE locus, a plasmid-borne heterohemolysin is known to cause highly virulent diarrhoeal infections. Involved in the transmission of hemorrhagic uremic syndrome caused by EHEC 0157:H7 which was first reported in South Africa (Browning, Botha, Sacho, & Moore, 1990), there have been series of its outbreak in other African countries due to contaminated food and water. They are characterized by the production of two potent cytotoxins denominated Shiga-like toxins 1 and 2 (stx_1 and stx_2) genes, as well as the LEE locus (*eae* gene)(Okeke, 2009; Vidal et al., 2005).

Diffuse adherent *E. coli* (DAEC) is another neonatal related diarrhoeal pathotype known to adhere to Hep-2 cells in a nonlocalized pattern, using a surface fimbria that has been known to be the virulence factor. The *daaE* gene is known to code for the expression of the F1845 fimbriae (the surface fimbriae)(Vidal et al., 2005). Until a recent study in Ghana, DAEC has been investigated to be associated with diarrhoeal disease in adults (Opintan et al., 2010).

Although, the occurrence of DEC among infected adults is mostly self-limiting and are managed with rehydration until the patient's immune system gets rid of the infection. Due to the increase of nosocomial infections, it would be ideal if bloody, persistent, and suspected outbreaks are investigated for DEC. Also, in the advent of growing resistance and the transfer of AMR to non-pathogenic bacteria in the gut, it would be expedient to perform routine AST procedures to track the performance of these antimicrobials on some of the *E. coli* strains that are recovered from diarrhoeal patients from some sentinel sites in both children and adults.

2.8 Street Food Vending

Various risk factors have been reported to cause diarrhoea and the other symptoms of foodborne infections. Weakened immune system, improper food storage or handling, cross-contamination of food due to contaminated surfaces, products and utensils and lack of personal hygiene by food handlers. Street food vending, a popular lucrative, economical and easily accessible venture have been implicated to be a major risk factor in the transmission of FBP in Ghana and other parts of the world (Ababio & Lovatt, 2015; Ameme et al., 2016; Danikuu et al., 2015; Dorny et al., 2009; Malm et al., 2015; Marras & Agbendech, 2016; Mensah et al., 2002; Rane, 2011). Due to the enormous increase of urban sprawl in most of the capital cities in Ghana, there is currently an increase in street food vending. The busy nature of the cosmopolitan lifestyle of most workers causes them to rely on street food. School children, market women and sometimes homebased residents patronize these street foods.

Convenience, affordable cost, variety, and availability of these foods are also reasons why people resort to them. These however put a lot of demand on these vendors contributing to their increase in numbers and most of the time, reducing their quality of services and technical know-how due to lack of education on food safety, personal hygiene and business accreditation/ licensing. Their increase in numbers is mostly evident in sudden and indiscriminate erection of stands, tabletops, stalls, kiosks and hawking in areas where demand and patronage are high. They can be located at many vantage places where their services are needed and highly indispensable. Most of these vendors lack education regarding the food vending business (Ababio & Lovatt, 2015; Danikuu et al., 2015; Marras & Agbendech, 2016; Mensah et al., 2002; Mosupye & Von Holy, 1999).

It is interesting to note that quite a number of the street food vendors in Ghana are mostly Basic school drop-outs and therefore with minimal exposure/ education (Amegah et al., 2020; Marras & Agbendech, 2016). Most of the vendors also lack the basic certification/ accreditation that allows them to partake in this venture (Agyei-Baffour, Sekyere, & Addy, 2013; Danikuu et al., 2015; Malm et al., 2015; Marras & Agbendech, 2016; Rane, 2011; Yeleliere et al., 2017) Some are not medically fit and those who are, do not have the health certificate of clearance to engage in this business (Danikuu et al., 2015; Marras & Agbendech, 2016).

Another important aspect of their quality of services and technical know-how is the lack of food hygiene and safety practices. Food hygiene refers to the conditions of food preparation and selling. This includes the preparation and cleaning of raw materials for cooking, i.e. the use of Sodium chloride preparation in water or the use of vinegar in washing vegetables, cleaning of utensils, the availability and use of good/ clean water for cooking and general cleanliness of the surroundings/ areas of cooking and vending. Hazard analysis and critical control point (HACCP) is a management system set up internationally and also adopted in Ghana by the Ghana Standard Authority (GSA) (GSA, 2016). This institution is to address food safety issues through the analysis and control of biological, chemical and physical hazards involved in the production of raw materials up to the consumption of the finished product (Agyei-Baffour et al., 2013; Rane, 2011). The Food and Drugs Authority, together with district assemblies, Public Health Units or Environmental Health Departments sees to it that the District Assembly Bye Laws, the Food and Drugs Act and the criminal code 1960 section 286 of the constitution are strictly observed (Act, 2012).

Unfortunately, there is paucity of knowledge from various food operators about this system and education. In view of this, some food vendors/ operators lack the requisite skills and practices that will enhance the viability of food prepared and sold (Agyei-Baffour et al., 2013). Some studies have raised concern about the unhygienic and unsanitary conditions of food

preparation and selling in some areas in Ghana (Abas et al., 2019; Ameme et al., 2016; Feglo & Sakyi, 2012; Marras & Agbendech, 2016; Mensah et al., 2002; Newman, 2005; Odonkor, Adom, Boatin, Bansah, & Odonkor, 2011).

Vending location is one of the most important aspects of consideration with regards to food handling and waste disposal. In that, the place of food preparation; with regards to good clean and assessible water for hand washing and cleaning of food elements is key to avoiding many hazards in food. Vendors may also be carriers of various pathogens (e.g. *Salmonella*, *E. coli* and *S. aureus, Shigella, Campylobacter*) (Hassan, Chakrabarty, Siddique, & Rahaman, 2018; Mensah et al., 1999). This is because the hands of vendors can transmit pathogens from the eyes, ears, mouth and nose to food (Rane, 2011). Also, there is the tendency of bacteria to thrive during scarcity/ unavailability of water, considering the inability to wash hands regularly with soap and running water. Lack of proper waste disposal facilities and the timely collection of waste are catalysts for houseflies and ants and even sometimes rodents at the food preparation and vending sites (Mensah et al., 2002). This can sometimes create a conducive situation for foodborne pathogens to spread and transmit to cause illness.

The poor quality and condition of raw materials for the preparation of food is also a means of transmission of foodborne pathogens. In that, due to the scarcity of water in some areas, vendors must reuse water for cleaning purposes for several hours before changing. This is likely to promote the transmission of foodborne pathogens in vended food. In a study conducted in Ouagadougou, Burkina Faso in 2006 by Barro et al., it was found that there were a lot of pathogens present in the water that was being used as washing water for pots, pans and plates that were used to serve customers (Barro et al., 2006). Also, due to the proximity of potable water to vending sites, there is storage of water over an excessive period, leaving molds, fungi and bacteria thriving in the storage facilities (gallons, water drums, tanks, and buckets). This can also cause foodborne infections to increase. Records of high rates of fecal

coliforms in water storage tanks have been recorded in some parts of India and Ghana (Ecklu-Mensah et al., 2019; Rane, 2011).

In a developing country like Ghana, farmers sometimes resort to unauthorized practices such as using unclean, contaminated water which normally have bacteria, parasites, sewage waste, factory/ chemical waste water in them to grow plants (Abakari et al., 2018; Newman, 2005). In doing that, farm produce such as vegetables and fruits remain contaminated when improperly washed before consumption. This is sometimes the FBP transmission pattern that occurs among some food vendors who use fresh farm produce like vegetables for cooking and vending (Abakari et al., 2018; Amoah, Drechsel, Abaidoo, & Ntow, 2006; Karikari, Frimpong, & Krogfelt, 2017).

Raw meat purchased from butcheries are sometimes kept in unhygienic conditions like car booth and bare floors exposing them to pathogens as well (Malm et al., 2015; Newman, 2005). Lack of maintenance of appropriate/ acceptable temperature conditions of meat and fish for cooking and vending serves as a risk for the containment and later spread of foodborne pathogens when present in these raw materials (Newman, 2005). The addition of condiments, spices, flavourings and unauthorized food additives to food can sometimes pose a threat to the transmission of foodborne pathogens in ready-to-eat foods as well (Kearney, 2018; Marras & Agbendech, 2016; Rane, 2011).

Utensils and equipment used in the cooking and serving of food have also been reported to sometimes be a vehicle for the transmission of foodborne bacterial pathogens. Chopping boards, pastry and working tables were implicated to have coliforms present suggesting the inadequate method of cleaning in some selected hotels in Ghana in a study conducted by Addo et al., in 2006 (Addo, Mensah, Bonsu, & Akyeh, 2007). Barro et al., 2006 also recorded bacterial organisms like *E. coli, S. aureus* and *Salmonella* on equipment like knives and utensils; and attributed it to the washing water used (Barro et al., 2006).

There have been reported cases of food poisoning and foodborne outbreaks due to the lack of proper storage and preservation of food (Ameme et al., 2016; Malm et al., 2015; Mensah et al., 2002). Due to the inconsistency of patronage and consumption by customers in the street food business, there are some instances when food needs to be stored for long periods onsite before sales, thus the tendency microbial entry and foodborne pathogen contamination. Also, one common practice that exist among vendors is the partial or full cooking of food in large quantities ahead of time before patronage, store and reheat them upon the requisition of customers (Barro et al., 2006; Mensah et al., 2002; Muinde & Kuria, 2005). This practice normally fails to eliminate all the FBPs that may emerge due to contamination and spoilage (Rane, 2011). Some vendors lack refrigeration facilities in preserving food for hours and rather resort to keeping them at ambient temperature and therefore one of the key factors in the transmission of foodborne pathogens (Malm et al., 2015). In such foods, the tendency of diarrhoea-causing organisms such as *B. cereus* is recorded to be high and can cause foodborne outbreaks as well (Ameme et al., 2016; Malm et al., 2015; Marras & Agbendech, 2016; Mensah et al., 2002; Newman, 2005; Rane, 2011).

Personal hygiene of street food vendors has been a long standing issue over the years in many countries in the food industry (Bhat & Waghray, 2000; Rane, 2011). Various conditions regarding the preparation of food may be strictly adhered to but negligence on the part of the vendors to exercise basic personal hygiene and safety practices like the washing of hands regularly, safe ways of coughing, sneezing, and handling money can contribute to the contamination of food. Money handling is one of the ways in which bacterial pathogens are transmitted to food leading to poisoning (Anning, Dugbatey, Kwakye-Nuako, & Asare, 2019). In such instances, the hand used in the collection of money from customers, unsanitized is once again used in the serving of food and therefore leading to the transmission. Coins have shown to harbor *S. aureus* in Barro et al's study conducted in Ouagadougou (Barro et al., 2006). Ghana

has recorded the occurrence of FBPs from money of various denominations (Amegah et al., 2020; Yar, 2020). A study conducted by Mensah et al., 1999 showed that street food vendors were a source of transmitting disease when various diarrhoeal pathogens were recovered from their stools and blood samples (Mensah et al., 1999). One major reason of diarrhoea caused by food is the issue of lack of personal hygiene among street food vendors (Danikuu et al., 2015).

General sanitation issues such as clearing of bushy areas, dirty gutters and surroundings where food is sold are also contributing factors in the transmission of FBPs (Ameme et al., 2016; Danikuu et al., 2015; Mensah et al., 2002).

2.8.1 Street food studies

Several studies investigating FBPs in street vended food have been conducted in different countries (Rane, 2011). Some have focused on bacterial identification as well as the total plate counts (TPC) and Total coliform counts (TCC) to ascertain the microbial quality of food that could lead to foodborne illness (Yeleliere et al., 2017). Others have also focused on bacteria identification and antimicrobial susceptibility testing to understand their AMR profiles (Abas et al., 2019; Amare et al., 2019; Anning et al., 2019; Dsani et al., 2020). In the advent of increasing AMR among many types of bacteria in stool, urine, blood and other human samples, food cannot be left out (Newman & Opintan, 2015). AMR can also be found among pathogens recovered in food (Amare et al., 2019), water (Barro et al., 2006) and palms of street food vendors (Anning et al., 2019; Hassan et al., 2018).

2.9 Pathogen recovery in food

Several pathogens have been noted to be present in food. There are standardized methods that are accepted and considered as analytical methods for routine food testing and official controls. In this regard, these methods are the traditional culture methods incorporating selective liquid

or solid culture media to grow, isolate and enumerate the organisms of interest whiles simultaneously preventing other organisms present in the food samples from growing (López-Campos, Martínez-Suárez, Aguado-Urda, & López-Alonso, 2012).

Various conventional culture methods are used to ascertain the presence or absence and enumeration of microorganisms in food samples. Conventional methods are time-consuming when pre-enrichment, enrichment and plating steps are needed to be considered for the optimum analysis of food samples. There is also a probability of false negative results being produced due to the presence of viable but nonculturable (VBNC) cells present. Observing all the microbiology steps could take between 48-72 hours for the preliminary identification of the organisms and up to about a week for confirmation of the pathogens. The two main methods; Qualitative culture method (presence or absence) for the identification of microorganisms and Quantitative culture method (enumeration) for the estimation of bacteria present in food samples are used in food microbiology (Baraketi et al., 2018).

2.9.1 Qualitative Culture methods

This type of culturing bacteria is not necessarily done to know the quantity, or the number of microorganisms present in the food samples but to know their presence or absence. They require the accurate weight of the food sample which is inoculated in a non-selective medium and incubated (pre-enrichment) to repair, recover and increase the injured cells present. This is also to gain high number of microorganisms. The pre-enrichment broth is then transferred to a selective enrichment broth and incubated to increase the specific microorganisms of interest whiles other associated microorganisms may not grow. The selective enrichment broth with the inoculum is then streaked on a selective differential agar medium which is incubated for a specific period to form colonies. The type of microbiological methods used, (for example, type of media) determines the kinds of colonies which are often called presumptive to be

recovered. For example, some organisms, like *Listeria monocytogenes* and *Campylobacter* spp., require specific selective media like PALCAM *Listeria* spp. selective agar (Gasanov, Hughes, & Hansbro, 2005) and Charcoal Cefoperazone Deoxycholate Agar (CCDA) respectively, in order to be recovered. Colonies recovered after incubation in selective agar are purified (normally on a general-purpose medium) and examined for morphological characteristics (usually using gram stain). Confirmation of the presumptive organisms are done using various biochemical and/ or serological tests using pure cultures (López-Campos et al., 2012). The use of this conventional method in the isolation of a pathogen can take between 10-12 days depending on the particular species of investigation (Erkmen & Bozoglu, 2016).

Common pathogens that have been recovered in Ghanaian foods include *Enterobacter* spp, *Escherichia* spp., *Staphylococcus* spp. and *Pseudomonas* spp. , *Klebsiella pneumoniae*, *Aeromonas hydrophila*, *E. coli*, *Citrobacter freundii* and *Bacillus* spp. occurring in food samples such as soup, stew, 'fufu', macaroni, salad, and 'waakye' (Feglo & Sakyi, 2012; Yeleliere et al., 2017). In the U.S., among 31 pathogens that have been identified as foodborne pathogens, *Salmonella*, *Campylobacter*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Clostridium perfringens*, and *Escherichia coli* O157:H7 have been recorded to cause majority of illnesses, hospitalizations, and deaths. Foods such as raw flour, seafood, raw eggs and milk, cheese, fruits, vegetables, chicken, beef, pork and turkey have been recorded to be those foods that 'most likely' cause majority of these foodborne illnesses. Common foodborne outbreaks causing several morbidities and mortalities that have ever occurred in many states in the U. S have also been caused by *Salmonella* spp., *L. monocytogenes*, *E. coli* O157:H7, and *S. aureus* (Baraketi et al., 2018).

2.9.2 *Klebsiella pneumoniae*

The presence of *Klebsiella pneumoniae* in food is an issue of public health concern due to the current properties it has been reported to have over the years. It is an opportunistic bacterium with a normal habitat of soil; skin, intestines, feces of mammals; and food. Aside causing bacteremia, pneumonia and urinary tract infections, cases of the gastrointestinal carriage of *K. pneumoniae* could lead to liver abscess which could lead to its hypervirulence as a result to AMR; an issue of concern which is gradually on the rise in Asia and other parts of the world (Lübbert, Wiegand, & Karlas, 2014). This bacterium has been acquired via nosocomial and community means with increasing cephalosporin and carbapenem resistance which has been noted to be the last line of antibiotic treatment among the Enterobacteriaceae family of bacteria (Cantón et al., 2008; Hassan et al., 2018; Sharma, 2013; Tansarli et al., 2014). Aside the bacterium's increasingly reported presence in nosocomial infections, raw and RTE food has also been listed as one of the media that acts as a vehicle for its transmission (Hartantyo et al., 2020). Several studies in Ghana and other parts of the world have reported the presence of *K. pneumoniae* in RTEs and other raw food substances on the market (Hassan et al., 2018; Nyenje, Odjadjare, Tanih, Green, & Ndip, 2012; Saba & Gonzalez-Zorn, 2012; Yeboah-Manu et al., 2010). They have been reported to be the cause/somewhat the contributing factors (their virulence and AMR properties) of various foodborne contamination and gastrointestinal disorders; a persistent issue of concern with regards to food hygiene, food safety and a major public health concern worldwide (Hartantyo et al., 2020).

2.9.3 *Citrobacter* spp

Citrobacter spp., more specifically, *Citrobacter freundii*, has been identified to be a causative agent of most nosocomial infections, sporadic infections, and outbreaks as well. They are FBPs that have been associated with infections from diarhoea patients as well as food sources and

have been reported to have both virulent and AMR properties (Amare et al., 2019; Banik, Abony, Datta, & Towhid, 2019; Duedu et al., 2017; Liu et al., 2017). They have also been investigated to possess ESBL as well as other MDR properties which increases the rate of transfer of resistance as well as virulence, having an adverse effect on not only nosocomial infections but environmental as well due to its pathogenesis (Liu et al., 2017; Tansarli et al., 2014). Several food related studies that have been conducted in Ghana and in other parts of the world have isolated this pathogen; some showing their AMR profiles (Amare et al., 2019; Banik et al., 2019; Saba & Gonzalez-Zorn, 2012) while others have focused on other molecular properties which causing virulence (Liu et al., 2017; Tansarli et al., 2014). Due to the One health perspective of disease surveillance and control, nosocomial infections are gradually shifting to becoming community-acquired, partly through food and water (Tansarli et al., 2014).

2.9.4 *Enterobacter* spp

Enterobacter species, specifically *Enterobacter cloacae*, is one of the most commonly isolated pathogens recovered from food samples in most food related works that have been conducted in Ghana and other parts of the world (Amare et al., 2019; Banik et al., 2019; Nyenje et al., 2012; Saba & Gonzalez-Zorn, 2012; Yeboah-Manu et al., 2010; Yeleliere et al., 2017). Some species of this bacteria can be found in the gastrointestinal tract, while others may be present on human skin surfaces, certain foods, soil and water (Ramirez & Giron, 2020). AMR is gradually on the rise among some of the species of the Enterobacter. Currently included among the WHO list of priority group of pathogens of urgent need of new antibiotics is the *Enterobacter* spp which was issued in 2017 with respect to increasing carbapenem resistance

aside the possession of ESBL genes (Annavajhala, Gomez-Simmonds, & Uhlemann, 2019; Ramirez & Giron, 2020).

2.9.5 *Listeria monocytogenes*

Listeria monocytogenes is a gram-positive bacterium that causes Listeriosis and is main transmitted through the consumption of contaminated food (Smith et al., 2019). It has been recorded to be the cause of several foodborne illnesses that has resulted to about 19% deaths in the United States of America (Scallan et al., 2011). Several foodborne related outbreaks have been attributed to RTE foods such as cheese, meat, frozen corn and vegetables, contaminated with *L. monocytogenes* in the US, Asia and Europe (Smith et al., 2019). Outcome of this bacteria infection often results in mild febrile gastroenteritis with severe cases characterized by bacteremia, meningitis, pneumonia, sepsis, and endocarditis (Scallan et al., 2011). Owing to their ability to form biofilms in food-processing plants, coupled with tolerance to sanitizers and ability to multiply even in low refrigeration temperatures, prevention from the contamination of RTE products is very difficult. Long incubation periods, complicated follow-up food tracing makes outbreak control difficult when it occurs (Smith et al., 2019). *L. monocytogenes* have been detected in RTE coleslaw from food vendors in the Accra metropolis due to inadequate and inappropriate vegetable washing techniques (Dogbe, 2010).

2.9.6 *Salmonella* spp

Salmonella spp., a known cause of Salmonellosis infection, is another example of a foodborne pathogen, with food and food handlers acting as major vehicles for the disease transfer. Known to exist in raw poultry and eggs and other dairy products, it has been shown to survive in adverse conditions over a period of time; being one of the causes of food contamination in imported and exported food worldwide (Dewey-Mattia et al., 2018; Rane, 2011; World Health

Organization, 2017c). The issue of AMR is increasing among salmonella strains and thus causing prolonged hospitalization in humans due to difficulty in treatment and death occurring in livestock (especially poultry) as well (Dewey-Mattia et al., 2018; Rane, 2011; Tadesse, Mitiku, Teklemariam, & Marami, 2019).

2.9.7 Confirmatory Identification of bacteria in food

Two main methods that have been used in confirming the identity of bacteria foodborne pathogens are Analytical Profile Index (API) and MALDI-TOF.

API involves biochemical tests that are used to identify different bacteria species based on their biochemical differences. API is a rapid way of identification of bacteria which incorporates the detection of enzymatic activity through strips of wells containing substrates (sugars) involved in the breaking down of proteins or amino acids when organisms are inoculated. FBPs have been identified/ confirmed using this mechanism and has helped in reducing the turnaround time in producing culture results in food (Banik et al., 2019; Mosupye & Von Holy, 1999).

The Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) is a soft ionization technique that works together with the MALDI Biotyper 2.0 (a dedicated software) and a constantly growing reference library provides a fast and more accurate platform for identification of bacteria (based on bacterial ribosomal protein) and determination of closely relatedness of isolates. This technology is able to identify microorganisms through the generation of fingerprints of numerous proteins which is followed by a linkage to reference spectra in a database (Pavlovic, Huber, Konrad, & Busch, 2013).

This technique has been tested and proven highly effective and accurate, especially in the clinical sector. Its use has recently been adopted in the veterinary diagnostics and in the

classification of environmental isolates. Its use in the food microbiology industry is gradually being explored and adopted and so far has proven reliable and highly effective in the identification of foodborne bacterial pathogens (Nicolaou, Xu, & Goodacre, 2012). Organisms including *Campylobacter* spp., *Staphylococcus* spp., *Listeria* spp., *Salmonella* spp. and *Vibrio* spp. and *Enterococcus* spp among others have been identified to the species level using this equipment. MALDI-TOF assay has proven to be reliable and fast in identifying organisms that are difficult to identify. An example is *Cronobacter* spp, and organism which has proven difficult to be isolated using both genotypic and phenotypic techniques (Stephan, Ziegler, Pflüger, Vogel, & Lehner, 2010).

This equipment helps in the testing of the quality and safety of food produced. The ability to produce the identification of foodborne bacteria pathogens when present in food within 48 hours makes it highly effective in determining product quality. This also helps in detecting contamination in food industry during large scale productions, to a large extent, strengthening the integrity of products when present during quality management systems (Nicolaou et al., 2012).

Another advantage of this technique is its ability to detect a wide array of organisms as compared to other biochemical methods such as the API kits that were specifically developed for clinical use. This is made possible by the database versions of the MALDI-TOF MS to be easily expanded to meet the specific needs of different laboratories and industries, aside its peculiar characteristic of the ability to detect specific species and variants of microorganisms in food (Pavlovic et al., 2013).

This extensive database system of this technology makes it possible to detect various kinds of organisms, both common and uncommon. In a classical foodborne surveillance and response system, this tool will be important in detecting prevalence, emergence, AMR, and outbreaks when they occur. There is paucity of data regarding the use of the MALD-TOF in food

microbiology in Ghana and Africa which exhibits another novelty in the use of this technique in this study.

2.10 Quantitative culture Methods (Total plate count)

This method is important in determining the viability of food in terms of monitoring hygiene due to handling and/ or food spoilage to measure the safety when consumed. Two methods are considered when performing quantitative culture analysis in food. These are; direct counting methods (Standard plate count, membrane filters, Howard mold count, agar droplet, dry film, direct microscopic count, spiral plate count) and indirect counting methods (Most probable number (MPN) method, turbidity, dry weight, methylene blue reduction test, amount of substance) (Erkmen & Bozoglu, 2016).

The standard plate counts also known as total plate count (TPC) or Aerobic Colony Count (ACC) is a useful tool in estimating the number of microbial populations present in food is commonly used. It is used in determining the number of viable cells, defined as coliform forming unit (CFU) in food. It is an indicator of quality but not safety (Center for Food Safety, 2014). In view of this, it cannot be considered as a safety assessment of RTE food when used during examination. The pour plate method is normally incorporated in this method in which specific quantities of food after preparation (diluted or nondiluted) are plated in an overlaid prepared selective medium to obtain and visualize visible colonies. This is incubated overnight at a particular temperature (37˚C) and counted to determine their significance. Plates having between 30-300 colonies are normally counted and considered statistically significant representing the measure of viable (living) cells present in the food samples. Even though cells are counted after the pour plate procedure, it is advised to desist from reporting them as viable cell counts, but coliform forming since all the microorganisms will manifest differently when subjected to this procedure. Several inherent characteristics such as morphology (shape, size,

form) and preparatory conditions (incubation time, temperature and other stressors) can prevent some microorganisms from growing (Baraketi et al., 2018; Erkmen & Bozoglu, 2016).

Various studies that have been conducted in Ghana to investigate the safety of RTE food sold on the streets have shown varying results of bacterial contamination in various food samples (Yeleliere et al., 2017). One of such studies is Feglo et al.'s study, conducted in 2012 in Kumasi which showed high levels of bacterial contamination at various levels in different kinds of food prepared and sold on the streets (Feglo & Sakyi, 2012). Most of the samples showed mean contamination levels of > 5.0 $\log_{10}$ CFU/ml as compared to reference figures of the Ghana Standard Authority, Center for Food Safety, Hong Kong and the New South Wales (NSW) Food Authority which recommends the standard limit for bacterial count of fully cooked RTE foods to be < 5.0 $\log_{10}$ CFU/ml (Center for Food Safety, 2014; GSA, 2016). The mean bacterial counts in the various food samples expressed in cfu/ml were as follows: ice-kenkey-5.58 l $\log_{10}$ CFU/ml, cocoa drink-6.16 $\log_{10}$ CFU/ml, macaroni-5.48 $\log_{10}$ CFU/g, salad-5.13 $\log_{10}$ CFU/g, 'fufu'-6.36 $\log_{10}$ CFU/g and 5.92 $\log_{10}$ CFU/ml for red-pepper (normally eaten with kenkey). These results shown were similar to the bacterial counts investigated in Mensah et al.'s study in 2002 that showed 6.2±1.57 $\log_{10}$ CFU/g in fufu (Mensah et al., 2002).

No ASTS were performed for the organisms recovered. Yeboah-Manu et al.'s study, 2010 on the fast food market on the University of Ghana, Legon campus, also showed mean bacterial counts which were also above acceptable limits in macaroni, salads and red pepper (Yeboah-Manu et al., 2010). A similar study conducted in Koforidua showed mean TPC in fufu of >1.0 x 10^3 Cfu/g which was at borderline but not satisfactory according to the food microbiology guidelines. The mean TPC of food samples in Gondar town in Ethiopia also showed unacceptable limits of bacterial contamination relating to poor hygiene and poor food handling practices (Amare et al., 2019). Another street-food study conducted in Alice, South Africa, showed high levels of mean TPC in vegetables (6.8 ± 0.07 $\log_{10}$ CFU/g) and rice (6.7 ± 1.7

$\log_{10}$ CFU/g) during the assessment of the microbiological quality of various RTE foods sold (Nyenje et al., 2012).

2.11 Pathogen recovery and Total coliform counts in water

Water plays a key role in the food industry and is mostly treated and investigated alongside. Water is used for drinking, cooking, washing/ cleansing and it therefore requires a level of purity/ quality to make it safe for these activities. The transmission of FBPs is the consumption of not only contaminated food, but water as well (Baraketi et al., 2018).

There have been several foodborne disease outbreaks recorded in Europe including waterborne outbreaks as well (Bintsis, 2017). In Ghana, cyclical outbreaks of cholera which has been occurring over the years since 1982 with the biggest outbreak occurring in 2014 is attributed to poor sanitation and the use of contaminated food and water (Mireku-Gyimah et al., 2018). *Salmonella spp, Campylobacter spp* and *E. coli* are some of the common pathogens that have been recovered in water worldwide and in resistant *Campylobacter* spp in some water sources in Ghana (Karikari, Obiri-danso, Frimpong, & Krogfelt, 2016; Rane, 2011).

During street food vending, accessibility of running water can sometimes be challenging as vendors are sometimes forced to fetch water from distant sources in gallons, buckets and bowls (Rane, 2011). The sources of the water are sometimes questionable since pipe-borne water may not be available in certain areas. Shortages sometimes warrant the need to acquire and store at inappropriate areas for long periods which could lead to contamination with FBPs (Mireku-Gyimah et al., 2018).

Another activity worth noting is the frequency of changing water used in washing hands and dishes at the vending sites. Some street food vendors do not change the water frequently and however repeatedly wash hand, utensils and other vending equipment in the same water for

hours causing contamination with foodborne pathogens (Mensah et al., 2002). Enteropathogens such as *Salmonella* spp and *Shigella* spp have been detected in dishwashing water that were used by some food vendors in Ouagadougou (Barro et al., 2006). The dishwashing water that was used showed high coliform counts which showed unacceptable levels of contamination and however classified as impure in the Barro et al. study. Coliform counts that were above acceptable limits according to WHO water standards (more than 16/100ml) were also detected in water that was kept in storage tanks among some food vendors in India with enteropathogenic *E. coli* (EPEC) being recovered as well (Bhat & Waghray, 2000). Some reasons attributed to this phenomenon was the lack of potable, running water, busy schedule in selling food and negligence.

Water plays a very critical role in the food industry due to its multifaceted functions. In view of this, when the basic requirements of care and management are not practiced, could lead to public health issues such as hospitalizations, treatment failures due to AMR and eventual deaths. Intensive education, law enforcements to ensure adherence of safety protocols and licensing are some of the interventions that can be implemented in ensuring proper water management among street food vendors (Bhat & Waghray, 2000). The provision of potable, running water to food vending areas is also key in minimizing foodborne infection spread through water (Mireku-Gyimah et al., 2018). There is much information about drinking/ sachet / bottled water but not much research into water used at vending sites in Ghana (Addo, Mensah, Bekoe, Bonsu, & Akyeh, 2009; Mosi, Adadey, Sowah, & Yeboah, 2019).

2.12 Pathogen recovery in palm

Pathways to the transmission of FBPs are numerous, especially in street food vending. Aside the food which has notably been established to be primary in the spread of foodborne pathogens, other routes of not much publicity are the hands/ palms and aprons of these food

vendors (Lues & Van Tonder, 2007). Defective practice in hand hygiene cause transmission of FBPs via food to consumers (Barro et al., 2006).

Best practices such as the use of quality raw materials for food preparation, clean utensils and other equipment for cooking and even general good sanitation are recommended to be observed during the production of food. There can still be a transmission of foodborne pathogens when personal hygiene among street food vendors is not observed. The serving stage after food preparation is very critical when standard recommended practices are not strictly and carefully adhered to (Mensah et al., 2002). Globally, there are reports of foodborne pathogens being transmitted due to the lack of proper personal hygiene of street food vendors (Rane, 2011). It is worth noting that the transmission of FBPs can be facilitated through the asymptomatic carriage of food vendors. However, when proper personal hygiene is not handled extensively, the transmission of pathogens such as *Salmonella* spp, *Shigella* spp and other enteric pathogens to consumers is a likelihood (Mensah et al., 1999; Tadesse et al., 2019).

One major practice in exercising personal hygiene in handling food is the hand/ palm hygiene involving its frequent washing with soap and water. Unfortunately, this exercise is not thoroughly practiced by most food vendors owing to negligence and other factors. Some are used to washing their hands but end up contaminating it again by cleaning their hands on dirty/ contaminated aprons and napkins (Lues & Van Tonder, 2007). Others are used to washing hands in water that has been fetched into buckets and bowls which are mostly used repeatedly in the washing of hands and eating bowls without frequent replacement (Mensah et al., 2002). These dishwashing and handwashing waters can be contaminated with several foodborne pathogens and as such, exposing consumers in getting infected (Barro et al., 2006).

Some vendors handle money with the same hands used to serve without proper cleaning which is a risk factor in acquiring and transferring FBPs (Amegah et al., 2020; Barro et al., 2006). Money is a means of buying and selling which causes it to circulate through different

environments and handlers, in that, reservoir of various kinds of bacteria like *E. coli* that can survive on inert surfaces for over 6 hours. A study conducted in the medical microbiology laboratory of Canisius Wilhelmina Ziekenhuis in 2012 showed the presence of Multidrug Resistant (MDR) bacteria like Extended Spectrum Beta Lactam (ESBL) *E. coli* and Vancomycin Resistant *Enterococcus* (VRE) on bank notes collected from different countries (Gedik et al., 2013). In a study performed in Ouagadougou in 2003, various coins were sampled to investigate their microbial status and the results showed the presence of coliforms and *S. aureus* (Barro et al., 2006). Their study showed that money handling was a significant risk factor in transmitting foodborne pathogens in street vended food. Multidrug resistant strains of *E. coli*, *Enterobacter* spp, *Proteus* spp, *Pseudomonas* spp were also recovered from Ghanaian coin currencies that were circulated in the Cape Coast metropolis aside the MDR bacteria that was recovered from raw meat (Anning et al., 2019).

Reports of greater risk of transmission of foodborne pathogens have also been recorded among vendors who serve with their bare hands instead of serving forks and spoons (Mensah et al., 2002). In cases where vendors were potential sources and carriers of foodborne pathogens like *Shigella spp* and pathogenic *E. coli*, the practice of serving food with bare hands could expose consumers to such foodborne pathogens (Mensah et al., 1999). This is because enteropathogens can survive for 3 hours and longer in the palms/ hands of food vendors and can multiply in food in a conducive temperature or at ambient temperature if proper hand hygiene is improperly practiced (Barro et al., 2006). Amoxicillin resistance in *Klebsiella* spp, *Acinetobacter* spp, and *E. coli*, has been reported among hand rinse samples that were investigated among street food vendors in Bangladesh, Dhaka, 2017 indicating the spread of AMR through the palms/hands of street food vendors (Hassan et al., 2018).

Proper hand washing should however be strongly encouraged among street food vendors to reduce the spread of FBPs which cause disease and the possible spread of AMR.

2.13 Antimicrobial susceptibility Testing

Investigating the microbiological quality of street-vended foods and other RTE food products have been carried out extensively in many countries including Ghana as well (Ababio & Lovatt, 2015; Banik et al., 2019; Barro et al., 2006; Mosupye & Von Holy, 1999; Nyenje et al., 2012; Pesavento, Calonico, Ducci, Magnanini, & Lo Nostro, 2014; Saba & Gonzalez-Zorn, 2012; Yeboah-Manu et al., 2010; Yeleliere et al., 2017). Most studies showed the level of safety that some of the common foods consumed in several countries have been contaminated and revealed the different foodborne pathogens they were exposed to.

In the advent of increasing resistance among FBPs, there is the need to investigate and understand their resistance profiles in order to manage their spread (Eromo, Tassew, Daka, & Kibru, 2016; Pesavento et al., 2014; Sivakumar et al., 2020; Ye et al., 2018). The spread of AMR among varying populations (humans, animals, and environment) can also be transmitted through contaminated RTE foods as well (O'Neill, 2015).

A few studies have investigated the AMR profiles of various RTE foods in several countries and have reported Multidrug resistant (MDR) FBPs, which could expose consumers to acquiring foodborne diseases (Amare et al., 2019; Banik et al., 2019; Mosupye & Von Holy, 1999; Pesavento et al., 2014; Sivakumar et al., 2020; Ye et al., 2018).

In Ghana, the focus of food work has been mostly on investigating their microbial quality and the causative organisms involved in contamination which may be due to unhygienic practices of food retailers (Yeleliere et al., 2017). However, there are records of the abuse and misuse of antimicrobials in plants and animals in Ghana which could lead to traces of resistance in their products (Addo, Adjei, Mensah, & Jackson-Sillah, 2015; Amoah et al., 2006; Yevutsey et al., 2017). Also, there are reports of *Salmonella spp*. and *Campylobacter spp*. showing resistance to fluroquinolones in Europe due to their antibiotic use in food animals. Treatment failures,

mortality and fatality rates have increased due to the quinolone resistance in Salmonella Typhimurium definitive phage type (DT) 104 and macrolide resistance in *Campylobacter spp.* (WHO/Europe, 2011).

Gradually, research being conducted on RTE food in Ghana is advancing from solely microbial quality and pathogen recovery to the investigation of the antibiotic susceptibility of the bacterial isolates recovered in these foods. One of such worth noting was the study carried out by Abas et al., 2019 that revealed 'Tuo zafi', a popular vended food in Ghana, which was sold in the central business district of Tamale showing high levels of microbial load and AMR among *E. coli* (gentamycin and erythromycin) and *S. aureus* (ampicillin, penicillin and chloramphenicol), isolated in the food (Abas et al., 2019). Ready-to-eat grilled meat (chevon, mutton, pork and guinea fowl), known as 'khebab' in Ghana showed an 85.7% multidrug resistance to several antibiotics in *S. aureus* that was recovered in Bolgatanga Municipality in Ghana (Adzitey et al., 2020). More of such studies regarding the AMR profiles of FBPs recovered in different RTE or street foods in Ghana are needed. This study sort to fill that gap of information.

Investigating the AMR profiles of FBPs recovered in the street food samples eliminates the repetitive nature of food research in Ghana as well as reveals the next step in food research in Ghana (Abakari et al., 2018).

3 CHAPTER THREE: MATERIALS AND GENERAL METHODS

3.1 Sample size calculation

Diarrhoea cases in Ghana are underreported, therefore underestimated due to the poor coordination, and testing capabilities in different health facilities. Many cases are treated symptomatically and therefore lack the proper laboratory diagnosis to ascertain the cause of the diarrhoea.

A diarrhoeal study that was conducted in the Central region to investigate the morbidity patterns of diarrhoea showed a 4% prevalence rate in the disease (Asamoah et al., 2016). This was used in calculating the sample size which gave a total of 59 diarrhoea patients to be enrolled in the study. The isolation rate was also calculated from another study which showed a 5% recovery rate of bacterial pathogens (Reither et al., 2007).

$$n = \frac{Z^2 * p(1-p)}{c^2}$$

Where:

Z = critical value of 95% confidence level which is 1.96

p = estimate prevalence of diarrhoea

c = margin of error at 5% (standard value of 0.05)

n= required sample size

n= $\{(1.96)^2 (0.04) (1-0.04)\}/ (0.05)^2$

= 59.00

For the isolation rate:

$$n = \frac{Z^2 * p(1-p)}{c^2}$$

n= $\{(1.96)^2 (0.05) (1-0.05)\}/ (0.05)^2$

=72.9

This means, a minimum of 73 bacteria isolates were expected to be collected from the 59 diarrhoea patients.

However, due to the possibilities of incomplete information from participants (adults and children), a 10% non-response rate was added to cater for the gap. Therefore, an addition of 6 participants for the total number 65 diarrhoea patients.

Therefore, to have an optimum enrollment rate, 65 stool samples were to be collected from each site making a total of 130 samples.

Purposive sampling was used to sample food vendors, where food, water and palm samples were collected. They were dependent on the ability of the patient to direct the study personnel to the food vending site and the willingness (consent) of the food vendor to provide the food samples for testing.

Due to the difficulty in getting precise locations of food vending sites, a community-based approach was adopted where food vendors in the Maamobi and Kaneshie communities, near the health facilities were enrolled in the study after giving their consent. They also provided food, water, and palm swabs for further investigations.

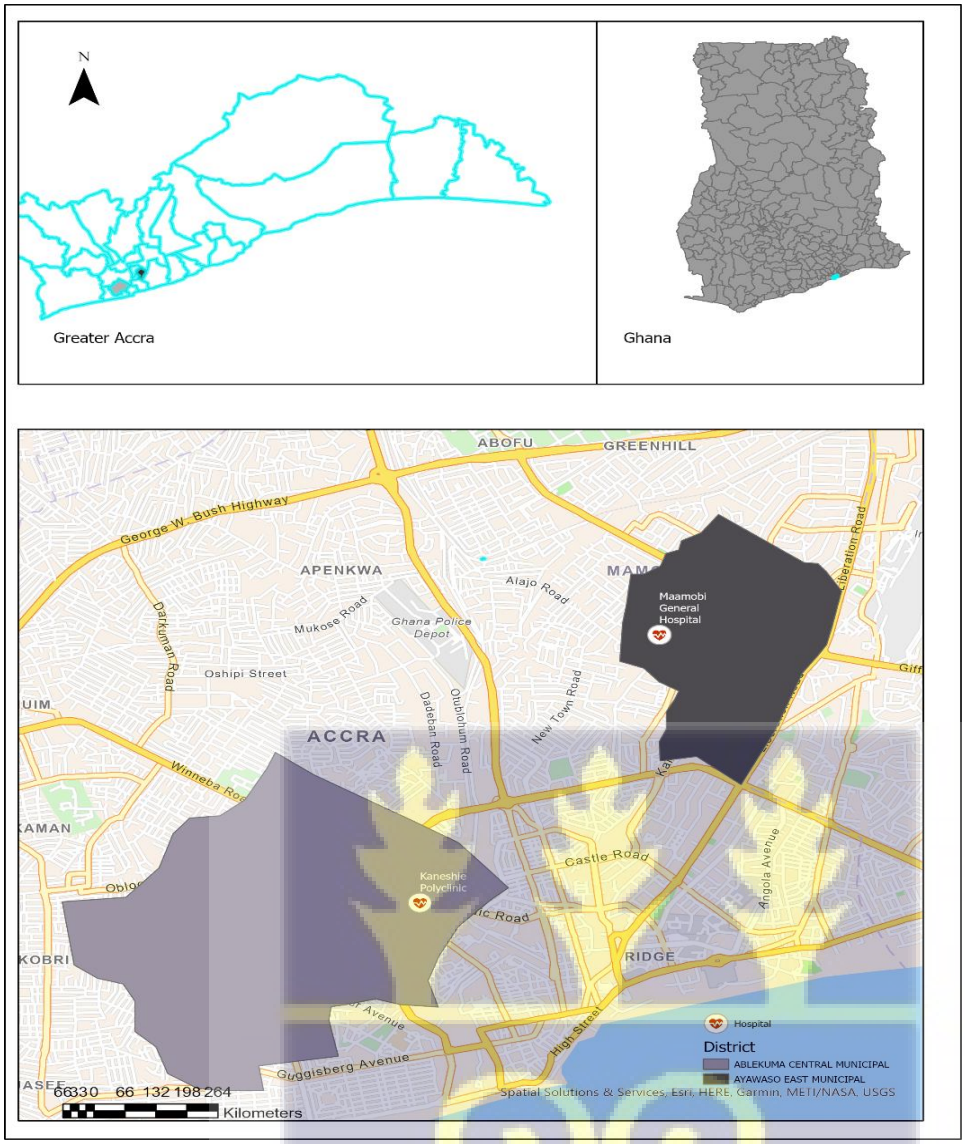


Figure 3.1: Map showing location of study sites

3.2 Study site selection

Maamobi General Hospital and Kaneshie polyclinic were the two main health facilities that this study was carried out (Figure 3.1). This was based on records of foodborne outbreaks that have occurred in the past (Dzotsi et al., 2015; Osei-Tutu & Anto, 2016). Maamobi General hospital served as one of the major referral hospitals during the 2011 cholera epidemic in Ghana (Dzotsi et al., 2015). Both hospitals serve as major point-of-care health facilities for all kinds of diseases and attends to all age groups as well. They both have laboratories that perform

routine laboratory tests but lack microbiology capacities and as such, unable to perform basic microbiology/ bacterial culture analysis and ASTs.

Both sites being peri-urban, are known for their business-oriented activities (market, kiosks, shops, retail outlets), busy streets, schools, recreational centers, and various religious establishments like churches and mosques. Both health facilities are centered in the heart of the community and therefore attend to majority of diarrhoea cases when home management fails.

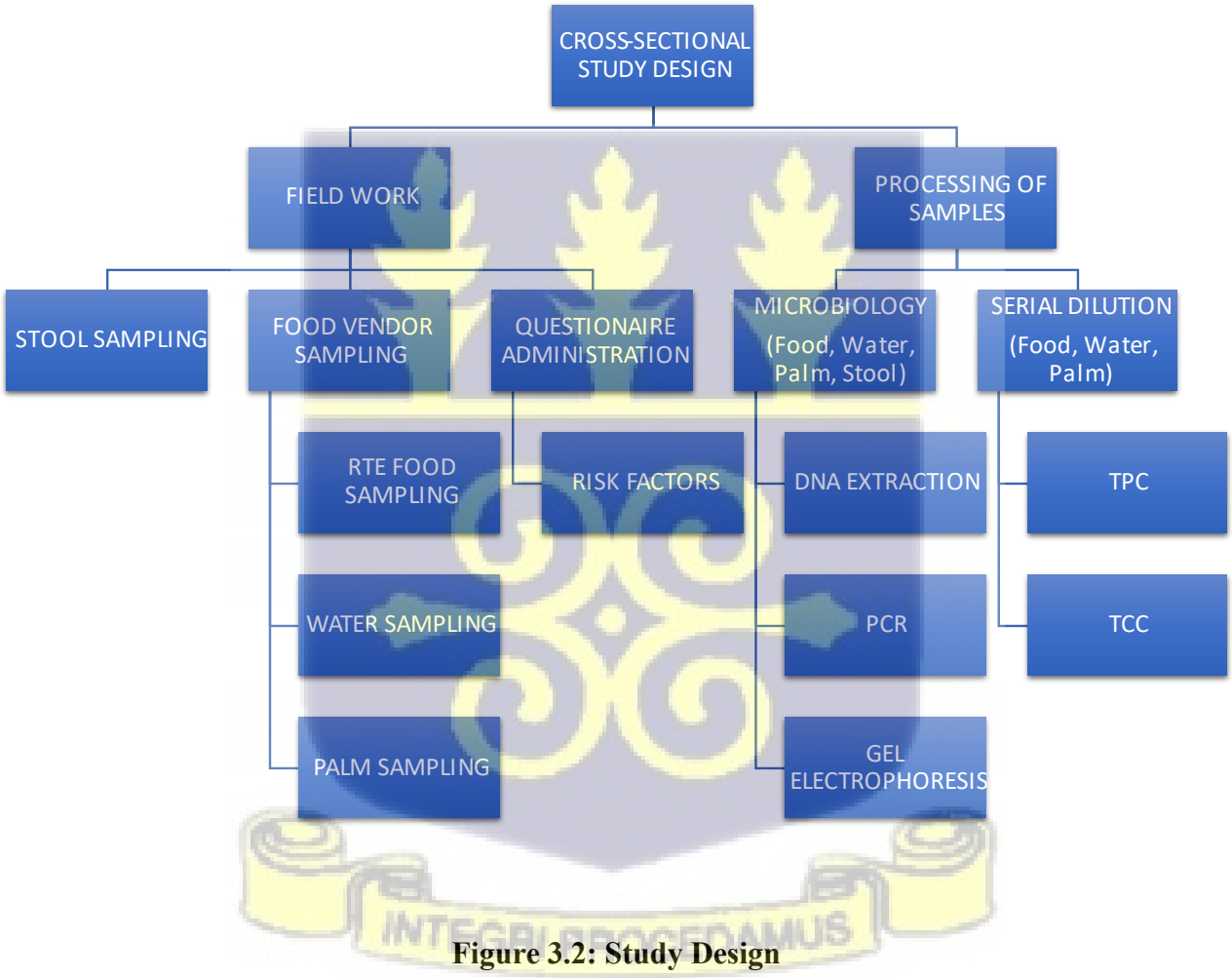


Figure 3.2: Study Design

3.3 Study Design

The study design is outlined in Figure 3.2

3.4 Ethical considerations

The study was taken through the Scientific and Technical Committee (STC) of the Noguchi Memorial Institute for Medical Research, Legon (NMIMR). After approval from the board, ethical clearance was sought from the NMIMR Institutional Review Board (IRB) with the approval number, CPN 097/17-18. Finally, approval was received from the Ghana Health Service Institutional Review Board (GHS-IRB) with the approval number, CPN 097/17-18 on the validation of the study design and informed consent procedures before the commencement of the study.

All participants (patients and food vendors) were informed about the study's purpose and procedures in accordance with the Declaration of Helsinki. In view of this, written consent was obtained from the parents or guardians prior to child enrolment during the diarrhoea study. Witnesses were allowed to be present and provide written authorization to confirm that nature, purpose, potential benefits, possible risks associated in participating in the study had been explained to the participants.

3.4.1 Consent procedures

The study personnel obtained written consent from every eligible subject and/or his or her responsible family members. If the patient was under 18 years of age (12-17 years) or unable to provide consent due to altered mental status or intubation etc., the study personnel requested permission to participate in the study from the patient's family member or guardian. Thus, a parental consent form was required from the parent/ guardian, as well as a child assent form from the patient.

The study personnel ensured that consent forms were available upon patient admission at the Outpatient Department (OPD) in the hospital and the consent forms were completed for

patients wishing to enroll in the study. Patients (or parents/guardians) were made to read the consent form and/or have the form read to them. The study personnel explained the risks, benefits, and procedures to the patient and answered all questions. Patients consented to participate by providing a signature or a thumbprint. Permission for the future use of biological specimens in other research studies required a separate patient signature or thumbprint. For patients that could not read the form, a witness signature was required, confirming that the consent form was read to the patient, explained fully, and all questions were answered.

There was also a patient logbook, which was kept at the hospital in a locked cabinet and used to track the specimen collection/transportation process. The procedures in the study were minimal risk and did not require consent in the context of clinical care.

3.4.2 Privacy and confidentiality

Each study participant was assigned a unique study identification number (study ID) that was used to ensure confidentiality during the study and analysis of data. The study ID was included on all of the questionnaires, clinical forms and samples.

Consent and clinical case forms were completed in a secure and private location within the various healthcare facilities. Patients were told that participation in the protocol was entirely voluntarily and that all patients were required to provide informed consent.

Also, the retail food operators were made aware that answers provided during the questionnaire/ interview session was strictly confidential. As such, business names were not used to provide any linkages, instead, study numbers were used as a means of identification.

3.4.3 Risks and benefit

Obtaining a stool sample is a common diagnostic procedure that contains no significant risks to any study participant. No sample was taken if the physician felt the patient was unable to provide a sample for any reason.

The results from this study will help in the selection of the appropriate treatment for the treatment in diarrhoea.

Benefits of this study in the food sector will help in finding out the microbial risks involved in food safety. This information will help inform policy makers to channel more efforts in enhancing good environmental sanitation, especially amongst people who are involved in food production, vending and consumption. Vendors and other stakeholders were taken through frequent hygiene and food safety education to help reduce the microbial risks. Consumers will in turn pay close attention to treatment measures of food processing to help reduce getting infected.

3.4.4 Voluntary Participation and Right to Leave the Research

A volunteer could withdraw from the study (withdraw consent to participate) at any time as wished. A volunteer could withdraw from the study for the following reasons:

a. Investigator or treating physician deemed it is in the volunteer's best interest of the volunteer's health to withdraw.

b. Volunteer failed to comply with the procedures outlined in this informed consent.

In view of this, the volunteer was made to understand that his/her refusal to participate in the study would not affect access to treatment.

Future use of biological specimen

Patients were allowed to agree or disagree that their samples will be tested in the future for other pathogens when ethical clearance is sought.

3.5 Consenting: Inclusion and Exclusion criteria

3.5.1 Inclusion criteria

The first part of the study was conducted in the health facilities to identify patients who meet the following eligibility criteria:

a. Parents who consent/ provide consent for children to participate in the study.

b. Patients who have passed out 3 loose or liquid stools within 24 hours, lasting less than 14 days and can provide stool sample.

c. Patients with clinical symptoms such as fever (axial temperature ≥ 38.0◦C), abdominal cramps, nausea, and vomiting.

d. Patients with acute watery or bloody diarrhoea without clinical symptoms.

e. Patients on admission at the In-patient and out-patient department of the selected health facilities.

3.5.2 Exclusion criteria

The following characteristics when identified prevented patients from getting enrolled:

a. Eligible patients who are less than 18 years old (12-17 years) without parental/ guardian consent and child assent.

b. Children with congenital diseases and severe neonatal diseases in newborns.

3.5.3 Consenting and questionnaire administration

Food vendors were required to also provide informed consent before food, water and palm samples were taken.

A detailed questionnaire on the demographics, general information on sanitation, knowledge of personal hygiene and risk factors were administered to patients and food vendors who consented to participate in the study. This information was analysed to understand the risk factors in contracting FBPs which could lead to diarrhoea transmission.

NB: Food samples was bought from various food vending sites based on the responses from the patients.

A community-based sampling approach was used to get food from vending sites due to difficulties in the first approach.

3.6 Sample collection and transportation

Stool

Eligible patients were given individual study numbers which was used throughout the study to identify the samples. Labels were printed and attached to each enrollment form and sample collection material. Stool samples were taken immediately after onset of illness or in the early stages of diarrhoea (preferably within 4 days of onset and before antimicrobial treatment has started). Patients were made to provide stool in a clean container which had no disinfectant with tight-fitting, leak-proof lids. Stool specimen was transferred into a tube containing Cary Blair media using sterile polyester-tipped swabs and stored at 2 - 8°C until transported to NMIMR. Specimen was transported using ice packs in ice chests (2 - 8°C) within the same day

to NMIMR and processed immediately on arrival. After culture leftover, stool specimen were kept at -80°C for further processing.

Food

Cooked food samples were purchased from the food vendors questioned earlier. Food samples ('kooko,' 'waakye', 'banku', etc.) were aseptically collected and kept in labelled sterile plastic bags and stored at 2 - 8°C, using ice packs and ice chests until transported to NMIMR and analysed within 12 hours.

Table 3.1 shows the different ready-to-eat food items, ingredients, and mode of preparation.

Water

Different types of water: storage water, cooking water and rinsing water were collected from the food vendors who consented to the study. Hundred milliliters were tested for the presence of coliforms and other Enterobacteriaceae species like *E. coli* and *K. pneumoniae*.

Palm swabs

Sterile cotton swabs dampened in 0.85% normal saline were used to take palm/ finger samples from consenting food vendors and transported in 30ml sterile containers to NMIMR. Samples were also tested for the presence of coliforms and the presence of foodborne bacteria

Table 3.1 Ready to eat foods encountered during the study

Food items	Ingredients	Description/Cooking method
Kooko (porridge)	Maize, millet, ginger, cloves, pepper, salt	Fermented, Boiling
Koose	Beans, onion, pepper, ginger, salt, vegetable oil	Fried Bean cake
Rice porridge	Rice, salt	Boiling
Kenkey	Maize dough, salt	Fermented, wrapped in corn husk and boiled
Banku, Akpler	Maize and cassava dough, salt	Fermented maize mixed with cassava, boiling
Fufu	Cassava, plantain, cocoyam	Boiling, pounding and turned with hand
Yam, plantain	Yam, plantain, salt, oil	Frying or boiling
Beans	Beans, salt, red oil	Boiling and mixed with oil
Rice	Rice, salt	Boiling
Fried rice	Rice, salt, vegetables, eggs, spices	Boiling and stir frying
Jollof	Rice, salt, vegetables, spices	Boiling, rice mixed with tomato sauce and boiled
Waakye	Rice, salt, beans, millet leaves	Boiling
Ground pepper	Red/green pepper, onion, tomatoes, salt	Grinding in earthenware
Fried pepper (shito)	Pepper, onion, dried fish and shrimps, oil, salt and spices	Frying
Soup	Okro, palm nut, peanut butter, salt, fish, meat, spices	Boiling
Indomie	Macaroni, fish/meat, salt, vegetables, oil	Boiling, frying

3.7 Data Analysis

Unique identification numbers were assigned to specimens/ patients. Cleaning and data analysis was performed in STATA, version 13.

Prevalence and AMR profiles in the foodborne pathogens in the stool, food, water, and palm swab samples were carried out using frequencies and percentages.

All categorical variables were described using figures, tables, pie charts, and bar graphs. Continuous variables were also described using mean and standard deviation. Bivariate analysis was done using Chi-square or Fishers' exact test where necessary. Statistical significance was set at $p<0.05$.

.

4 CHAPTER FOUR: OCCURENCE OF BACTERIAL FOODBORNE PATHOGENS IN STOOL, FOOD, WATER AND PALM SWAB SAMPLES

4.1 Introduction

Foodborne bacteria have been the cause of many food-related outbreaks worldwide leading morbidity and mortality (Bintsis, 2017; Dewey-Mattia et al., 2018). Several gastroenteritis infections have resulted in various clinical manifestations with diarrhoea being one of the major symptoms (Humphries & Linscott, 2015; Sattar & Singh, 2018; Turgeon & Fritsche, 2001). Owing to this, this study sought to investigate the major bacteria pathogens that could have led to diarrhoea cases that were presented at the two health facilities. Also, since the consumption of contaminated food and water are one of the main causes of diarrhoea (Baraketi et al., 2018; Bintsis, 2017; Yeleliere et al., 2017), RTE food sources that were implicated by patients were located and food sampled to investigate the presence of FBPs coupled with water from the vending sites and palm swabs of the food vendors.

The identification of various foodborne bacteria, especially, among hard-to-identify bacteria with the help of the MALDI-TOF made it easy and reduced the turn-around time as compared to the basic microbiological culture methods (Christner et al., 2014; Pavlovic et al., 2013). Coupled with its ability to identify a varied array of organisms, reduced turn-around time and precision, it has been recommended by the World Health Organization (WHO) when conducting integrated foodborne surveillance studies (World Health Organization, 2017b).

The results derived from this approach will help detect the stage or source at which food samples get contaminated in the food chain (preparation-to-fork).

This chapter is divided into two parts; the first part showing the occurrence of the foodborne pathogens while the second part looked at the demographics, general information on sanitation,

personal hygiene and knowledge of risk factors that could lead to diarrhoea among patients and food vendors.

4.2 Materials and Methods

4.2.1 Laboratory stool processing

Initially, the study sought to isolate and recover five major bacterial pathogens (*Vibrio cholerae, Shigella spp, Salmonella spp*, *E. coli spp, Staphylococcus spp)* in the stool samples. Therefore, various selective media including Thiosulfate citrate bile salts sucrose (TCBS) agar, Selenite F broth, Salmonella-Shigella agar, Alkaline peptone water, Trypticase soy broth (TSB), mannitol salt agar and MacConkey agar were used. However, due to the extensive array of bacteria that were bound to be recovered, the MALDI-TOF was used to identify other bacteria apart from the five pathogens.

Stool samples in Cary Blair medium were cultured on MacConkey (MaC), Deoxychocolate (DCA), Hekton agar (HK), Chocolate agar and Thiosulfate Citrate-Bile-Sucrose (TCBS) agar (Beckton Dickinson BD, USA). Swabs were cultured in Selenite F broth (SF) and Alkaline Peptone Water (APW), for the enrichment of *Salmonella* spp and *Vibrio* spp, respectively. Agar plates and broths were incubated under aerobic conditions at 37°C for 18 – 24h. Broth cultures in SF and APW were then inoculated onto DCA and TCBS plates, respectively, after 18-24h incubation. Species of all bacteria isolates were confirmed by application of the Matrix Assisted Laser Desorption Ionization Time of Flight (MALDI-TOF) method from fresh overnight cultures. Colonies that were also not identified by selective media were plated on nonselective media such as Nutrient agar and confirmed using MALDI-TOF.

Identified bacteria were stored in Tryptic Soy Broth (TSB) +15% glycerol at -80°C for further testing and long-term storage.

4.2.2 Isolation Procedure for various bacteria

Various agar were prepared according to the manufacturers' instructions. The choice of agar that was used in the study were based on the bacteria that were sought to be investigated. Slide preparation from all bacterial growth for Gram staining were prepared for initial pathogen identification.

All isolates were stored in Trypticase soy broth (TSB) with 10% glycerol after isolation.

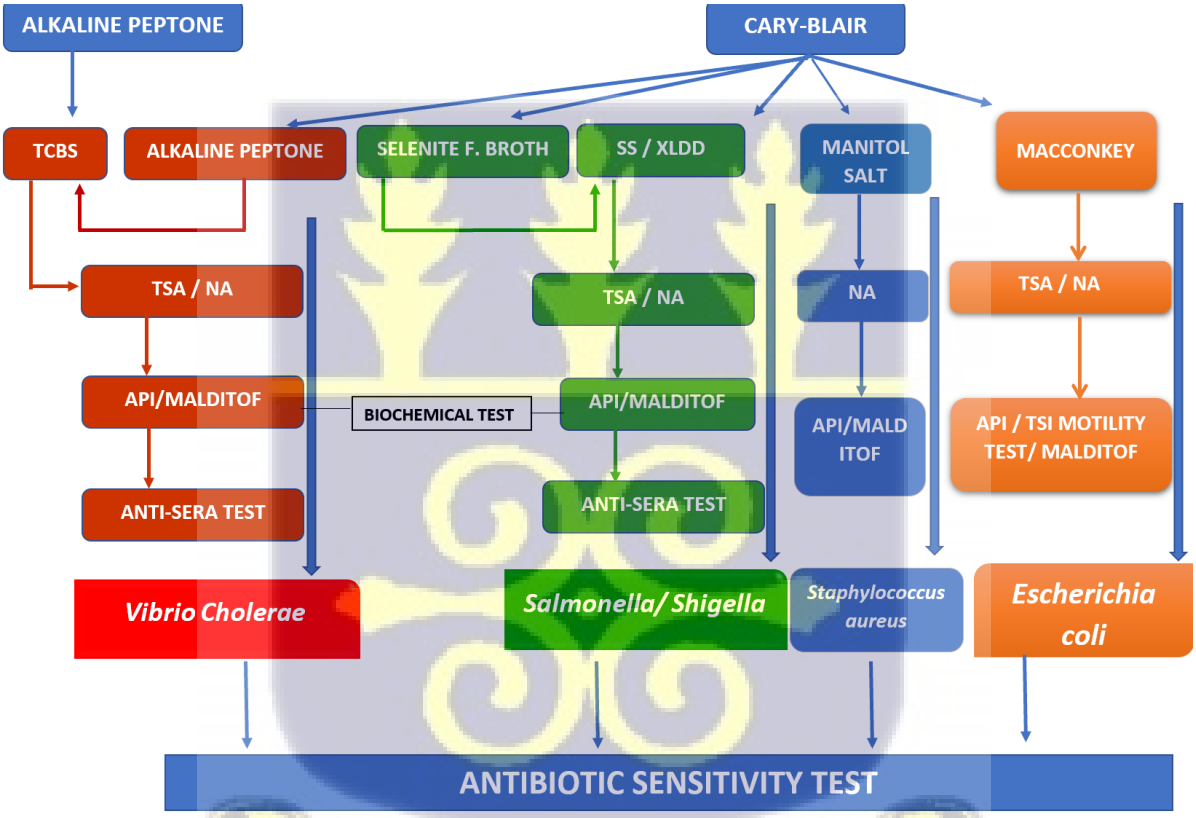


Figure 4.1: Stool flowchart of general laboratory procedure

Figure 4.1 shows the workplan of the bacteria recovery from stool using various enrichment broths and selective media.

Isolation Procedure for *V. cholerae:* Fecal swabs were inoculated in 15ml centrifuge tube containing alkaline peptone water (APW) for enrichment and isolation of *Vibrio* spp. (The stool inoculum did not exceed 10% of the volume of the APW broth). After incubation with loosened cap at 35-37ºC for 6-8hours, inoculum was subcultured on TCBS agar plate (direct streaking/plating) using one to two loopfuls from the surface and topmost portion of the APW broth (*Vibrio* spp preferentially grow in this area). Care was taken not to shake or mix the tube before sub-culturing. After incubation at 37ºC for 24hours, the amount and type of growth (e.g., sucrose-fermenting or sucrose-non-fermenting) on the TCBS plate was recorded. *V. cholerae* is sucrose fermenting and therefore a production of yellow 2-3mm in diameter shiny colonies on TCBS agar.

Isolation Procedure for *Escherichia coli:* A loopful of the fecal sample was picked in Cary Blair medium and streaked directly on MacConkey Agar /plate by making a uniform smear at the edge of the plate with the inoculum on the surface of the media. Extensions from the streaks were made from the inoculum by making at least three different streaks with overlaps at each beginning section to form individual colonies and incubated at 37ºC for 24hours.The amount and type of growth (e.g., lactose-fermenting, or non-lactose-fermenting) on the plate were recorded. Lactose fermenting organisms (e.g., *E. coli*) grew as pink to brick red colonies with or without a zone of precipitated bile. Non lactose fermenting organisms grew as colourless or clear colonies.

Isolation Procedure for *Salmonella* spp or/and *Shigella* spp*:* Fresh fecal swabs were inoculated in 15ml centrifuge tube containing Selenite F broth for the enrichment and isolation of *Salmonella* spp *or Shigella* spp. Incubation was done with the cap of the tube loosened at 35-37ºC for 6-8hours. The broth was mixed and subcultured on Salmonella-Shigella agar plate using one to two loopfuls. Concurrently, the same sample was plated on nutrient agar. The S-S Agar plate and the nutrient agar plate were incubated at 37ºC for 24hours.The amount and

type of growth (e.g., lactose-fermenting, or non-lactose-fermenting) on the plate were recorded and slides prepared for gram staining. Positive Salmonella samples did not ferment lactose but produce hydrogen sulfide (H_2S). The resulting bacterial colonies appeared colourless with black centers.

Enterobacter and *Klebsiella* appeared larger than *E. coli*, mucoid, pale, opaque cream to pink.

Isolation procedure of *S. aureus:* A loopful of the fecal sample was picked in Cary Blair medium and streaked directly on Mannitol Salt Agar /plate by making a uniform smear at the edge of the plate with the inoculum on the surface of the media. Extensions from the streaks were made from the inoculum by making at least three different streaks with overlaps at each beginning section to form individual colonies. Mannitol plates were incubated at 37^{o}C for 24hours. The amount and type of growth (e.g., mannitol-fermenting, or non-mannitol-fermenting) on the plate were recorded and slide prepared for Gram stain after subculturing and growth on Nutrient agar. S. aureus due to mannitol fermentation turned the red colour of the medium yellow (yellow colonies with yellow zone). Non mannitol fermenting organisms like *S. epidermidis* and *Micrococci* spp showed pink or red colonies while *E. coli* had no growth.

4.2.3 Gram Staining Principle

Most bacteria can be differentiated by their gram reaction due to differences in their cell wall structure. Those organisms are called Gram positive which after being stained dark purple with crystal violet are not decolorized by acetone or ethanol. Those organisms are called Gram negative which after being stained with the crystal violet lose their colour when treated with acetone or ethanol, and stain red with neutral red, safranin, etc. The iodine solution used in the technique acts as a mordant for the crystal violet (Humphries & Linscott, 2015).

Procedure for Gram Staining

All isolates recovered were transferred unto slides, primarily fixed on a Bunsen burner, and stained with crystal violet stain for 30-60 seconds. After washing the stain with clean water, the sides were tipped off to remove all water and covered with gram iodine for another 30-60 seconds. With acetone-alcohol, the iodine was decolorized rapidly after being washed off with water. Water was used to wash off the acetone-alcohol and covered completely with neutral red stain for 2 minutes. The stain was washed off with clean water, the back of the slide wiped clean and placed in a draining rack for the smear to air dry before microscopy.

The smear was examined microscopically, first with the 40X objective to check the staining and to see the distribution of material and the with the oil immersion objective to look for bacteria and cells. The condenser iris was opened fully when using the oil immersion lens.

4.2.4 Principle and use of the MALDI TOF

While all bacteria were identified by their colonial morphology, confirmation of all isolates was conducted using the Matrix Assisted Laser Desorption/ Ionization Time of Flight (MALDI TOF) (Bruker, USA). The MALDI TOF equipment is comprised of three main components; the ionization source, the analyser and detector as well as an inlet to load the samples. The MALDI (matrix-assisted laser desorption ionization) method ionises the sample once it is loaded whiles the Time of Flight (TOF) spectrometer analyses the gas phase analytes ions. The machine generates results within a short period of time based on the quality and purity of the sample, the database complexity as well as the number of reference spectra that is generated in the database (Pavlovic et al., 2013).

Colonies harvested from fresh overnight cultures were placed on a stainless-steel plate known as the target plate using a 1µ loop and allowed to air-dry. One (1µl) of formic acid was then

added and allowed to air-dry as well for 15 minutes, after which 1µl matrix preparation (α-cyano-4-hydorxy-cinnamic acid and trifluoroacetic acid)was added and allowed to air-dry for another 15 minutes. The plate was placed into the MALDI TOF machine to identify and confirm bacteria by producing ionization peaks (spectra) that were generated and matched against the integrated reference library of the equipment (Egyir, Nkrumah-Obeng, et al., 2020). The machine uses a database of known organisms to match the unknown isolate on the target place while providing a matching score which is also known as logarithmic score value. Scores of >2 are considered as very good matches for species identification while score values between 1.7 and 2.0 are sufficient for identification of the genus. Score values below 1.7 show an outcome of no identification of an isolate (Pavlovic et al., 2013).

Figure 4.2: MALDITOF being used in the confirmation of bacteria IDs

Note: The MALDI TOF equipment was used in the confirmation of all bacteria from fresh overnight cultures of isolates from food, water, palm of food vendors and stool samples from patients (Figure 4.2).

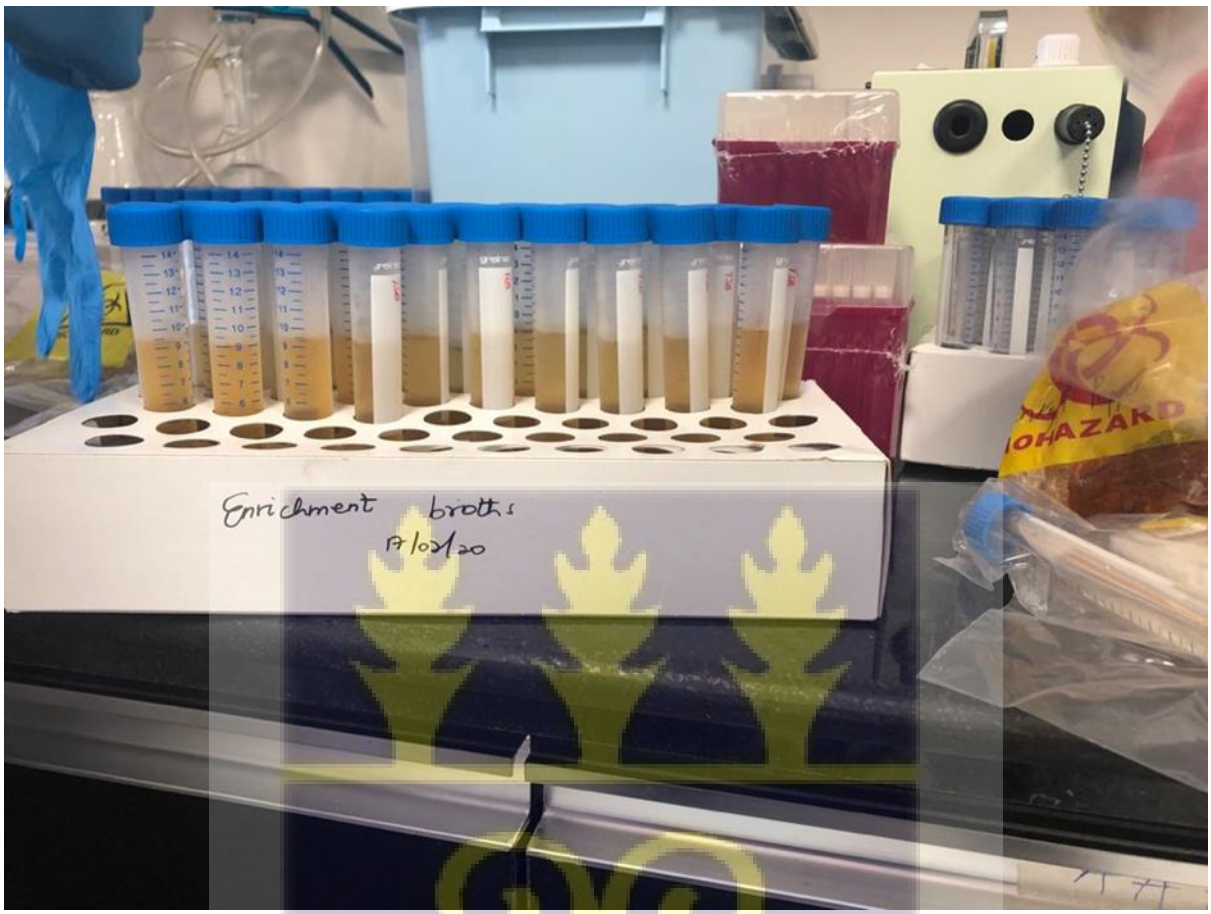


Figure 4.3: Enrichment broths being prepared for the inoculation of samples

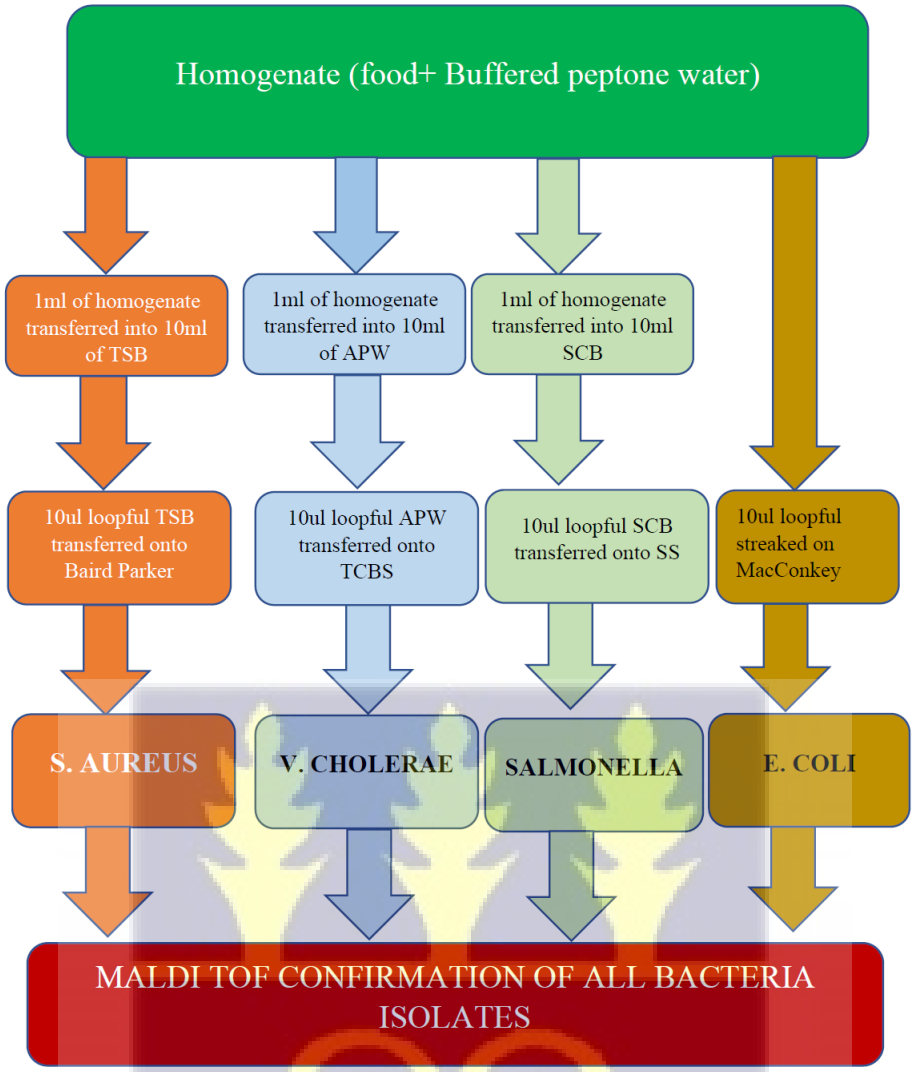


Figure 4.4: Flowchart of Food laboratory bacteria identification

4.2.5 General sample preparation and pre-enrichment

Food sample (25g/ml) is weighed into a sterile empty wide mouth container with screw cap or suitable closure and add 225ml of Buffered peptone water. A uniform suspension is made by blending using a homogeniser if necessary and incubated at 35^{O}C for 24 hours (homogenate). Figure 4.3 shows the various enrichment broths for the isolation of bacteria from food and water samples. Figure 4.4 illustrates the workplan that was used in isolating specific pathogens from food.

Selective Enrichment, detection, and confirmation of *Salmonella* spp in food: The homogenate was gently shaken and 1ml transferred to 10 ml freshly prepared selenite cysteine broth and incubated at 35^{O}C for 24 hours. The broth was vortexed and 10ul loopful streaked on Salmonella-Shigella agar (SS agar). Plates were incubated at 35^{O}C for 24 hours and 48 hours. Plates were observed for Salmonella colonies (transparent colonies with or without black centers). Suspicious colonies were subcultured on Nutrient agar for confirmatory testing using the MALDI TOF after the performance of gram stain, catalase, and oxidase tests.

Selective Enrichment, detection, and confirmation of *S. aureus* in food: An amount of the homogenate (1ml) was transferred into 10ml tryptic soya broth (TSB) on Baird parker agar and incubated at 35℃ for 48 h. After vortexing the tube, 10ul loopful of tryptic soya broth was streaked on Baird parker agar and incubated at 35℃ for 24 h. Colonies of *S. aureus* are typically grey black to jet black, circular, smooth, convex, moist and 2-3 mm on agar plates. After the performing gram stain and catalase test, the isolates were confirmed using coagulase test and MALDI TOF.

Selective Enrichment, detection, and confirmation of *V. cholerae* in food: The homogenate (1ml) was transferred into 10ml Alkaline Peptone Water (APW) and incubated at 35℃ for 24 h. The tube was vortexed and a 10ul loopful of APW streaked on Thiosulfate Citrate bile salts sucrose agar (TCBS) and incubated at 35℃ for 24 h. *V. cholerae* is sucrose fermenting and therefore produces yellow 2-3mm in diameter shiny colonies on TCBS agar. Subculturing was done on Nutrient agar and confirmation was done using MALDI TOF.

Detection and confirmation of *E. coli*

A 10µl loopful of homogenate (food + buffered peptone water) was streaked on MacConkey agar and incubated at 37℃ for 24 h. *E. coli* appears pink on the agar. After subculturing on

Nutrient agar, gram stain, catalase and oxidase tests were performed before their final confirmation using the MALDI TOF.

4.2.6 Laboratory processing of water samples

Bacteria identification involved taking 10mls of the concentrate or initial 1: 10 suspension, incubating and centrifuging at 10,000 rpm for 30 mins in a centrifuge using Eppendorf centrifuge, 5804. After the supernatant was decanted, a loop-full of the pellet was inoculated into SCB for selective enrichment of *Salmonella* and *Shigella* and streaked on MCA for the detection of *E. coli* and other Enterobacteriaceae. Two loop-fulls from the SCB was subcultured on SSA and incubated further to enable the detection of *Salmonella* and *Shigella* (Addo et al., 2007).

Figure 4.5: RTE food, water and palm samples being prepared for laboratory processing

4.2.7 Laboratory processing of palm swab samples

Saline solutions containing palm swabs were subcultured on MacConkey and 5% sheep blood agar at 35-37˚C within 24-48 hours for coliform counts bacterial growth determination (Figure 4.5). Presumptive tests such as gram staining, catalase and coagulase were performed prior to final subculture on Nutrient Agar for the confirmation of the bacteria using MALDI-TOF.

Note: All identified bacteria were stored in Tryptic Soy Broth (TSB) +15% glycerol at -80°C for further testing and long-term storage.

4.2.8 Questionnaire administration

A standardized questionnaire was used to collect the socio-demographic characteristics (age, sex), clinical presentation (dehydration, fever, and vomiting), medical care (rehydration treatment, hospitalization) and outcome (deceased or discharged).

Questionnaire were administered to the retail food operators to provide information of various practices in food preparation and vending. Their basic knowledge in food safety was also investigated based on the questionnaire.

4.2.9 Data analysis

Descriptive statistics was carried out using percentages, frequencies, bar graphs and pie charts. Also, association between the hospital sites with demographic characteristics, clinical presentation, behavioural traits, and knowledge of the risks of contracting diarrhoea was carried out using Chi-square and Fishers' exact where necessary.

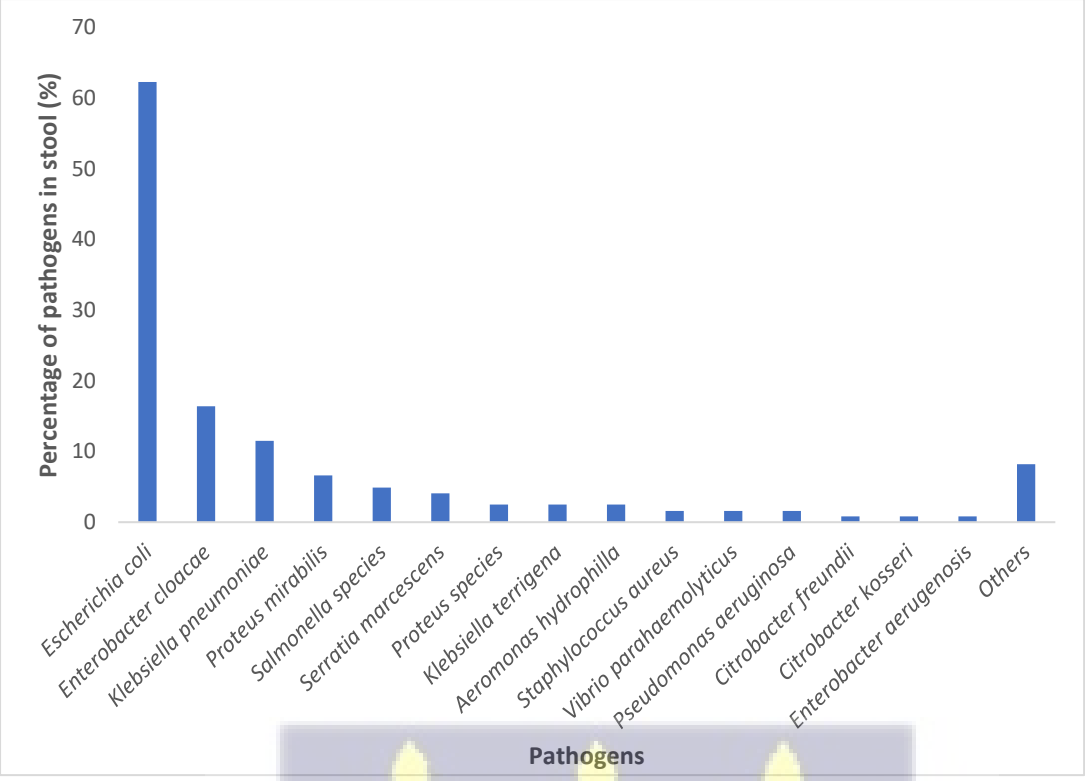


Figure 4.6: Isolated pathogens from stool samples

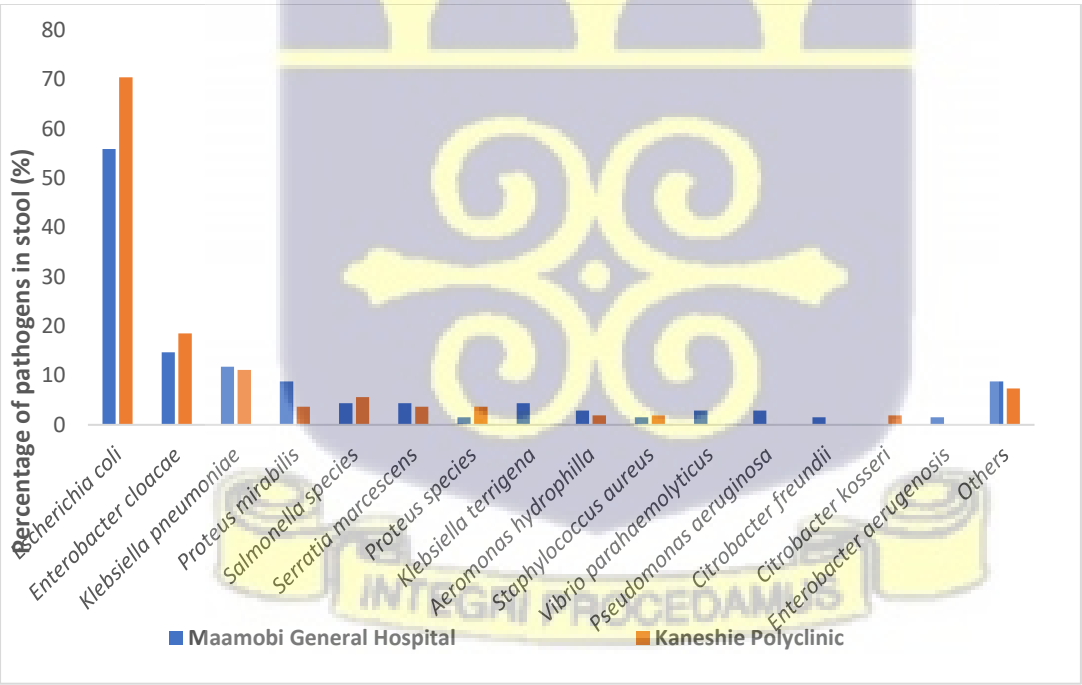


Figure 4.7: Isolated pathogens from Maamobi General Hospital and Kaneshie Polyclinic

4.3 Results (Occurrence of foodborne pathogens)

4.3.1 Occurrence of foodborne pathogens in stool

A total of 122 stool samples were collected during the study. Several pathogens were isolated which included both gram negative and gram-positive bacteria using the MALDI-TOF. Figures 4.6 and Figures 4.7 show the total bacteria recovered per site.

E. coli (62.3%) was the most frequently isolated bacteria, followed by *E. cloacae* (16.4%) and *K. pneumoniae* (11.5%). Apart from these three frequently isolated bacteria, the following bacteria were also recovered; *Salmonella spp.* (4.9%), *E. aerogenosis* (0.8%), *P. mirabilis* (6.6%), *Proteus spp.* (2.5%), *C. freundii* (0.8%), *Serratia marcescens* (4.1%), *Aeromonas hydrophilla* (2.5%), *S. aureus* (1.6%), *K. terrigena* (2.5%), *V. parahaemolyticus* (1.6%) and *P. aeruginosa* (1.6%). However, majority of *E. coli* and *E. cloacae* (70.4%; 18.5%) that were recovered were from the Kaneshie polyclinic. Apart from *C. koseri* which was isolated from only Kaneshie polyclinic and *V. parahaemolyticus*, *P. aeruginosa*, *C. freundii*, *E. aeruginosa* from the Maamobi General hospital only, all other pathogens were recovered from both sites.

Other pathogens included *Aeromonas jandei, Klebsiella oxytoca, Morganella morganii, Pantea spp, Providencia stuartii, Pseudomonas mosseli* and *Staphylococcus carnosus* (8.2%) that have not been listed in the pathogens were also recovered from stool.

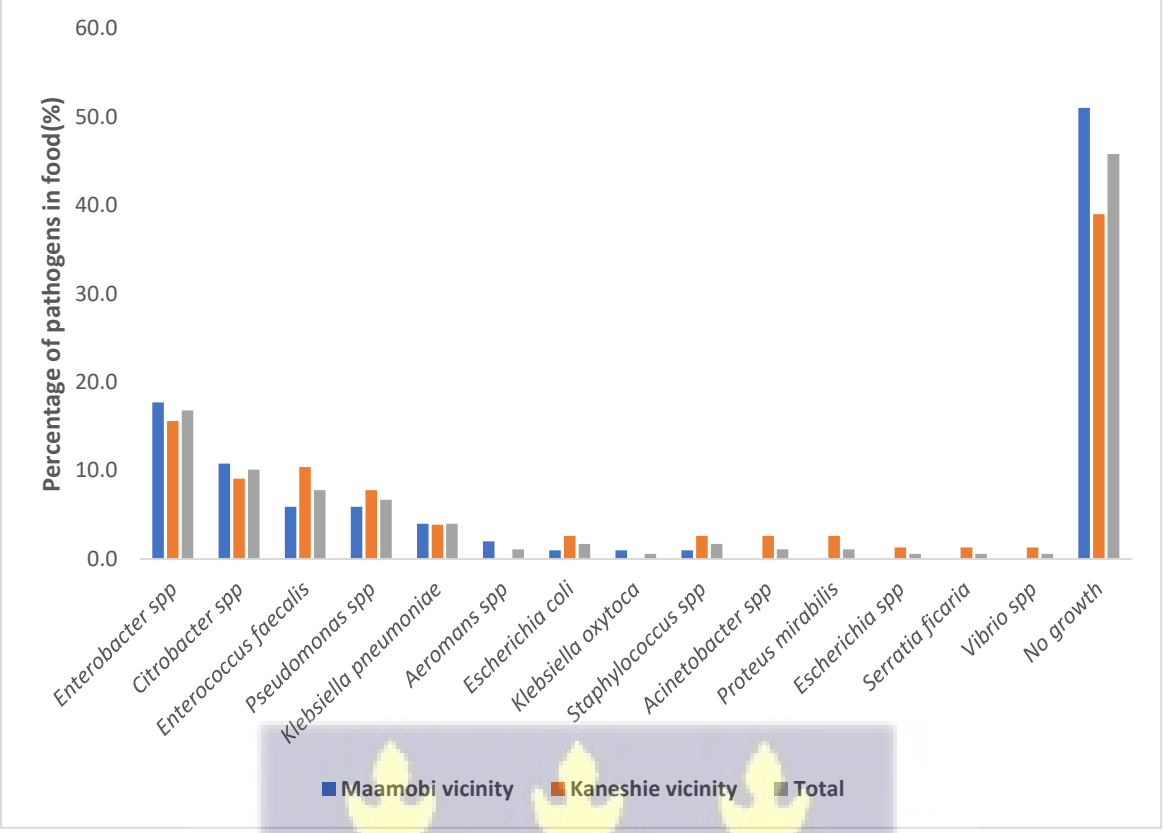


Figure 4.8: Isolated pathogens from food items from both vicinities

4.3.2 Occurrence of foodborne pathogens in food

Various FBPs were recovered from the food items in the study. *Enterobacter* spp. (16.8%) was the most common, followed by *Citrobacter* spp. (10.1%), *Enterococcus faecalis* (7.8%), *Pseudomonas* spp. (6.7%), and *Klebsiella pneumoniae* (4.0%). *Acinetobacter* spp., *Proteus mirabilis*, *Escherichia* spp., *Serratia ficaria* and *Vibrio* spp. were recovered only at Kaneshie vicinity and not Maamobi vicinity (Figure 4.8). Two *E. coli* isolates were recovered from a rice sample from the Kaneshie vicinity and porridge ('kooko') from the Maamobi vicinity.

4.3.3 Bacteria and the food items of isolation in Maamobi and Kaneshie vicinities

Five food items (beans, gari, kenkey, salad and soup) contained one bacterium each, while two food samples ('banku' and fried egg) had two bacteria (Table 4.1). Stews had the highest number of bacteria isolated (eleven pathogens) followed by Jollof, porridge, rice, waakye,

Indomie/ spaghetti and ground pepper. A total of 179 food items were collected from both study vicinities with 102 food items from Maamobi and 77 food items from Kaneshie.

Table 4.1 Isolated bacteria from food items in Maamobi and Kaneshie vicinities

Food item	**Bacteria isolated**
Ampesi	*Acinetobacter baumanii, Staphylococcus sciuri, Enterobacter asburiae*
Banku	*Enterobacter cloacae, Citrobacter freundii*
Beans	*Enterobacter cloacae*
Fried egg	*Enterobacter cloacae, Enterococcus faecalis*
Gari	*Enterobacter cloacae*
Ground pepper	*Aeromonas caviae, Citrobacter braakii, Enterobacter cloacae, Enterococcus faecalis*
Indomie/ spaghetti	*Acinetobacter baumannii, Citrobacter freundii* (ESBL-*TEM*), *Enterococcus faecalis, Pseudomonas aeruginosa*
Jollof	*Citrobacter freundii, Enterobacter cloacae, Enterococcus faecalis, Escherichia* spp., *Proteus mirabilis, Pseudomonas aeruginosa, Serratia ficaria, Pseudomonas mendoncina, Citrobacter braakii*
Kenkey	*Citrobacter freundii* (ESBL-*TEM*)
Porridge	*Citrobacter koseri, Enterobacter cloacae, Enterococcus faecalis, Escherichia coli* (ETEC-*Lt* gene), *Klebsiella pneumoniae, Pseudomonas aeruginosa, Pseudomonas plecoglossicida*
Rice	*Citrobacter freundii, Enterobacter cloacae, Enterococcus faecalis, Escherichia coli* (ETEC-*Lt* gene), *Staphylococcus epidermidis, Pseudomonas aeruginosa, Pseudomonas mendoncina*
Salad	*Proteus mirabilis*
Soup	*Enterobacter cloacae*
Stew	*Citrobacter freundii, Enterobacter cloacae, Enterococcus faecalis, Escherichia hermanii, Proteus mirabilis, Pseudomonas aeruginosa, Serratia ficaria, Pseudomonas mendoncina, Citrobacter braakii, Pseudomonas putida*
Waakye	*Citrobacter freundii, Enterobacter cloacae, Enterococcus faecalis, Pseudomonas aeruginosa, Staphylococcus* spp., *Vibrio fluvalis*

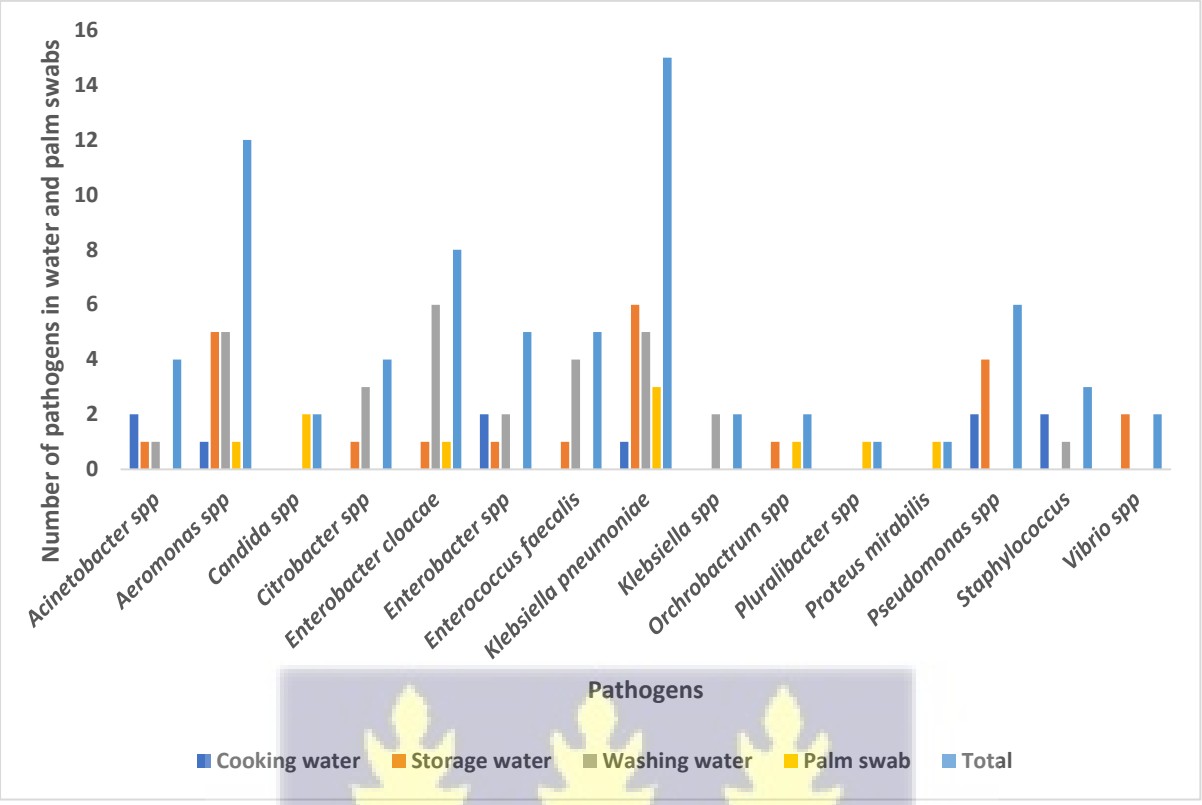


Figure 4.9: Isolated pathogens from water and palm swab samples

4.3.4 Occurrence of foodborne pathogens in the water and palm samples

A vast array of pathogens was found in the palm swabs as well as the different water samples. *Klebsiella pneumoniae* (20.8%) occurred most and frequently among all the sample types, followed by *Aeromonas* spp (16.7%) and *Enterobacter cloacae* (11.1%). Other organisms included *Acinetobacter* spp, *Candida* spp, *Enterobacter* spp, *Enterococcus faecalis*, *Pseudomonas* spp, *Vibrio* spp, *Proteus mirabilis*, *Orchrobactrum* spp, *Pluralibacter* spp and *Staphylococcus* spp, *Aeromonas* spp and *Klebsiella pneumoniae* were found in all four samples (Figure 4.9).

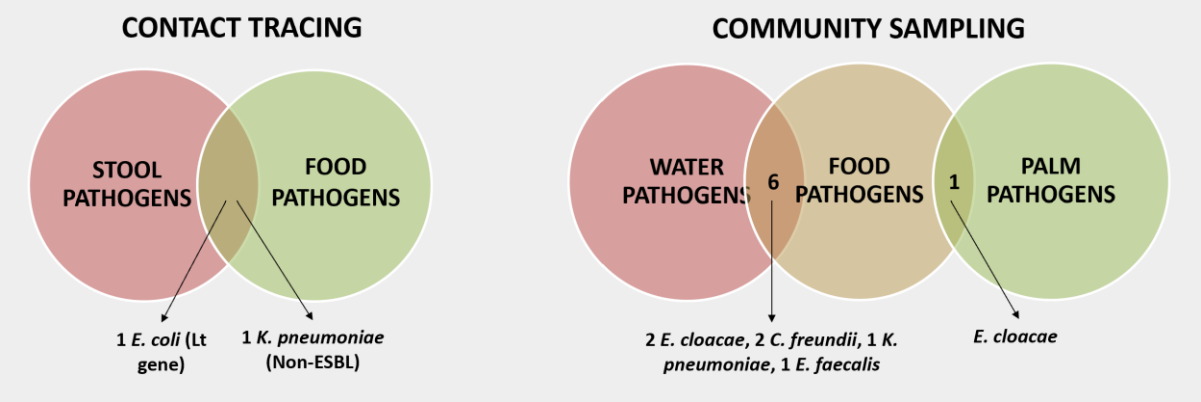


Figure 4.10: Common Pathogens

4.3.5 Common pathogens recovered during Contact tracing and Community sampling

During the first phase of sampling which involved contact tracing, 1 *E. coli* isolate with the *Lt* gene and *K. pneumoniae* were found in both stool and related food sample (Hausa kooko). During the community sampling, six pathogens consisting of 2 isolates of *E. cloacae* and *C. freundii*, 1 isolate of *K. pneumoniae* and *E. faecalis* were common between water and their respective food samples. One *E. cloacae* isolate was found in both food and the palm of the food vendor.

4.4 Results (Demographics, general information on sanitation, personal, and Risk factors)

The second section of this chapter focused on understanding the demographics, general information on sanitation, personal, and risk factor knowledge of contracting diarrhoea from the two groups of respondents in the study (patients and food vendors).

4.4.1 Patient Demographics

Over the study period of February 2019 to March 2020, a total of one hundred and twenty-two patients were enrolled at Maamobi General hospital and Kaneshie polyclinic with more females showing up in both facilities than males (67.2% ,32.8%) respectively. Patients below

18years formed part of minority (17.2%) of patients who were enrolled in the study at both sites where the study was undertaken. Summary of the enrolment by study site, gender, age, marital status, education, and ethnicity is illustrated in Table 4.2. Patients who were single showed the highest (59%) occurrence followed by patients who were married (36.9%) as compared to the other categories of marital status. However, majority of the patients enrolled reported being educated (80.3%) as compared to those who had no formal education (19.7%).

Table 4.2 Demographic information (N=122)

Characteristics	Total N(%)	Maamobi General Hospital n(%)	Kaneshie Polyclinic n(%)	*p*-value
Gender				
Male	40(32.8)	20(29.4)	20(37.0)	0.373
Female	82(67.2)	48(70.6)	34(63.0)	
Age				
Infant (<5 yrs)	12(9.8)	7(10.3)	5(9.3)	0.825
Children (5-17yrs)	9(7.4)	4(5.9)	5(9.3)	
Adult (≥18 yrs)	101(82.8)	57(83.8)	44(81.5)	
Marital Status				
Single	72(59.0)	39(57.4)	33(61.1)	
Married	45(36.9)	28(41.2)	17(31.5)	
Divorced	1(0.8)	0(0.0)	1(1.9)	0.094
Widowed	3(2.46)	0(0.0)	3(5.6)	
Separated	1(0.8)	1(1.5)	0(0.0)	
Education				
No School	24(19.7)	15(22.1)	9(16.7)	
Primary	23(18.9)	14(20.6)	9(16.7)	
JHS	26(21.3)	14(20.6)	12(22.2)	0.223
SHS	26(21.3)	17(25.0)	9(16.7)	
Tertiary	23(18.9)	8(11.8)	15(27.8)	
Ethnicity				
Akan	33(27.1)	1(1.5)	32(59.3)	
Ewe	35(28.7)	32(47.1)	3(5.6)	
Ga/ Dangme	15(12.3)	5(7.4)	10(18.5)	<0.001
Northner	38(31.2)	29(42.7)	9(16.7)	
Nzema	1(0.8)	1(1.5)	0(0.0)	

4.4.2 Clinical Presentation of patients

Majority of patients (89.9%) reporting to both Maamobi General hospital and Kaneshie polyclinic reported having temperatures that were less than 38°C as compared to patients having temperatures greater or equal to 38°C (13.1%). More than half of patients (24%) suffering from diarrhoea who had temperatures of ≥38°C were from Kaneshie polyclinic. Majority of patients reported passing stool more than three times within 24 hours with a greater

occurrence at Kaneshie polyclinic (92.6%) as compared to Maamobi General hospital (85.3%). Majority of stool samples collected from both sites were liquid/ loose with majority coming from Maamobi General hospital (68;100%). There were more cases of traces of blood/mucous in the stool of patients from Kaneshie polyclinic (61.1%) than Maamobi General hospital (27.9%) during the study (Table 4.3).

Table 4.3 Clinical Presentation of patients

Characteristics	Total N(%)	Maamobi General Hospital n(%)	Kaneshie Polyclinic n(%)	*p*-value
Clinical Presentation				
Temperature				
<38°C	106(89.9)	65(95.6)	41(75.9)	0.001
≥38°C	16(13.1)	3(4.4)	13(24.0)	
Times passed stool within 24hours				
2-3 times	14(11.5)	10(14.7)	4(7.4)	0.209
More than 3 times	108(88.5)	58(85.3)	50(92.6)	
Stool form				
Liquid/Loose	120(98.4)	68(100.0)	52(96.3)	0.11
Soft/greasy	2(1.6)	0(0.0)	2(3.7)	
Traces of Blood/mucous				
Yes	52(42.6)	19(27.9)	33(61.1)	<0.001
No	70(57.4)	49(72.1)	21(38.9)	

4.4.3 Presenting symptoms of patients

Patients who admitted to having fever (44%) during diarrhoea, headache (50%), flatulence (16.7%), abdominal pain/ cramps (72.2%), bloating (25.9%), nausea (68.5%), vomiting (61.1%) and fatigue (57.4%) were more in Kaneshie Polyclinic as compared to Maamobi General hospital, except the urgency to go to toilet which had majority of the cases (83.8%) coming from Maamobi General hospital (Table 4.4). Also, among the various symptoms presented by the patients during the study, apart from the frequency of passing stool, its urgency, form, and the days of onset of various symptoms, all the other symptoms had *p*- values (<0.05) that were significant. This implies that having traces of blood/mucous, fever, flatulence, headache, abdominal pain/cramps, bloating, nausea, vomiting, and fatigue are clinical presentations that have a significant association with a patient with diarrhoea.

More than half of the patients enrolled in the study, reported having symptoms lasting less than 24 hours or within a day.

Table 4.4 Presenting symptoms of patients

Characteristics	Total N(%)	Maamobi General Hospital n(%)	Kaneshie Polyclinic n(%)	*p*-value
Presenting Symptoms				
Fever during illness				
Yes	25(20.5)	1(1.5)	24(44.4)	
No	97(79.5)	67(99.0)	30(55.6)	<0.001
Urgency to go to toilet				
Yes	105(86.1)	57(83.8)	48(88.9)	
No	17(14.0)	11(16.2)	6(11.1)	0.422
Headache				
Yes	36(29.5)	9(13.2)	27(50.0)	
No	86(70.5)	59(86.8)	27(50.0)	<0.001
Flatulence				
Yes	10(8.2)	1(1.5)	9(16.7)	
No	112(91.8)	67(98.5)	45(83.3)	0.002
Abdominal pain/cramps				
Yes	54(44.3)	15(22.1)	39(72.2)	
No	68(55.7)	53(78.0)	15(27.8)	<0.001
Bloating				
Yes	16(13.1)	2(2.9)	14(25.9)	
No	106(86.9)	66(97.06)	40(74.1)	<0.001
Nausea				
Yes	49(40.16)	12(17.7)	37(68.5)	
No	73(60.0)	56(82.4)	17(31.5)	<0.001
Vomiting				
Yes	56(45.9)	23(23.8)	33(61.1)	
No	66(54.1)	45(66.2)	21(38.9)	0.003
Fatigue				
Yes	34(27.9)	3(4.4)	31(57.4)	
No	88(72.1)	65(96.0)	23(42.6)	<0.001
Days had symptoms				
<1day	20(16.4)	9(13.2)	11(20.4)	
1day	45(36.9)	27(39.7)	18(33.3)	
2days	17(13.9)	13(19.1)	4(7.4)	0.062
3days	21(17.2)	13(19.1)	8(14.8)	
>3days	19(15.6)	6(8.8)	13(24.1)	

4.4.4 Behavioral traits of diarrhoea patients

One of the basic behavioral traits prior to the onset of diarrhoea was whether the patients had eaten or drank. Out of the total of 122 responses, over 95% admitted having eaten or drank before the onset of the illness with more than 90% of such cases occurring in both hospital facilities. The source of drinking water for almost all the patients enrolled (97.6%) was sachet water and over ninety percent responding to washing their hands with soap and water. More than half of the participants took medications upon the onset of diarrhoea prior to attending the hospitals. Regarding the destination of waste disposal by patients enrolled during the study, many of them reported the use of dustbins as compared to vehicles, pits, lagoon, or river or burning of the waste. Over 75% of patients described the general sanitation conditions of their source of food consumption to be tidy (Table 4.5).

4.4.5 Patient's knowledge about the causes of diarrhoea

Patients' knowledge about the causes of diarrhoea were investigated and over 50% responded germs to be the cause as compared to the other options of dirty food, water and hands that were given. Table 4.5 and Table 4.6 show a detailed account of the behavioral traits and the knowledge of patients about the cause of diarrhoea.

Table 4.5 Behavioral traits about the cause of diarrhoea

Characteristics	Total N(%)	Maamobi General Hospital n(%)	Kaneshie Polyclinic n(%)	*p*-value
Behavioral traits				
Ate or drank before illness				
Yes	116(95.9)	63(94.0)	53(98.2)	
No	5(4.1)	4(6.0)	1(1.9)	0.379
Source of drinking water				
Sachet water	119(97.6)	65(95.6)	54(100.0)	
Tap	3(2.5)	3(4.4)	0(0.0)	0.254
Taking medication upon onset of illness				
Yes	65(53.3)	33(48.5)	32(59.3)	
No	57(46.7)	35(51.5)	22(40.74)	0.238
Wash hands with soap and water				
Yes	110(90.2)	65(95.6)	45(83.3)	
No	12(9.8)	3(4.4)	9(16.7)	0.024
Destination of waste disposal Vehicle				
Yes	10(8.2)	8(11.8)	2(3.7)	
No	112(91.8)	60(88.2)	52(96.3)	0.107
Pit				
Yes	3(2.5)	2(3.0)	1(1.9)	
No	119(97.5)	66(97.1)	53(98.2)	0.7
Lagoon or river				
Yes	1(0.8)	1(1.5)	0(0.0)	
No	121(99.2)	67(98.5)	54(100.0)	0.371
Burning				
Yes	27(22.1)	23(33.8)	4(7.4)	
No	95(77.9)	45(66.2)	50(92.6)	<0.001
Dustbin				
Yes	98(80.3)	60(88.2)	38(70.4)	
No	24(19.7)	8(11.8)	16(30.0)	0.014
Sanitation of source of food				
Very tidy	19(15.6)	12(17.7)	7(13.0)	
Tidy	88(72.1)	43(63.2)	45(83.33)	0.017
Not tidy	15(12.3)	13(19.12)	2(3.7)	

Table 4.6 Patients' knowledge about the cause of diarrhoea

Characteristics	Total N(%)	Maamobi General Hospital n(%)	Kaneshie Polyclinic n(%)	*p*-value
Knowledge about the cause of diarrhoea				
Dirty food				
Yes	40(32.8)	26(38.2)	14(25.9)	0.15
No	82(67.2)	42(61.8)	40(74.1)	
Dirty water				
Yes	24(19.7)	21(30.9)	3(5.6)	<0.001
No	98(80.3)	47(69.1)	51(94.4)	
Germs				
Yes	78(63.9)	54(79.4)	24(44.4)	<0.001
No	44(36.1)	14(20.6)	30(55.6)	
Dirty hands				
Yes	18(14.8)	14(20.6)	4(7.4)	0.041
No	104(85.3)	54(79.4)	50(92.6)	

4.4.6 Food vendor demographic information (tracing)

Patients who could attribute the cause of their diarrhoea to the food they ate from vending sites were made to provide directions to the food vending sites during consenting and the administration of the questionnaire. During the sample collection of the 122 stool samples, only 25 food vendors were traced based on the patients' information for purposive sampling of food. Food vending gender consisted of solely females with more than 50% above the age of 40 years and married. Majority (92%) of the food vendors had received formal education ranging from Primary, Junior High School (JHS), Senior High School (SHS) and tertiary (Table 4.7).

Table 4.7 Food vendor demographic information (tracing)

Characteristics	Total N(%)	Maamobi Vicinity n(%)	Kaneshie Vicinity n(%)
Gender			
Male	0(0)	0(0)	0(0)
Female	25(100.00)	14(100.00)	11(100.00)
Age			
>30 years	6(24.00)	2(14.29)	4(36.36)
31-39 years	6(24.00)	6(42.86)	0(0.00)
≥40 years	13(52.00)	6(42.86)	7(63.64)
Marital Status			
Single	7(28.00)	2(14.29)	5(45.45)
Married	14(56.00)	11(78.57)	3(27.27)
Divorced	1(4.00)	0(0.00)	1(9.09)
Widowed	2(8.00)	0(0.00)	2(18.18)
Separated	1(4.00)	1(7.14)	0.00)
Education			
No School	2(8.00)	1(7.14)	1(9.09)
Primary	8(32.00)	7(50.00)	1(9.09)
JHS	7(28.00)	2(14.29)	1(9.09)
SHS	7(28.00)	3(21.43)	4(36.36)
Tertiary	1(4.00)	1(7.14)	0(0.00)

4.4.7 General information on sanitation, personal hygiene, risk factors

All food vendors had access to pipe water with majority (80%) having access to sanitation at the vending sites. All food vendors responded in the affirmative to washing their hands with soap and water. Over 70% of hand wash with soap and water was highly practiced after toilet use and after touching dirty things, although the practice was below 50% after sneezing (Table 4.8.1). Heavy littering and stagnant water were not observed at the vending sites. However, more than half of the food vendors wash and cook vegetables at the vending sites. Almost all ingredients for the preparation of food were purchased from the market with fish being the most frequently used (64%) as compared to chicken, cow, and goat. Food vendor knowledge on the causes of diarrhoea showed over 50% of the responses attributed to germs and dirty

food (Table 4.8.2). Finally, the use of dustbins in the disposal of waste was high (60%) against the other destinations; vehicle, pit, lagoon and burning (Table 4.8.3).

Table 4.8 General information on sanitation, personal hygiene, risk factors

Characteristics	Total n(%)	Maamobi Vicinity n(%)	Kaneshie Vicinity n(%)
Source of water for food preparation			
Pipe	25(0.00)	14(100.00)	11(100.00)
Access to sanitation at facilities			
Yes	20(80.00)	13(92.86)	7(63.64)
No	5(20.00)	1(7.14)	4(36.36)
Wash hands with soap and water			
Yes	25(100.00)	14(100.00)	11(100.00)
After toilet use			
Yes	21(84.00)	11(78.57)	10(90.91)
No	4(16.00)	3(21.43)	1(9.09)
After sneezing			
Yes	12(48.00)	10(71.43)	2(18.18)
No	13(52.00)	4(28.57)	9(81.82)
After touching dirty things			
Yes	18(72.00)	11(78.57)	7(63.64)
No	7(28.00)	3(21.43)	4(36.36)
Littered vicinity			
Heavily littered			
No	25(100.00)	14(100.00)	11(100.00)
Some litter			
Yes	19(76.00)	11(78.57)	8(72.73)
No	6(24.00)	3(21.43)	3(27.27)
No litter			
Yes	5(25.00)	2(14.29)	3(27.27)
No	20(80.00)	12(85.00)	8(72.73)
Flies present at vicinity			
Yes	3(12.00)	2(14.29)	1(9.09)
No	22(88.00)	12(85.71)	10(90.91)
Stagnant water at vicinity			
No	25(100.00)	14(100.00)	11(100.00)
Cook vegetables at vicinity			
Yes	13(52.00)	7(50.00)	6(54.55)
No	12(48.00)	7(50.00)	5(45.45)
Wash before cooking at vicinity			
Yes	16(64.00)	8(57.14)	8(72.73)
No	9(36.00)	6(42.86)	3(27.27)

Table 4.9 General information on sanitation, personal hygiene, risk factors

Characteristics	Total N(%)	Maamobi vicinity n(%)	Kaneshie vicinity n(%)
Where ingredients are purchased before cooking			
Market			
Yes	24(96.00)	13(92.86)	11(100.00)
No	1(4.00)	1(7.14)	0(0.00)
Slaughterhouse			
No	25(100.00)	14(100.00)	11(100.00)
Street hawking			
Yes	1(4.00)	1(7.14)	0(0.00)
No	24(96.00)	13(92.86)	11(100.00)
Supermarket			
Yes	1(4.00)	1(7.14)	0(0.00)
No	24(96.00)	13(92.86)	11(100.00)
Type of meat/fish used in cooking			
Goat			
Yes	2(8.00)	1(7.14)	1(9.09)
No	23(92.00)	13(92.86)	10(90.91)
Cow			
Yes	6(24.00)	1(7.14)	5(45.45)
No	19(76.00)	13(92.86)	6(54.55)
Chicken			
Yes	10(40.00)	4(28.57)	6(54.55)
No	15(60.00)	10(71.43)	5(45.45)
Fish			
Yes	16(64.00)	8(57.14)	8(72.73)
No	9(36.00)	6(42.86)	3(27.27)
Vendor knowledge on causes of diarrhoea			
Dirty food			
Yes	13(52.00)	7(50.00)	6(54.00)
No	12(48.00)	7(50.00)	5(45.45)
Dirty water			
Yes	9(36.00)	6(42.86)	3(27.27)
No	16(64.00)	8(57.14)	8(72.73)
Germs			
Yes	16(64.00)	9(64.29)	7(63.64)
No	9(36.00)	5(35.71)	4(36.36)
Dirty hands			
Yes	4(16.00)	2(14.29)	2(18.18)
No	21(84.00)	12(85.71)	9(81.82)

Table 4.10 General information on sanitation, personal hygiene, risk factors

Characteristics	Total N(%)	Maamobi vicinity n(%)	Kaneshie vicinity n(%)
Destination of waste disposal at business center			
Vehicle			
Yes	3(12.00)	2(14.29)	1(9.09)
No	22(88.00)	12(85.71)	10(90.91)
Pit			
Yes	1(4.00)	0(0.00)	1(9.09)
No	24(96.00)	14(100.00)	10(90.91)
Lagoon			
No	25(100.00)	14(100.00)	11(100.00)
Burning			
Yes	5(20.00)	4(28.57)	1(9.09)
No	20(80.00)	10(71.43)	10(90.91)
Dustbin			
Yes	15(60.00)	11(78.57)	4(36.36)
No	10(40.00)	3(21.43)	7(63.64)

4.4.8 Food vendor demographic information (community)

During the study, there was difficulty in tracing the source of food due to various reasons. Some of the reasons included difficulty in locating the food vending sites because of non-precision regarding directions, lack of consent from the food vendors, unavailability of food samples upon locating the vending site and mobile vending/ street hawking.

In acquiring more samples, community sampling of food from food vendors near the Maamobi General hospital and Kaneshie polyclinics was adopted. A total of 65 food vendors consented to being part of the study. Only one male from the Kaneshie vicinity consented to the study with over 50% of the food vendors being below 40 years. Over 50% of the vendors were married with majority of the vendors who reported to have formal education ranging from Primary, Juniour High School (JHS), Senior High School (SHS) and tertiary. Food vendor

demographic information for the community sampling of the study is shown in Table 4.9 below.

Table 4.11 Food vendor demographic information (community)

Characteristics	Total N(%)	Maamobi Vicinity n(%)	Kaneshie Vicinity n(%)
Gender			
Male	1(1.54)	0(0.00)	1(3.70)
Female	64(98.46)	38(100.00)	26(96.30)
Age			
>30 years	23(35.38)	18(47.37)	5(18.52)
31-39 years	14(21.54)	10(26.32)	4(14.81)
≥40 years	28(43.08)	10(26.32)	18(66.67)
Marital Status			
Single	26(40.00)	16(42.11)	10(37.04)
Married	35(53.85)	22(57.89)	13(48.15)
Divorced	1(1.54)	0(0.00)	1(3.70)
Widowed	3(4.62)	0(0.00)	3(11.11)
Education			
No School	4(6.15)	2(5.26)	2(7.41)
Primary	11(16.92)	7(18.42)	4(14.81)
JHS	32(49.23)	19(50.00)	13(48.15)
SHS	14(21.54)	9(23.68)	5(18.52)
Tertiary	4(6.15)	1(2.63)	3(11.11)

4.4.9 General information on sanitation, personal hygiene, risk factors

All food vendors had access to pipe water with majority (80%) having access to sanitation at the vending sites. Over 80% of the food vendors responded in the affirmative to washing their hands with soap and water. Regarding the preparation of food, over 70% was prepared by other people and not the vendors. Majority (over 85%) of the vendors confirmed to have undergone medical examination. Preparation of food was equally carried out at home or at the vending sites. Transportation of food stuff to where food was prepared was mostly carried out by carrying on the head followed by an automobile/ car depending on the proximity of the cooking sites (Table 4.10.1). Regarding the food operators' consideration when choosing foodstuff for cooking, majority of them affirmed quantity to be primary, followed by cost (affordability) before quality. Food operators' preferred buying foodstuff from their respective vicinities' markets (Nima or Kaneshie) as compared to other popular markets. More than half of the operators cook vegetables during food preparation as well as admitted washing them with either plain water or salty water before use. A high percentage of the operators purchase ingredients from the markets for cooking as compared to the farms. Source of meat for cooking was normally either from the open market or cold store with over 60% of respondents admitting using food additives and condiments. Storage of cooked food in saucepans were most resorted to (over 40%) during food vending. Regarding the frequency of washing eating plates, majority (over 80%) claimed they did so after each use while the rest did so either at the beginning of sale or at the end of the day (Table 4.10.2)

Table 4.12 Food vendor responses on sanitation, personal hygiene, and risk factors

Characteristics	Total N(%)	Maamobi Vicinity n(%)	Kaneshie Vicinity n(%)
Source of water for food preparation			
Pipe	65(100.00)	38(100.00)	27(100.00)
Access to sanitation at facilities			
Yes	52(80.00)	37(97.37)	15(55.56)
No	13(20.00)	1(2.63)	12(44.44)
Wash hands with soap and water			
Yes	52(80.00)	34(89.47)	18(66.67)
No	13(20.00)	4(10.53)	9(33.33)
Person who prepares food for sale			
Another	46(70.77)	22(57.89)	24(88.89)
Self	23(35.38)	17(44.74)	6(22.22)
Vendor medically examined			
Yes	57(87.69)	32(84.21)	25(92.59)
No	8(12.31)	6(15.79)	2(7.41)
Where food is cooked			
At home	32(49.23)	19(50.00)	13(48.15)
At selling place	33(50.77)	19(50.00)	14(51.85)
Transportation of food stuff/ food to cooking sites			
At the spot	11(18.33)	9(27.27)	2(7.41)
Car	20(33.33)	7(21.21)	13(48.15)
Carriage truck	3(5.00)	0(0.00)	3(11.11)
Carrying on head	26(43.33)	17(51.52)	9(33.33)

Table 4.13 Food vendor general information on sanitation and risk factors

Characteristics	Total N(%)	Maamobi Vicinity n(%)	Kaneshie Vicinity n(%)
Consideration when choosing foodstuff			
Quality	25(38.46)	5(13.16)	20(74.07)
Cost	26(40.00)	10(26.32)	16(59.26)
Quantity	27(41.54)	5(13.16)	22(81.48)
Where foodstuff is bought			
Accra	4(6.15)	4(10.53)	0(0.00)
Agbogbloshie	7(10.77)	0(0.00)	7(25.93)
CMB-Accra	2(3.08)	0(0.00)	2(7.41)
Kaneshie	15(23.08)	0(0.00)	15(55.56)
Nima	28(43.08)	26(68.42)	2(7.41)
Maamobi	8(12.31)	8(21.05)	0(0.00)
Cook vegetables			
Yes	39(60.00)	21(55.26)	18(66.67)
No	26(40.00)	17(44.74)	9(33.33)
Treatment of vegetables before use			
Wash with salty water	20(30.77)	10(26.32)	10(37.04)
Wash with water	20(30.77)	11(28.95)	9(33.33)
Wash with vinegar	3(4.62)	1(2.63)	2(7.41)
Where ingredients are purchased for cooking			
Market	59(90.77)	32(84.21)	27(100.00)
Farms	3(4.62)	1(2.63)	2(7.41)
Source of meat for cooking			
Open market	20(30.77)	4(10.53)	16(59.26)
Abattoir	2(3.08)	1(2.63)	1(3.70)
Cold store	24(36.92)	17(44.74)	7(25.93)
Usage of food additives and condiments			
Yes	42(64.62)	18(47.37)	24(88.89)
No	23(35.38)	20(52.63)	3(11.11)
Place for storing cooked food			
Ice Chest	14(21.54)	8(21.05)	6(22.22)
Saucepan	29(44.62)	19(50.00)	10(37.04)
Plain rubber sack	16(24.62)	5(13.16)	11(40.74)
Sieve	11(16.92)	9(23.68)	2(7.41)
Frequency of washing eating plates			
After each use	45(83.33)	25(78.13)	20(90.91)
At the beginning of sale	2(3.70)	1(3.13)	1(4.55)
At the end of the day	7(12.96)	6(18.75)	1(4.55)

4.5 Discussion (Occurrence of foodborne pathogens)

4.5.1 Specific foodborne pathogens recovered from stool

Although less frequently sort out for in most enteric studies, *E. coli* is the most common isolated bacteria pathogen in Africa (Okeke, 2009) due to being a normal and most abundant gastrointestinal flora (Akhtar & Sutjita, 2006). Generally, apart from *K. pneumoniae* which is also common, several studies that have been conducted in Africa have ascertained this phenomenon (Diriba, Awulachew, Tekele, & Ashuro, 2020; Ouchar Mahamat et al., 2019). It was not surprising that several enteric studies that have been conducted in Ghana have also shown similar outcomes (Akuffo et al., 2017; Duedu et al., 2017; Opintan et al., 2010; Reither et al., 2007). In a 6-month nationwide surveillance conducted to investigate AMR and to identify the possible gaps that could prevent future collaboration and sharing of data locally and nationally at Korle-bu Teaching hospital and Kwame Nkrumah University of Science and Technology, it was observed that 27.53% of the bacteria isolates were *E. coli* (Opintan et al., 2015).

4.5.2 Specific foodborne pathogens recovered from food

4.5.2.1 *Enterobacter cloacae*

Members of the Enterobacteriaceae family have been implicated both nationally and internationally as the most recurrent group to cause foodborne outbreaks (Banik et al., 2019; Nyenje et al., 2012). They include *Enterobacter* spp, *Citrobacter* spp, *Klebsiella* spp, *Aeromonas* spp, *Escherichia* spp, among others. Of the groups mentioned, *Enterobacter* spp, specifically, *E. cloacae* is the most common fecal-orally transmitted pathogen in food and simultaneously, is the sixth most common cause of nosocomial infections in health care settings with clinical symptoms such as intravascular device-associated infections, surgical site

infections, UTIs, bacteremia and lower respiratory tract infections (Ramirez & Giron, 2020). Their presence in the RTEs could pose serious health risks and further medical implications; especially among consumers with underlying conditions.

Enterobacter cloacae being the most common pathogen, was recovered in almost all the RTE food types, especially in stew and waakye during the study. This is similar to the findings of Nyenje and colleagues in some selected roadside cafeterias and retail outlets in Alice, South Africa who isolated 24% of *E. cloacae* from beef stew (Nyenje et al., 2012). Several street vended food studies that have been carried out in Ghana and other African countries have reported *Enterobacter cloacae* as the most common pathogen to be recovered (Eromo et al., 2016; Mensah et al., 2002; Yeboah-Manu et al., 2010; Yeleliere et al., 2017). Lack of proper personal hygiene has been the major problem among street food vending for several years all over the world (Rane, 2011).

4.5.2.2 *Citrobacter freundii*

Stews recorded the highest number of *Citrobacter freundii* occurrence and was second to *Enterobacter cloacae*. Several street food studies in Ghana have recorded the presence of *C. freundii* in many RTEs which is consistent with this study (Mensah et al., 2002; Saba & Gonzalez-Zorn, 2012; Yeleliere et al., 2017). They are commensal inhabitants of the human and animal intestinal tracts with acquired virulence properties that can cause food poisoning, hence leading to diarrhoea in humans when transmitted (Liu et al., 2017). In view of this, their presence in eight different food items (banku, Indomie/ spaghetti, jollof, kenkey, porridge, rice, stew, and waakye) is an indication of lack of proper personal hygiene by the vendors which requires some form of education, monitoring and control by the required regulatory authorities.

C. freundii is known to be an emerging opportunistic pathogen to be involved in causing several nosocomial infections like UTIs, gastrointestinal infections, septicemia, meningitis, and wound infections, mostly among immunocompromised patients (Liu et al., 2017).

4.5.2.3 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa, an opportunistic pathogen, able to survive in different environments, is normally associated with nosocomial infections, pneumonia and high mortality rate among Cystic fibrosis patients as well as in people who are immunocompromised (Pang, Raudonis, Glick, Lin, & Cheng, 2019). Their presence in RTEs is of considerable concern which could be due to lack of proper personal hygiene or general sanitation of the vending sites leading to diarrhoea. They have also been isolated in RTE food among various street vending studies in Ghana, with the finding of being a precursor to food spoilage, especially when kept at ambient temperature for a long time, causing an increase of the bacteria load (Mensah et al., 2002; Saba & Gonzalez-Zorn, 2012; Yeboah-Manu et al., 2010; Yeleliere et al., 2017).

4.5.2.4 *Proteus mirabilis*

P. mirabilis, a common opportunistic pathogen which is normally associated with food poisoning outbreaks was isolated from salad and stew. They are normally found in water, soil, and the intestines of humans, implying their presence in food to be of a public health risk due to the ability to cause diarrhoea when consumed. They have been implicated to cause several food poisoning episodes in several countries like China, United Kingdom, Turkey and other parts of the world (Gong et al., 2019). Their putrefaction ability in protein-related foods like meat, which leads to food spoilage have implicated them to be associated to food spoilage, especially in dairy products and cold dishes (Gong et al., 2019; Nyenje et al., 2012).

Rapid spoilage of food occurs because of their ability to swarm and other virulence factors like type IV secretion system (T4SS), causing rapid spread and multiplication. Diarrhoea occurs as a result of this food poisoning incident with other clinical symptoms such as abdominal pain, nausea and dizziness (Gong et al., 2019).

Several food spoilage and poisoning incidents in Ghana and other countries based on the consumption of RTEs like meat, vegetables and seafood have been related to *P. mirabilis* (Gong et al., 2019; Nyenje et al., 2012; Yeboah-Manu et al., 2010). However, their presence in salad poses a health risk to consumers since salads are eaten cold and may not be subjected to heating prior to consumption. Rapid deterioration and spoilage can occur due to the presence of this pathogen in the stew as well since most vendors sell them for prolonged hours at ambient temperature and hardly reheat stews onsite (Mensah et al., 2002). Lack of proper hand washing by vendors could also result in the transmission of *P. mirabilis* as some studies have shown their presence in the hands of vendors and money used in purchasing (Anning et al., 2019; Yar, 2020).

4.5.2.5 *Escherichia coli*

E. coli, a bacterium which is normally present in the gastrointestinal tract of human and warm-blooded animals can also survive outside its natural habitat and it is therefore considered a clear indication of fecal contamination when it occurs in either food or water. In view of that, their presence in food (rice and 'hausa kooko-porridge) could be attributed to contaminated raw materials used in food preparation, unhygienic conditions in food preparation environments and the presence of other enteric pathogens (Lima et al., 2017). *E. coli* in rice and Hausa kooko was an indication of fecal contamination and lack of proper hand hygiene by the food vendors.

4.5.2.6 *Klebsiella pneumoniae*

Klebsiella pneumoniae, an opportunistic gram-negative bacterium which is found in the soil, gastrointestinal tract, faeces and skin of humans can also sometimes be in food (Hartantyo et al., 2020). It is one of the most common FBPs recovered in RTE food items in Ghana (Abakari et al., 2018; Anning et al., 2019; Mensah et al., 2002; Tellevik et al., 2016; Yeboah-Manu et al., 2010) and other parts of the world (Hartantyo et al., 2020; Hassan et al., 2018; Nyenje et

al., 2012; O'Neill, 2016; Rane, 2011). In view of this, their presence in most of the RTE food is an indication of a possible fecal contamination which could be attributed to post preparation conditions such as improper handling, lack of personal hygiene and other sanitation factors.

4.5.2.7 *Acinetobacter baumannii*

Acinetobacter spp are opportunistic gram-negative bacilli, normally non-motile and commonly found in the environment like soil and water. They are attributed to nosocomial spread and cause infections in the blood, wounds, lungs (pneumonia) and urinary tract (UTIs) among humans (O'Neill, 2015). Since they can survive for long durations on environmental surfaces, they are able to spread from one contaminated object/infected person to another, normally through contaminated hands. No wonder *A. baumannii* was found in the hands of street food vendors in Dhaka city in Bangladesh (Hassan et al., 2018). In this study, the pathogen was found in two RTE food items ('Ampesi'-boiled plantain and spaghetti) which indicates fecal contamination, possibly via the vendors' hands. This is because these meals are prepared at boiling temperature but during serving, some vendors use their bare hands which predisposes the meals to getting contaminated with this bacteria (Mensah et al., 2002).

4.5.2.8 *Aeromonas* spp

Aeromonas spp are ubiquitous waterborne bacteria which are normally isolated from water but gradually, is being isolated from food such as vegetables, meat, fish and other processed food. They are able to grow in high salt concentrations (pH 4-10) and release exotoxins which enable them to thrive in low temperatures as well (O'Neill, 2015). Based on their properties, they pose a serious public health threat, especially in the RTE food industry.

In this study, *Aeromonas spp* was found in ground pepper which is a common RTE which serves as an accompaniment for majority of foods like 'kenkey', 'banku', akpler, rice and many others. It is normally prepared after washing fresh organic vegetables like tomatoes, onions and pepper in salt water and ground in earthenware pots or in blenders. It is not surprising that

Aeromonas spp was recovered in this RTE since the contamination is likely to have been from the vegetables that were used in its preparation despite the use of salt water that is normally used by most food operators to wash vegetables before grinding. This is similar to findings from Northern Ireland where *Aeromonas* spp was the only bacteria that was found in RTE vegetables after undergoing some minimal processing (washing) before retailing (McMahon & Wilson, 2001). This can cause food poisoning after consumption which can cause gastroenteritis as well.

4.5.2.9 *Staphylococcus* spp

Staphylococcus spp are gram-positive cocci facultative aerobes and anaerobes, found in many natural habitats such as the skin of humans, mammals and birds, upper respiratory tracts of infected hosts, blood, mouth, mammary glands, genitourinary and intestinal tracts of infected hosts (Bintsis, 2017). Their presence in RTE food could lead to food poisoning through inadequate cooking or heating, inadequate refrigeration of food or poor personal hygiene through contact from the nose, mouth, clothing or skin of food vendors (Eromo et al., 2016).

Although *S. aureus* to be precise was not found in the food samples in the study, *S. sciuri* and *S. epidermidis* were recorded. Other RTE food studies in Ghana have reported the occurrence of *S. aureus* in food (Abas et al., 2019; Mensah et al., 2002; Yeboah-Manu et al., 2010; Yeleliere et al., 2017), the hands of food vendors and different denominations of money used in business transaction (Anning et al., 2019; Hassan et al., 2018). This indicates that foods that are frequently handled during preparation and serving could be prone to being contaminated with *Staphylococcus* spp. (Nyenje et al., 2012).

4.5.2.10 *Enterococcus faecalis*

Enterococci are ubiquitous bacteria that can be found in both the human and animal gastrointestinal tract. They can also be found in soil and sometimes in crops and water by way of transfer through faecal contamination by either humans (sewage) or animal. Their presence

in food (cheese, vegetables, poultry and pork) and water could lead to gastroenteritis in humans when transmitted (Pesavento et al., 2014). It was the third most abundant bacteria (7.8%) after *Enterobacter* spp and *Citrobacter spp* to be recovered in six different types of food items (fried egg, ground pepper, porridge, rice, stew, 'waakye') which is inconsistent with several RTE studies in Ghana, apart from Yeboah-Manu et al.'s work that was conducted on and around University of Ghana campus, Legon (Yeboah-Manu et al., 2010).

4.5.2.11 Other organisms (*Serratia* spp and *Vibrio fluvialis*)

Serratia spp is a gram-negative facultative anaerobe and an opportunistic pathogen which has been reported to cause many hospital-acquired infections (HAIs) like UTIs, catheter-associated bacteremia and wound infections. They can also be found in the gastrointestinal systems of children and thrive abundantly on damp surfaces like bathroom tiles and shower corners in the home (Bintsis, 2017). *Serratia* spp used to be considered as a harmless pathogen to the gastrointestinal tract but it is gradually changing and has been implicated to cause foodborne infections when isolated in food. In this study, it was found in stew. It normally shows as a red pigmentation, commonly in dairy foods and can occur concurrently with other FBPs like *Salmonella enterica*, *E. coli* and *Acinetobacter* spp.

Vibrio fluvialis on the other hand was isolated from 'waakye' from the Kaneshie vicinity with no resistance to all the antibiotics. They are halophilic gram-negative bacteria, mostly occurring in the aquatic environment and currently recognized as an emerging FBP due to its deceptive microbiological resemblance to *V. cholerae* and *Aeromonas* spp, widespread presence (clinic and environment) and their unknown virulence factors (Ramamurthy, Chowdhury, Pazhani, & Shinoda, 2014). Apart from various diarrhoea cases recorded as a result of raw sea food contaminated with *V. fluvialis*, it has been known to cause a variety of infections like biliary tract infection, bacteremia and acute diarrhoea among immune-

competent HIV patients (Ramamurthy et al., 2014). The presence of this bacterium in waakye could be because of fecal contamination due lack of proper handling after preparation.

4.5.3 Pathogens found in water and the palms of food vendors

According to WHO standards, pathogens such as *Citrobacter*, *Edwardsiella*, *Aeromonas*, *Aerobacter*, *Erwinia*, *Enterobacter, Escherichia*, *Klebsiella*, *Proteus*, *Hafnia*, *Morganella*, *Kluyvera*, *Serratia*, *Yersinia*, *Shigella,* and *Vibrio* are considered as genera of bacteria that are commensals in soil or water (WHO, 2011). Although organisms such as *Salmonella*, *Shigella* and *E. coli* are directly attributed to fecal contamination which could lead to gastroenteritis when ingested, transmission of non-gastrointestinal pathogens to susceptible individuals can occur through hospitals and healthcare facilities. In this case, genera considered human microbial flora like *Acinetobacter* spp and *Staphylococcus* spp displaced into water or food when ingested poses the risk of possible food or waterborne contamination. It is in this vain that personal hygiene and environmental sanitation is emphasized as control measures to reduce the transmission of these pathogens (Manjaya, Tilley, & Marks, 2019). Reports of re-use of water in the cleaning and washing of vegetables and utensils for long periods at vending sites have also been identified as one of the reasons for the persistence of pathogens in the various water sources used (Bintsis, 2017; Rane, 2011). In a study conducted among street food vendors in Johannesburg, South Africa, 78% of dishwashing water was identified to have *E. coli* present in them owing to the lack of potable/ running water at the vending sites (Mosupye & Von Holy, 1999). Organisms such as *Salmonella* spp and *Shigella* spp were recovered from dishwashing waters of food vendors sampled in the streets, markets and schools of some districts in Ouagadougou (Barro et al., 2006).

In Ghana, there is paucity of data regarding the state of the different water types used in the various activities such as cooking, washing and storage at food vending sites. However, several

studies have been carried out on the state of drinking and bottled water in various parts of the country (Addo et al., 2009; Dzotsi et al., 2015; Mosi et al., 2019). Although Mensah and colleagues had identified the use of dirty water for washing crockery as a factor that could influence food contamination, further investigation to ascertain the suspicion was not carried out (Mensah et al., 2002). Addo and colleagues did not identify mesophilic bacteria during a study that was carried out in food, water and fruit juice samples in Accra among ten selected hotels to determine their safety and microbial analysis (Addo et al., 2007).

Majority of the pathogens isolated from the different water types belong to the commensals of water and soil and as such, could be easily overlooked as expected. Their presence does not also suggest their transmission capability, especially among the storage and cooking water due to the process of rigorous cooking at high temperatures which is known to get rid of certain bacterial organisms (Newman, 2005). However, since not all RTEs are cooked at high temperatures (vegetables, porridges- 'kooko'), the presence of some of these bacteria cannot be overlooked. Outbreaks of infections such as urethritis, bacteremia and pneumonia are sometimes the result of *Acinetobacter* spp persistence in the environment whiles opportunistic nosocomial infections, meningitis, pneumonia and septicemia could occur due to *Aeromonas* spp and *Klebsiella* spp infections (WHO, 2011).

It is to be noted that the presence of some of these organisms in the washing water indicates their unrenewal during the vending activity (Barro et al., 2006; Mensah et al., 2002). This may happen due to the busy schedules attached to the sale of RTEs, unavailability of potable/ running water or lack of adherence to personal hygiene or environmental sanitation due to laziness or negligence. Washing water that was sampled during the study were collected from the last water (after washing with soap and water) that is used in rinsing the crockery and it is therefore expected to be clean and frequently changed to avoid contamination.

Also, the presence of *Vibrio* spp, in storage water shows a possible fecal contamination which could also be attributed to the source of water, cleanliness of water containers for storage, or the hand hygiene of the users. Their presence is not indicative of infection but possible transmission if care is not taken. Reports of cholera outbreaks have been reported in Ghana and several parts of the world (Mireku-Gyimah et al., 2018; Rane, 2011)

Apart from *Acinetobacter* spp, *Citrobacter* spp, *Enterobacter* spp, *E. faecalis*, *Klebsiella* spp, *Pseudomonas* spp, *Staphylococcus* spp and *Vibrio* spp, the rest of the pathogens were identified among the palm swabs that was tested from both vicinities. The presence of some of the bacteria known to be commensals of soil and water but found in the palms of the vendors was an indication of a possible transfer which could be because of lack of personal hygiene of the food vendors. Thus, further transmission to food or water when ingested by consumers could lead to possible gastrointestinal infections due to their survival, replication, transfer of infectious genetic materials to other non-infectious bacteria rendering them pathogenic. Several studies have implicated the hand hygiene of food vendors as one of the causes of food borne infection (Ababio & Lovatt, 2015; Abakari et al., 2018; Hassan et al., 2018; Okareh & Erhahon, 2015; Rane, 2011). Pathogens belonging to the genus *Escherichia*, *Klebsiella* and *Acinetobacter* were isolated in the palms of street food vendors from various parts of Dhaka University campus and surrounding areas in Bangladesh during an RTE food study (Hassan et al., 2018). Also, Barro and colleagues also identified *Salmonella* and *Shigella* in the hands of street foods in Ouagadougou, Burkina Faso (Barro et al., 2006).

In a cross-sectional survey among food handlers situated at various educational institutions in the Sagnarigu Municipality in Ghana showed 85.4% of food vendors did not wash their hands after touching money and as such the possible of transfer of bacteria from the contaminated money to the hands and finally to the food (Amegah et al., 2020). Money has been confirmed to be a vehicle of the transfer of resistant bacteria worldwide and in Ghana through humans to

food when proper hand washing is not exercised after they are handled (Anning et al. 2019; Barro et al. 2006; Gedik, Voss, and Voss 2013; Yar 2020).

4.6 Demographics, general information on sanitation, personal, and patients' knowledge of diarrhoea risk factors

4.6.1 Presenting symptoms of patients

Due to the limited resource of many hospitals in Ghana, diagnosis and treatment of diarrhoea is mostly syndromic. In a diarrhoea study carried out by Ameme et al. in Ghana, abdominal pain, fever, nausea, bloody diarrhoea, vomiting and headache were identified as symptoms commonly reported during the foodborne outbreak among a group of Senior High School students from Fanteakwa district presented to the emergency unit of the district hospital (Ameme et al., 2016). Other diarrhoea related studies carried out in Ghana also showed similar symptoms (Akuffo et al., 2017; Danikuu et al., 2015). Common symptoms (blood/mucous in stool, fever, flatulence, headache, abdominal pain/cramps, bloating, nausea, vomiting, and fatigue) reported in this study were concurrent with the previous diarrhoea studies.

Various bacterial and viral associated infections, such as *Bacillus cereus*, *Campylobacter jejeni*, *Clostridium spp*, *E. coli* (travelers' diarrhoea/0157 strain), Hepatitis A, *Listeria moncytogenes*, Norovirus, Rotavirus, *Salmonella* spp, *Shigella* spp, *Staphylococcus* spp and *Vibrio* spp., have shown abdominal pain/cramps, vomiting, watery diarrhoea and fever to be common symptoms associated with having those infections (Akhtar & Sutjita, 2006; Humphries & Linscott, 2015; Okeke, 2009; Sattar & Singh, 2018; Switaj & Christensen, 2015). Several clinical symptoms such as fever, history of blood in stool, vomiting, abdominal pain/cramps have been recorded in the past in the US in a prospective study to determine the etiology of diarrhoea among patients in Baltimore, Maryland and New Haven, Connecticut

(Nataro et al., 2006). Abdominal cramps, headache, loss of appetite and fever were some of the most common symptoms presented during a cross-sectional, questionnaire-based, survey that was undertaken in a household/hospital diarrhoeal-based study which enrolled cases across all ages in Soweto, South Africa (Johnstone et al., 2021). In view of this, based on commonly reported symptoms, syndromic management of diarrhoea could be more effective based on previous study outcomes.

4.6.2 Behavioral traits of patients

Worldwide estimates have established contaminated food to be the major cause of diarrhoea in Africa and the world at large (Havelaar et al., 2015).

Although the behavioural trait of taking medication upon the onset of illness was not a suspected cause of diarrhoea illness based on the responses of patients from both hospitals, it is worth noting that most respondents responded in the affirmative (53.3%). Reasons for self-medication in Ghana are diverse; ranging from high costs when prescribed and hospital delays when attended (Jimah, Fenny, & Ogunseitan, 2020). This has contributed to the issue of AMR due to majority of self-medication drugs being antibiotics, followed by analgesics (Donkor, Tetteh-Quarcoo, Nartey, & Agyeman, 2012). Abuse of antibiotics due to the aforementioned reasons are very common in Ghana based on several studies on the knowledge and attitudes of the populace (Effah et al., 2020; Elong Ekambi et al., 2019; Jimah et al., 2020; Yevutsey et al., 2017).

Several studies that have been conducted in Ghana have also implicated food and water to be the major sources of FBPs which could lead to diarrhoea (Ababio & Lovatt, 2015; Abakari et al., 2018; Abas et al., 2019; Anyorikeya, Ameme, Nyarko, Sackey, & Afari, 2016; Asamoah et al., 2016; Dzotsi et al., 2015; Marras & Agbendech, 2016; Mensah et al., 2002; Mireku-Gyimah et al., 2018; Yeleliere et al., 2017). In view of this, it was not surprising for majority

of the diarrhoea patients in the study to admit eating food or drinking prior to the onset of symptoms leading to hospital attendance. Several illnesses, deaths and hospitalizations have been reported in the United States due to instances of the consumption of contaminated food and water (Mead et al., 1999).

Over 95% of the patients reported having their source of drinking water to being sachet water in the study. Several studies in Ghana that have been carried out in the past have implicated sachet water in general to be a factor in diarrhoea diseases based on its reduced quality despite its national acceptance and popularity because of its affordability and availability on the market (Addo et al., 2009; Dzotsi et al., 2015; Mosi et al., 2019). A similar study conducted in Nigeria revealed sachet water to possess microbial contaminant which could lead to transmission of FBPs (Epundu et al., 2017). Further targeted studies on sachet water analysis should be encouraged to ascertain the transmission of FBPs leading to diarrhoea in Ghana.

On the other hand, it was recorded in the study that majority of the diarrhoea patients attested to washing their hands with soap and water, which implies an adherence to personal hygiene, a way of alleviating foodborne bacteria transmission. This response is however subject to individual bias due to the general expectancy of the need to adhere to personal hygiene guidelines because in a recent study that was conducted to investigate diarrhoea incidence among various households, reports showed that majority did not wash hands with soap and water before food preparation and consumption (Larbi et al., 2021). In view of this, fewer cases of diarrhoea were recorded among households who practiced hand washing with soap and water as compared to those who did not.

Also, the study reported the practice of hand washing with soap and water to have a significant association ($p<0.05$) with determining the cause of diarrhoea among patients who attended Maamobi General Hospital and Kaneshie polyclinic. Continuous advocacy of proper hand washing as a key personal hygiene practice still needs to be encouraged in curbing the

transmission of foodborne pathogens leading to diarrhoea. Also, reported cases of the lack of water and/or soap at canteens and vending sites which could lead to diarrhoea have been reported in Ghana (Malm et al., 2015). Transmission of foodborne pathogens leading to diarrhoea can however be transmitted due to improper hand hygiene, either by the handling of cooked food, especially the 'staple balls', ('banku', 'Tuozafi', 'akpler', 'fufu' and 'kokonte') by the hands of a vendor or by the consumer (Larbi et al., 2021; Malm et al., 2015; Mensah et al., 2002; Tadesse et al., 2019). This is indicative of proper handwashing as an important means of preventing diarrhoea in the community.

In this study, the use of dustbins as the most preferred option in disposing waste suggests a more orderly destination of waste which is usually collected by the paid services of ad hoc motorized cart operators or company trucks, concurrent with the study conducted by Larbi and colleagues in Ghana (Larbi et al., 2021). This finding shows an improvement to improper waste disposal sites, personnel and equipment in 2014 and 2015, which contributed to the cyclical transmission and surge of cholera epidemics in Accra, Ghana (Mireku-Gyimah et al., 2018).

Majority (80.3%) of the patients' choice of dustbin as the destination of waste disposal is one of the safest and environmentally friendly means. The use of dustbin, a behavioral trait that could cause diarrhoea had a significant association (p<0.05) with the response from both hospital sites. In this, patients from Maamobi General Hospital (88.2%) were more likely to dispose of their waste using dustbins as compared to patients from Kaneshie polyclinic (70.4%). This implies that the chances of one having the transmission of foodborne infection among the patients who responded to the use of dustbins was lower than those who resorted to waste disposal via lagoon/river, pit, or vehicle. Although there was a significant association between burning wastes, a behavioral trait that could lead to diarrhoea infection and patients' response from both sites, only few patients responded in the affirmative (22.1%). It was observed that patients from Maamobi General hospital were more likely (33.8%) to burn their

solid waste as compared to Kaneshie polyclinic (7.4%). Response about burning of waste in this study is somehow a reflection of a report on solid waste and health in Accra, Ghana which showed an increase of burning of solid waste from 10.7% in 2010 to 19.5% in 2017, especially in low to middle-income communities (Mudu, Nartey, Kanhai, Spadaro, & Fobil, 2021). About 87% of the surveyed households in this same study did not attribute means of waste disposal as a factor that can cause infection among people. There is a tendency of spillover of foodborne pathogens that could lead to diarrhoea in the community and air pollution as well based on open burning when waste disposal issues are not addressed properly.

As expected, good sanitation/ environmental hygiene reduces the chances of diarrhoea infection in the community. Responses of source food having very tidy environment purchase as compared to not tidy showed significant association with being the cause of diarrhoea or not. One of the key means of environmental transmission is by dust blown into food being sold or eaten. The dust could carry FBPs from the surrounding (not tidy) environment and transmitted to the consumer (Mensah et al., 2002). Inspection of clean environment where food is sold should be strongly encouraged to manage this transmission route.

When patients were interviewed to investigate their knowledge about the possible causes of diarrhoea, out of the various variables such as dirty food, water, germs, and dirty hands, dirty food was less likely to be associated with diarrhoea infection ($p > 0.05$). Also, patients from Maamobi were more likely to associate dirty water, germs and dirty hands to diarrhoea infection as compared to Kaneshie polyclinic ($p<0.05$). Several foodborne disease outbreaks have been associated with these possible causes in various reported studies (Barro et al., 2006; Hassan et al., 2018; Marras & Agbendech, 2016; Mensah et al., 2002; Newman, 2005; Rane, 2011). Education on the causes of foodborne transmission should be intensified to spread the awareness.

4.6.3 Food vendor demographic information (tracing and community)

Similar demographic information regarding the food vendor demographic occurred during the community sampling with only two exceptions: gender and educational background. At the end of sample collection, one male enrolled in the study with majority of food vendors attaining JHS level. Overall, majority of the food vendors were females, which concurs to the general notion of women being primarily responsible for cooking; which is consistent with several street food studies that have been conducted in Ghana and other parts of the world (Amare et al., 2019; Amegah et al., 2020; Marras & Agbendech, 2016; Mensah et al., 2002). This is contrary to street food vending activities that are normally carried out by males in India and Bangladesh (Bhat & Waghray, 2000; Odonkor et al., 2011).

Generally, both phases of the study recorded majority of the vendors being 40 years and above which is inconsistent with majority of street food studies with records of vendors being normally between 25 to 39 years. Contrary to several street food vending studies that have been carried out in the past, food vending has been associated wtih younger and middle-aged women in Ghana (Amare et al., 2019; Marras & Agbendech, 2016; Mensah et al., 2002; Mosupye & Von Holy, 1999). Due to the mandate of taking proper care of the home by gaining a meaningful livelihood, majority of the food vendors were married, which corroborates findings described elsewhere (Amegah et al., 2020; Marras & Agbendech, 2016).

Pertaining to education, many of street food vendors in this study had received some basic education (Primary and JHS) consistent with Mensah et al., 2002's street food vendor study identifying similar trends but with a higher percentage of the vendors having no formal education (Mensah et al., 2002). Most studies have reported majority of street food vendors who did not have any formal education which could be a factor leading to negligence or non-adherence to basic personal hygiene and food safety practices (Amegah et al., 2020).

4.6.4 Food vendors general information on sanitation, personal hygiene, and risk factors

All food vendors responded in the affirmative regarding getting water from pipes/ tap water as their source of water for food preparation. This water after fetching was stored, used in cooking and washing activities at the vending sites. Most participants also responded to washing their hands with soap and water although later findings from their palms, food and water contamination indicated their positive response might not have been translated to good practice. Similar street food vending studies have also recorded the use of tap water, and handwashing activities in their studies (Amegah et al., 2020; Diriba et al., 2020; Yeleliere et al., 2017).

Majority of the vendors had access to sanitation at the vending sites as was observed in street food vendors in Ghana and Ethiopia (Danikuu et al., 2015; Yeleliere et al., 2017). Although there were bins available for waste disposal, most of the vending sites were described to be heavily littered, comparable to Mensah et al.'s street vending work in Accra which reported some litter (57.3%) at the food vending sites (Mensah et al., 2002). The absence of stagnant water and flies at the vending sites is inconsistent with Mensah et al.'s findings where there were stagnant water and flies at the vending sites.

In understanding the knowledge of Policy on Hazard Analysis and Critical Control Point (HACCP) and adherence to food preparation guidelines by food vendors and operators in Kumasi, it was observed that RTE food was mostly prepared by another person and not the vendor (Agyei-Baffour et al., 2013). This supports the finding in this study where over 70% of the vendors were not responsible for cooking of the RTE food. Venue of the preparation was either at the vending site or brought from home as reported by other studies (Marras & Agbendech, 2016; Odonkor et al., 2011).

The subject of vendors being medically examined plays a very important role in the safety of street food vending. Similar foodborne disease outbreaks occurrences in Ghana and other countries could have been attributed to possible transmission from the vendors to the consumers due to failure and non-adherence to basic hygiene and sanitations practices (Choudhury, Mahanta, Goswami, Mazumder, & Pegoo, 2011; Danikuu et al., 2015; Diriba et al., 2020; Hassan et al., 2018; Marras & Agbendech, 2016; Mensah et al., 1999; Mosupye & Von Holy, 1999; Rane, 2011; Tadesse et al., 2019; Yeleliere et al., 2017). Although most of the vendors responded in the affirmative of being medically examined, positive response on the subject does not guarantee adequate implementation and effective food safety and personal hygiene practices; similar studies have attested to this (Amare et al., 2019; Danikuu et al., 2015; Diriba et al., 2020).

Equal probabilities of where food was cooked (home or vending site) was reported with more than 30% transporting food either in a car or head carriage in cases of food preparation sites being distant from the vending site. This is a popular occurrence in Ghana and has been identified to be a potential vehicle for transmission of FBPs when proper care is not taken during their transportation, especially in car boots (Malm et al., 2015; Newman, 2005).

Due to economic hardship and efforts to make maximum profit during food vending activities, most vendors and street food operators in this study chose cost (low pricing) and quantity over quality to meet the demands of RTE food consumers. Majority of the vendors preferred buying foodstuff from nearby satellite markets for convenience and economic reasons than from supermarkets, slaughterhouses or streets (Amoah et al., 2006; Karikari et al., 2017; Marras & Agbendech, 2016). Due to the busy lifestyle, and low-middle socioeconomic standard of living of many Ghanaians, the patronage of RTE food is increasingly gaining more grounds and popularity.

Majority of the food vendors and operators resorted to using chicken or meat (40% and 64%) in preparing food more than chevron or mutton. This could be due to the availability and affordability of these staples that are normally purchased from cold stores in Ghana (Saba & Gonzalez-Zorn, 2012).

The knowledge of food vendors on the causes of diarrhoea is very important in influencing their practice of personal hygiene and sanitation during vending. It was encouraging to note that more than 50% of the vendors attributed dirty food and germs to cause diarrhoea. However, there is the need for more education on the risks leading to the transmission of foodborne disease since most vendors did not know that FBPs could be transmitted through dirty hands and germs as well. The knowledge of vendors on the causes of foodborne disease transmission is shallow and therefore more targeted education and awareness creation should be implemented. Mensah et al., 2002 witnessed similar responses by vendors in their study, as they also attributed diarrhoea to be caused by dirty food (29.1%) and germs (17.9%) in Accra (Mensah et al., 2002).

The use of dustbins at the vending sites to dispose of waste was well resorted to against other means (vehicle, pit, lagoon and burning) of waste disposal. This practice helped in reducing the spread of foodborne bacteria as a result of spill-over from improperly disposed waste (Mudu et al., 2021). Many food vending studies and reviews have confirmed increasing usage of waste bins at vending sites (Marras & Agbendech, 2016; Mensah et al., 2002; Odonkor et al., 2011).

More than 60% of food vendors resorted to using condiments in the RTE foods. The use of condiments such as ketchup, salad creams/ mayonnaise, especially hot pepper sauce (shitor) was affirmed to be commonly used in the sale of food items during food vending activities and in most Ghanaian street vending outlets (Marras & Agbendech, 2016). They complement RTE foods in making them more spicy, tasty, and nutritious. Despite their importance in the food

industry, they are likely to pose risks in transmitting foodborne pathogens when used in RTE foods like salads (Okeke, 2009).

Cooked food was commonly sold (44.6%) from the same cooking pots that were used in the preparation of food which is considered the safest method since the transfer of cooked food into other containers could lead to the transmission of FBPs if they are contaminated (Marras & Agbendech, 2016; Mensah et al., 2002).

Food vendors responded to washing serving plates after each use and not after the end of the day which was not what the surveillance team observed. It shows the knowledge of food vendors in proper washing of bowls/plates and other culinary apparatus but was clearly not being converted to practice. There is a tendency of foodborne disease transmission being high. RTE vendors in major parts of Accra have been identified to practice wrong handling of plates/ bowls and resort to quick washes in contaminated/ dirty water in haste of serving consumers (Marras & Agbendech, 2016; Mensah et al., 2002).

5 CHAPTER FIVE: DETERMINATION OF ANTIMICROBIAL RESISTANCE (AMR) PROFILES OF BACTERIA ISOLATES

5.1 Introduction

Superbugs in stool, food and water are continually causing economic and public health problems globally due to the difficulty in acquiring the required treatment regimen (Qadri et al., 2005). Most diarrhoea cases are self-resolving without the use of antibiotics (Humphries & Linscott, 2015). In cases of severity, preceding treatment should have been the performance of antimicrobial susceptibility testing (AST) in almost all foodborne implicated diarrhoea cases. Unfortunately, owing to the urgency to treat diarrhoea cases and the burden of doctor-to-patient ratio which is mostly high in almost all the health facilities in Ghana, clinicians normally resort to the syndromic approach of treatment (Ghana National Drugs Programme (GNDP), 2004).

AST results helped determine the AMR profiles in the isolates recovered from the different types of samples (stool, food, water, and palms) processed in the study. Breakpoints of various selected antibiotics peculiar to the bacteria investigated were interpreted from Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2017). Various resistance patterns like Multidrug Resistant (MDR) isolates, preliminary antibiogram testing for ESBLs, and other peculiar resistance groups were thoroughly examined and explained among the isolates recovered (Magiorakos et al., 2012).

5.2 Materials and Methods

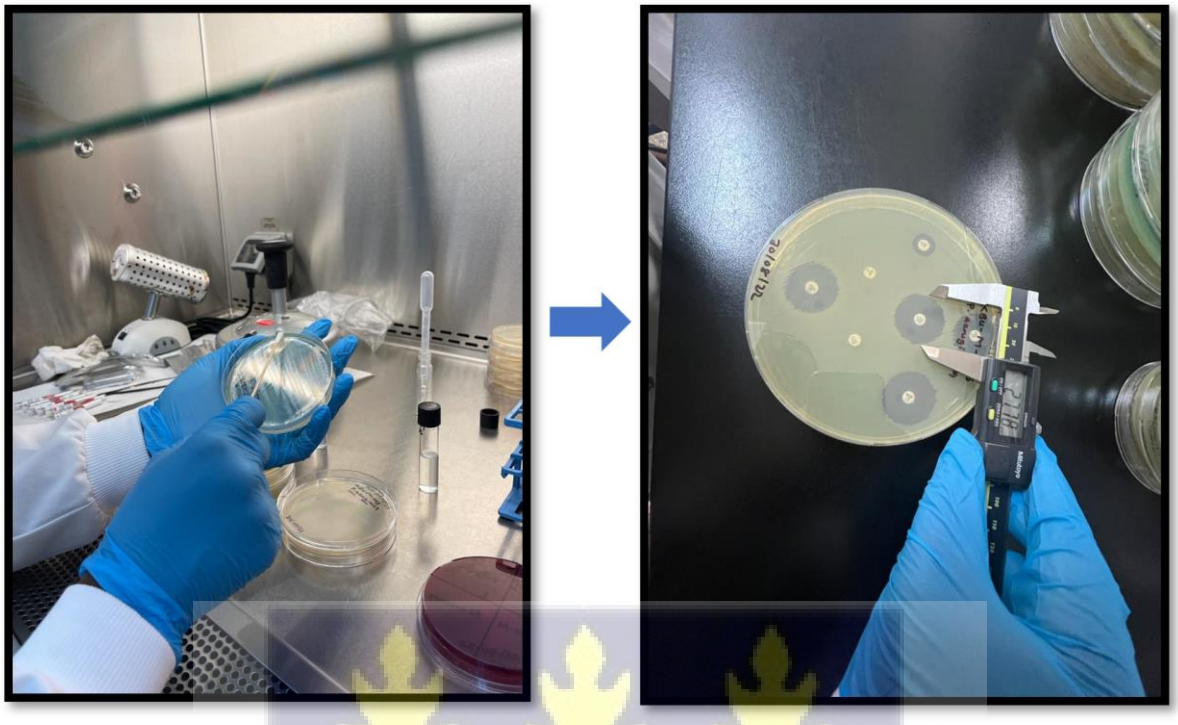


Figure 5.1: Procedure for AST (Kirby Bauer disc diffusion method)

5.2.1 Antimicrobial susceptibility testing (AST)

The performance of AST was carried out on all isolates recovered from stool, food, water, and palm swabs. This was carried out based on the Kirby Bauer disk diffusion method on Mueller-Hinton (MH) agar, using the Clinical and Laboratory Standard Institute (CLSI) guidelines (M100, 28th edition) for the interpretation of results (CLSI, 2017). Three to five colonies of overnight growth were inoculated in 5 ml saline which was mixed gently to form a homogenous mixture, referenced with 0.5 McFarland turbidity standard. The bacteria inoculum was uniformly smeared on the entire surface of Mueller Hinton agar plates using sterile cotton swabs. The entire plate is covered with the inoculum by rotating the plate at approximately 60° to ensure an even distribution of the inoculum. Antibiotics were placed on

plates within fifteen minutes using sterile forceps (using a Bacti-cinerator), inverted, and incubated at 35-37˚C for 18-24 hrs. The procedure for AST is shown in Figure 5.1.

The choice of the antibiotics was based on the type of bacteria that was being tested, that is, Enterobacteriaceae spp, *Staphylococcus* spp, *Pseudomonas* spp and *Enterococcus* spp. The following antibiotics were used for isolates that belonged to the Enterobacteriaceae family in general; Ceftriaxone (30µg), Sulfamethoxazole-trimethoprim (25µg), Piperacillin-tazobactam (110 µg), Ticarcillin-clavulanate (85µg), Tetracycline (30µg), Amikacin (30µg), Gentamicin (10µg), Ciprofloxacin (5µg), Meropenem (10µg), Azithromycin (15µg), Chloramphenicol (30µg), Nitrofurantoin (300µg), Nalidixic acid (30µg), Ceftazidime (30µg) and Amoxicillin-clavulanate (20µg). Vancomycin (30µg), Erythromycin (15µg), Rifampin (5µg), Penicillin (10units) and Linezolid (30µg) were exclusively used to test for Staphylococcus and Enterococcus species of isolates that were recovered in addition to Chloramphenicol, Sulfamethoxazole-trimethoprim, Ciprofloxacin, Gentamicin, Tetracycline and Gentamicin. Piperacillin-tazobactam, Ticarcillin-clavulanate, Meropenem, Gentamicin, Amikacin, Ciprofloxacin, and Ceftazidime were the antibiotics that were used in testing isolates that belonged to the Pseudomonas species.

All ASTs were performed using quality control strains that were adequate for each bacteria group in determining their susceptibility or otherwise in all the isolates. Clearing around each disc (inhibition zones) was measured in millimeters using either a ruler or a caliper and interpreted according to standards as sensitive, intermediate, or susceptible based on the CLSI standards M100S. Standard microbiological procedures were strictly followed and handled in a biosafety level 2 (BSL-2) facility.

Multidrug resistance (MDR) was defined as the resistance to at least one agent in three or more categories of antimicrobial agents. The categories based on the aforementioned list of antibiotics included; β-lactam or β-lactamase inhibitors (Amoxicillin-clavulanate,

Piperacillin-tazobactam, Ticarcillin-clavulanate), Quinolones/ Fluoroquinolones (Ciprofloxacin, Nalidixic acid), Cephalosporins (Ceftazidime, Ceftriaxone), Aminoglycosides (Gentamicin, Amikacin), Tetracycline, Macrolides (Azithromycin, Erythromycin), Sulfonamides (Sulfamethoxazole-trimetropim), Phenicols (Chloramphenicol), Nitofurans (Nitrofurantoin), Carbapenems (Meropenem), Glycopeptides (Vancomycin), Penicillinase (Penicillin), Ansamycins (Rifampim) and Oxazolidinose (Linezolid) (Magiorakos et al., 2012).

5.3 Results

5.3.1 Antimicrobial Susceptibility Testing (AST) from both sites

All pathogens recovered (both Enterobacteriaceae and non-Enterobacteriaceae) were taken through Antimicrobial Susceptibility testing (AST) to investigate their AMR properties. Generally, *E. coli* isolates were resistant to all antimicrobial agents with over 50% showing resistance to Sulfamethoxazole-trimethoprim, Tetracycline and Azithromycin. Apart from *E. coli* isolates, all other Enterobacteriaceae pathogens that were recovered were resistant to Amikacin as illustrated in Figure 5.1. As illustrated in Tables 5.1 (a) and (b), some *Salmonella spp*, *K. pneumoniae*, *Proteus spp*., *Serratia marcescens* and *Aeromonas hydrophilla* isolates were susceptible to Meropenem, except *E. coli* (4%), *E. cloacae* (10%), *K. terrigena* (67%) and *Proteus mirabilis* (13%). All *Salmonella species, Serratia marcescens* and *Aeromonas hydrophilla* showed susceptibility to the aminoglycosides (Amikacin and Gentamicin), penicillin-type (Piperacillin-tazobactam and Ticarcillin-clavulanate), Sulfonamide (Sulfamethoxazole-trimethoprim), Cephalosporins (Ceftriaxone, Ceftazidime), Carbapenem (Meropenem) and fluoroquinolone (Ciprofloxacin) but were resistant to the macrolide (Azithromycin) antibiotics.

Table 5.1 Tables showing AST patterns of Enterobacteriaceae recovered from both Maamobi General hospital and Kaneshie polyclinic

Table 5.1 (a)

Pathogens	Antibiotics							
	SXT n (%)	TIM n (%)	TE n (%)	F n (%)	CN n (%)	NA n(%)	TZP n (%)	AZM n (%)
E. coli(n=76)	53(69.7)	17(22.4)	56(73.7)	13(17.1)	7(9.2)	33(43.4)	10(13.2)	45(59.2)
Salmonella spp(n=6)	0(0.0)	0(0.0)	2(33.3)	2(33.3)	0(0.0)	0(0.0)	0(0.0)	4(66.7)
Enterobacter cloacae(n=20)	10(50.0)	2(10.0)	11(55.0)	16(80.0)	0(0.0)	4(20.0)	0(0.0)	12(60.0)
*K. pneumoniae(*n=14)	8(57.1)	2(14.3)	7(50.0)	10(71.4)	1(7.1)	1(7.1)	1(7.1)	6(42.9)
*K. terrigena(*n=3)	2(66.7)	2(66.7)	2(66.7)	3(100.0)	3(100)	2(66.7)	0(0.0)	3(100.0)
Proteus mirabilis(n=8)	6(75.0)	2(25.0)	7(87.5)	7(87.5)	0(0.0)	2(25.0)	1(12.5)	7(87.50)
Proteus species(n=3)	1(33.3)	2(66.7)	3(100.0)	2(66.7)	0(0.0)	3(100)	1(33.3)	3(100)
*Serratia marcescens(*n=5)	0(0.0)	0(0.0)	3(60.0)	4(80.0)	0(0.0)	0(0.0)	0(0.0)	4(80.0)
Aeromonas hydrophila(n=3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(33.3)	0(0.0)	2(66.7)

Key: SXT- Sulfamethoxazole-trimethoprim; TIM- Ticarcillin-clavulanate; TE-Tetracycline; F-Nitrofurantoin; CN-Gentamicin; NA- Nalidixic acid; TZP- Piperacillin-tazobactam; AZM- Azithromycin

Table 5.2 (b)

Pathogens	Antibiotics							
	AK n (%)	C n(%)	MEM n (%)	CIP n (%)	CRO n (%)	CAZ n (%)	AMC n (%)	MDR n (%)
E. coli(n=76)	4(5.3)	19(25.0)	3(4.0)	26(34.2)	14(18.4)	10(13.2)	9(11.8)	50(65.8)
Salmonella spp(n=6)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.00)	0(0.0)	2(33.3)	0(0.0)
Enterobacter cloacae(n=20)	0(0.0)	7(35.0)	2(10.0)	0(0.0)	1(5.00)	0(0.0)	12(60.0)	14(70.0)
*K. pneumoniae(*n=14)	0(0.0)	4(28.6)	0(0.0)	2(14.3)	3(21.4)	2(14.3)	3(21.4)	6(42.9)
*K. terrigena(*n=3)	0(0.0)	2(66.7)	2(66.7)	0(0.0)	1(33.3)	0(0.0)	1(33.3)	3(100)
Proteus mirabilis(n=8)	0(0.0)	4(50.0)	1(12.5)	1(12.5)	2(25.0)	1(12.5)	1(12.5)	7(87.5)
Proteus species(n=3)	0(0.0)	2(66.7)	0(0.0)	2(66.7)	1(33.3)	1(33.3)	3(100)	3(100)
*Serratia marcescens(*n=5)	0(0.00)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(20.0)	3(60.0)
Aeromonas hydrophila(n=3)	0(0.00)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(100.0)	0(0.00)

Key: AK-Amikacin; C- Chloramphenicol; MEM-Meropenem; CIP- Ciprofloxacin; CRO-Ceftriaxone; CAZ- Ceftazidime; AMC- Amoxicillin-clavulanate; MDR-Multidrug resistant

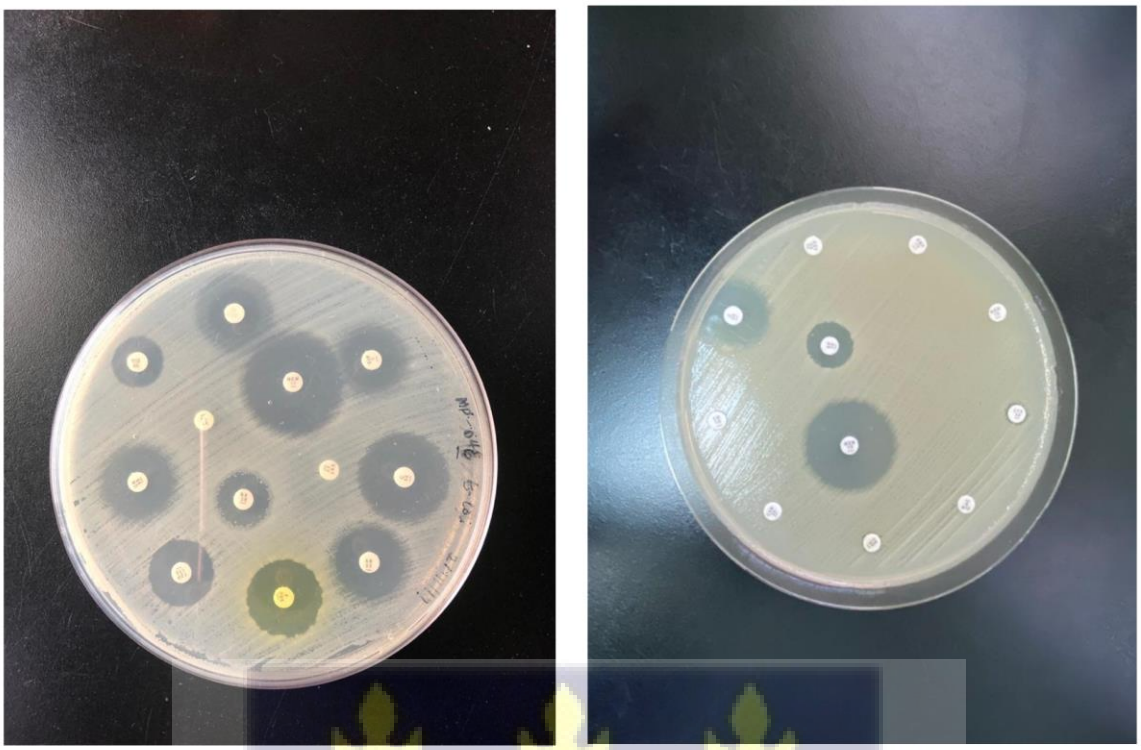


Figure 5.2: *E. coli* AST plates showing resistance

Two *S. aureus* isolates were recovered; in which one each were obtained from both sites. Vancomycin was the only resistance observed in one of the isolates, recovered from Kaneshie polyclinic. Also, two *P. aeruginosa* isolate, another enteric commensal was recovered from the stool samples that were collected at the Maamobi General hospital and not from the Kaneshie polyclinic. One out of two of the isolates was resistant to Tetracycline, Chloramphenicol, Ceftazidime, Nalidixic acid and Ceftriaxone and all the isolates were resistant to Nitrofurantoin and Sulfamethoxazole-trimethoprim. Figure 5.2 shows an *E. coli* isolate showing resistance to some antibiotics.

5.3.2 Antimicrobial Susceptibility Testing of stool isolates from Maamobi General Hospital

No resistance to Piperacillin-tazobactam and Ceftazidime were observed for in all the Enterobacteriaceae organisms recovered at Maamobi General Hospital, except *E. coli* which showed resistance (18.42%; 15.79%) as illustrated in Tables 5.2 (a) and (b). Similarly, apart from *E. coli* and *K. terrigena* which showed resistance to Gentamicin (7.89%; 100%) and Amikacin (12.63; 100%), the other Enterobacteriaceae organisms were susceptible. All *Salmonella* spp. Isolates were susceptible to all the antibiotics tested apart from Azithromycin (100%) and Amoxicillin-Clavulanate (33.33%).

Table 5.2 AST among the Enterobacteriaceae pathogens of stool samples from Maamobi General hospital

Table 5.2 (a)

Pathogens	Antibiotics							
	SXT n (%)	TIM n (%)	TE n (%)	F n (%)	CN n (%)	NA n (%)	TZP n (%)	AZM n (%)
***E. coli* (n=38)**	25(65.8)	10(26.3)	27(71.1)	5(13.2)	3(7.9)	16(42.1)	7(18.4)	26(68.4)
***Salmonella* spp (n=3)**	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	3(100.0)
***Enterobacter cloacae*(n=10)**	6(60.0)	1(10.0)	6(60.0)	9(90.0)	0(0.0)	2(20.0)	0(0.0)	5(50.0)
***K. pneumoniae*(n=8)**	5(62.5)	0(0.0)	4(50.0)	6(75.0)	0(0.00)	0(0.0)	0(0.0)	3(37.5)
***K. terrigena*(n=3)**	2(66.7)	2(66.7)	2(66.7)	3(100.0)	3(100.0)	2(66.7)	0(0.0)	3(100.0)
***Proteus mirabilis*(n=6)**	5(83.3)	1(16.7)	5(83.3)	6(100.0)	0(0.0)	1(16.7)	0(0.0)	5(83.3)
***Proteus species*(n=1)**	0(0.0)	0(0.0)	1(100.0)	1(100.0)	0(0.0)	1(100.0)	0(0.0)	1(100.0)
***Serratia marcescens*(n=3)**	0(0.0)	0(0.0)	2(66.7)	2(66.7)	0(0.0)	0(0.0)	0(0.0)	2(66.7)
***Aeromonas hydrophila*(n=2)**	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(50.0)

Key: SXT- Sulfamethoxazole-trimethoprim; TIM- Ticarcillin-clavulanate; TE-Tetracycline; F-Nitrofurantoin; CN-Gentamicin; NA- Nalidixic acid; TZP- Piperacillin-tazobactam; AZM-Azithromycin

Table 5.2 (b)

Pathogens	Antibiotics							
	AK n (%)	C n (%)	MEM n (%)	CIP n (%)	CRO n (%)	CAZ n (%)	AMC n (%)	MDR n (%)
***E. coli* (n=38)**	1(2.63)	9(23.7)	0(0.0)	12(31.6)	8(21.1)	6(15.8)	2(5.3)	26(68.4)
***Salmonella* spp (n=3)**	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(33.3)	0(0.0)
***Enterobacter cloacae*(n=10)**	0(0.0)	4(40.0)	1(10.0)	0(0.0)	1(10.0)	0(0.0)	5(50.0)	7(70.0)
***K. pneumoniae*(n=8)**	0(0.0)	1(12.5)	0(0.0)	0(0.0)	1(12.5)	0(0.0)	2(25.0)	3(37.5)
***K. terrigena*(n=3)**	3(100.0)	2(66.7)	2(66.7)	0(0.0)	1(33.3)	0(0.0)	1(33.3)	3(100)
***Proteus mirabilis*(n=6)**	0(0.0)	3(50.0)	1(16.7)	0(0.0)	1(16.7)	0(0.0)	0(0.0)	5(83.3)
***Proteus species*(n=1)**	0(0.0)	0(0.0)	0(0.0)	1(100.0)	0(0.0)	0(0.0)	1(100)	1(100.0)
***Serratia marcescens*(n=3)**	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(33.3)	2(66.7)
***Aeromonas hydrophila*(n=2)**	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(100)	0(0.0)

Key: AK-Amikacin; C- Chloramphenicol; MEM-Meropenem; CIP- Ciprofloxacin; CRO-Ceftriaxone; AMC- Amoxicillin-clavulanate; CAZ- Ceftazidime; MDR- Multidrug resistant

5.3.3 Antimicrobial Susceptibility Testing of stool isolates from Kaneshie Polyclinic

Majority of the Enterobacteriaceae isolates recovered from the Kaneshie Polyclinic were resistant to Azithromycin while apart from some *E. coli* isolates (7.89%), all the Enterobacteriaceae recovered were susceptible to Amikacin. As illustrated in Table 5.3 (a) and (b), only some *E. coli* (7.89%) and *E. cloacae* (100%) showed resistance to Amikacin among the Enterobacteriaceae organisms.

Table 5.3 AST among the Enterobacteriaceae pathogens from Kaneshie Polyclinic

Table 5.3 (a)

Pathogens	Antibiotics							
	SXT n (%)	TIM n (%)	TE n (%)	F n (%)	CN n (%)	NA n (%)	TZP n (%)	AZM n (%)
E. coli(n=38)	28(73.7)	7(18.4)	29(76.3)	8(21.1)	4(10.5)	17(44.7)	3(7.9)	19(50.0)
Salmonella species(n=3)	0(0.0)	0(0.00)	2(66.7)	2(66.7)	0(0.0)	0(0.0)	0(0.0)	1(33.3)
Enterobacter cloacae(n=10)	4(40.0)	1(10.0)	5(50.0)	7(70.0)	0(0.0)	2(20.0)	0(0.0)	7(70.0)
K. pneumoniae(n=6)	3(50.0)	2(33.3)	3(50.0)	4(66.7)	1(16.7)	1(16.7)	1(16.7)	3(50.0)
Proteus mirabilis(n=2)	1(50.0)	1(50.0)	2(100.0)	1(50.0)	0(0.0)	1(50.0)	1(50.0)	2(100.0)
Proteus species(n=2)	1(50.0)	2(100.0)	2(100.0)	1(50.0)	0(0.0)	2(100.0)	1(50.0)	2(100.0)
***Serratia marcescens*(n=2)**	0(0.0)	0(0.0)	1(50.0)	2(100.0)	0(0.0)	0(0.0)	0(0.0)	2(100.0)
***Aeromonas hydrophila*(n=1)**	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(100.0)	0(0.0)	1(100.0)

Key: SXT- Sulfamethoxazole-trimethoprim; TIM- Ticarcillin-clavulanate; TE-Tetracycline; F-Nitrofurantoin; CN-Gentamicin; NA- Nalidixic acid; TZP- Piperacillin-tazobactam; AZM- Azithromycin

Table 5.3 (b)

Pathogens	Antibiotics							
	AK n (%)	**C n (%)**	**MEM n(%)**	**CIP n (%)**	**CRO n (%)**	**CAZ n (%)**	**AMC n (%)**	**MDR n (%)**
E. coli(n=38)	3(7.9)	10(26.3)	3(7.9)	14(36.8)	6(15.8)	4(10.5)	7(18.4)	24(63.2)
Salmonella species(n=3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(33.3)	0(0.0)
Enterobacter cloacae(n=10)	0(0.0)	3(30.0)	10(100.0)	0(0.0)	0(0.0)	0(0.0)	7(70.0)	7(70.0)
K. pneumoniae(n=6)	0(0.0)	3(50.0)	0(0.0)	2(33.3)	2(3333)	2(33.3)	1(16.7)	3(50.0)
Proteus mirabilis(n=2)	0(0.0)	1(50.0)	0(0.0)	1(50.0)	1(50.0)	1(50.0)	1(50.0)	2(100.0)
Proteus species(n=2)	0(0.0)	2(100.0)	0(0.0)	1(50.0)	1(50.0)	1(50.0)	2(100.0)	2(100.0)
***Serratia marcescens*(n=2)**	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(50.0)
***Aeromonas hydrophila*(n=1)**	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

Key: AK-Amikacin; C- Chloramphenicol; MEM-Meropenem; CIP- Ciprofloxacin; CRO- Ceftriaxone; AMC- Amoxicillin-clavulanate; CAZ- Ceftazidime; MDR-Multidrug resistant

5.3.4 Antibiotic Resistance profiles of Enterobacteriaceae isolates from Maamobi and Kaneshie vicinities

Resistance to antibiotics among the Enterobacteriaceae isolates in the food items was minimal during the AST profiling. Generally, the highest cases of resistance among the Enterobacteriaceae organisms were in Tetracycline and Nitrofurantoin, followed by Azithromycin and Amoxicillin-clavulanate as shown in Tables 5.4 (a) and (b). Few cases of resistance to the Cephalosporins were recorded whereas Carbapenem resistance was recorded in only one *Citrobacter spp* isolate. *Enterobacter* spp., the most common recovered isolate showed over 60% resistance to Nitrofurantoin and Amoxicillin-clavulanate and varying resistance rates in Azithromycin, Tetracycline, Sulfamethoxazole-trimethoprim, Gentamicin, Nalidixic acid, Amikacin, Chloramphenicol and Ciprofloxacin. No resistance was observed among all the Enterobacteriaceae isolates to Ticarcillin-clavulanate and Piperacillin-tazobactam. The two *E. coli* isolates were susceptible to all the antibiotics that were tested apart from resistance to Nalidixic acid by the *E. coli* recovered from rice from Kaneshie vicinity.

Table 5.4 Antibiotic Resistance profiles of Enterobacteriaceae isolates of food items in both Maamobi and Kaneshie vicinities

Table 5.4 (a)

Pathogens	Antibiotics							
	SXT n (%)	TIM n(%)	TE n (%)	F n (%)	CN n (%)	NA n (%)	TZP n (%)	AZM n (%)
Enterobacter cloacae(n=30)	4(13.3)	0(0.0)	10(30.3)	23(76.7)	1(3.3)	1(3.3)	0(0.0)	11(36.7)
Citrobacter spp(n=18)	14(77.8)	0(0.0)	12(66.7)	8(44.4)	1(5.6)	3(16.7)	0(0.0)	4(22.4)
Klebsiella pneumoniae(n=7)	0(0.0)	0(0.0)	0(0.0)	3(42.9)	0(0.0)	0(0.0)	0(0.0)	2(28.6)
Aeromonas spp(n=2)	0(0.0)	0(0.0)	0(0.0)	1(50.0)	0(0.0)	1(50.0)	0(0.0)	1(50.0)
Escherichia coli(n=3)	0(0.0)	0(0.0)	1(33.3)	0(0.0)	0(0.0)	2(66.7)	0(0.0)	0(0.0)
Klebsiella oxytoca	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(100.0)
Acinetobacter baumannii(n=2)	0(0.0)	0(0.0)	1(50.0)	2(100.0)	0(0.0)	0(0.0)	0(0.00)	2(100.0)
Proteus mirabilis(n=1)	0(0.0)	0(0.0)	1(100.0)	1(100.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Escherichia spp(n=1)	1(100.0)	0(0.0)	1(100.0)	1(100.0)	0(0.0)	0(0.0)	0(0.00)	0(0.0)
Serratia spp(n=1)	0(0.0)	0(0.0)	1(100.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Vibrio fluvialis	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

Key: SXT- Sulfamethoxazole-trimethoprim; TIM- Ticarcillin-clavulanate; TE-Tetracycline; F-Nitrofurantoin; CN-Gentamicin; NA- Nalidixic acid; TZP- Piperacillin-tazobactam; AZM- Azithromycin

Table 5.4 (b)

Pathogens	Antibiotics						
	AK n (%)	C n (%)	MEM n (%)	CIP n (%)	CRO n (%)	CAZ n (%)	AMC n (%)
Enterobacter cloacae(n=30)	2(6.7)	2(6.7)	0(0.0)	1(3.3)	0(0.0)	0(0.0)	18(60.0)
Citrobacter spp(n=18)	1(5.6)	1(5.6)	1(5.6)	0(0.0)	1(5.6)	1(5.6)	12(66.7)
Klebsiella pneumoniae(n=7)	1(14.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(14.3)
Aeromonas spp(n=2)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(50.0)
Escherichia coli(n=3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Klebsiella oxytoca	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Acinetobacter baumannii(n=2)	0(0.0)	1(50.0)	0(0.0)	0(0.00)	1(50.0)	0(0.0)	1(50.0)
Proteus mirabilis(n=1)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Escherichia spp(n=1)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(100.0)
Serratia spp(n=1)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Vibrio fluvialis	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

Key: AK-Amikacin; C- Chloramphenicol; MEM-Meropenem; CIP- Ciprofloxacin; CRO- Ceftriaxone; CAZ- Ceftazidime; AMC- Amoxicillin-clavulanate

5.3.5 Antibiotic Resistance profiles of non-Enterobacteriaceae isolates from Maamobi and Kaneshie vicinities

Non-Enterobacteriaceae isolates that were recovered from the two vicinities included *Enterococcus faecalis*, *Pseudomonas* spp., and *Staphylococcus* spp. Apart from *E. faecalis*, resistance to few antibiotics was observed among the other non-Enterobacteriaceae. Majority of the isolates were susceptible to almost all the antibiotics that were tested. More than 60% resistance to Rifampin was observed among the *E. faecalis* isolates (Table 5.5).

Table 5.5 Antibiotic Resistance profiles of Enterococcus faecalis isolates from both Maamobi and Kaneshie vicinities

	Pathogens/ sites *Enterococcus faecalis (n=14)*		
Antibiotics	**Total n(%)**	***Maamobi vicinity n(%)**	***Kaneshie vicinity n(%)**
VA	5(35.7)	2(33.3)	3(37.5)
E	4(28.6)	3(50.0)	1(12.5)
TE	3(21.4)	2(33.3)	1(12.5)
CIP	2(14.3)	1(16.7)	1(12.50)
F	2(14.3)	1(16.7)	1(12.5)
C	3(21.4)	2(33.3)	1(12.5)
RD	10(71.4)	3(50.0)	7(87.5)
P	2(14.3)	0(0.0)	2(25.0)
LZD	3(21.4)	1(16.7)	2(25.0)

Key: VA-Vancomycin; E-Erythromycin, TE-Tetracycline; CIP-Ciprofloxacin; F-Nitrofurantoin; C-Chloramphenicol; RD-Rifampin; P-Penicillin; LZD-Linezolid

*Six *E. faecalis* isolates were recovered from Maamobi General hospital while eight were recovered from Kaneshie polyclinic

5.3.6 Antimicrobial resistance profiles of the Enterobacteriaceae isolates from water samples and palm swabs

Among the Enterobacteriaceae organisms, no resistance was recorded in Gentamycin, Ticarcillin-clavulanate, Amikacin, Meropenem and Ciprofloxacin. There was resistance to the rest of the antibiotics among the *Klebsiella pneumoniae* pathogens. One of the *K. pneumoniae*

isolates that was derived in the palm of a food vendor possessed the *bla*$_{TEM}$ gene but was susceptible to all the antibiotics apart from Nitrofurantoin and Azithromycin (Table 5.6).

Table 5.6 Antibiotic resistance profiles of Enterobacteriaceae isolates from both vicinities

Pathogens	Antibiotics							
	SXT n (%)	TE n (%)	F n (%)	NA n (%)	AZM n (%)	C n (%)	CRO n (%)	AMC n (%)
***Acinetobacter* spp(n=4)**	0(0.0)	0(0.0)	3(75.0)	1(25.0)	0(0.0)	3(75.0)	2(50.0)	0(0.0)
***Enterobacter cloacae*(n=8)**	3(37.5)	2(25.0)	0(0.0)	0(0.0)	2(25.0)	0(0.0)	0(0.0)	6(75.0)
***Enterobacter* spp(n=5)**	0(0.0)	3(60.0)	1(20.0)	1(20.0)	2(40.0)	1(20.0)	0(0.0)	3(60.0)
***Citrobacter* spp(n=4)**	3(75.0)	3(75.0)	2(50.0)	1(25.0)	2(50.0)	1(25.0)	1(25.0)	3(75.0)
***Klebsiella pneumoniae*(n=15)**	1(6.7)	5(33.3)	8(53.3)	1(6.7)	7(46.7)	2(13.0)	1(6.7)	2(13.3)
***Aeromonas* spp(n=12)**	2(16.7)	1(8.3)	1(8.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

Key: SXT- Sulfamethoxazole-trimethoprim; TE-Tetracycline; F-Nitrofurantoin; NA- Nalidixic acid; AZM- Azithromycin; C- Chloramphenicol; CRO- Ceftriaxone; AMC- Amoxicillin-clavulanate

5.3.7 Antimicrobial resistance profiles of the non-Enterobacteriaceae isolates from water samples and palm swabs

All *Staphylococcus* spp isolates were resistant to Linezolid, Penicillin, Erythromycin, and Rifampin apart from reduced resistance in Erythromycin and no resistance in Ciprofloxacin. *E. faecalis* showed over 40% resistance to the antibiotics tested apart from Ciprofloxacin. *Pseudomonas* spp showed susceptibility to all the antibiotics apart from a low resistance (16.67%) that was reported in Ciprofloxacin (Table 5.7).

Table 5.7 Antibiotic resistance profiles of non-Enterobacteriaceae isolates from both vicinities

Pathogens	Antibiotics						
	LZD n (%)	TE n (%)	P n (%)	VA n (%)	E n (%)	RD n (%)	CIP n (%)
***Enterococcus faecalis*(n=8)**	2(40.00)	2(40.00)	2(40.00)	3(60.00)	2(40.00)	4(80.00)	0(0.00)
***Staphylococcus* spp(n=3)**	3(100.00)	1(33.33)	3(100.00)	2(66.67)	3(100.000	3(100.00)	0(0.00)
***Pseudomonas* spp(n=6)**	-	-	0(0.00)	-	-	0(0.00)	1(16.67)

Key: VA-Vancomycin; E-Erythromycin, TE-Tetracycline; CIP-Ciprofloxacin; RD-Rifampin; P-Penicillin; LZD-Linezolid

5.4 Discussion

5.4.1 Antimicrobial resistance of the stool isolates

The problem about increasing AMR in Ghana among common enteric pathogens like *E. coli*, *Salmonella* spp and *Shigella* spp have persisted over the years. A previous study conducted in 2005 and 2006 had shown resistance of *E.coli* to antibiotics like Ampicillin or amoxicillin, trimethoprim/ sulfamethoxazole and chloramphenicol among stool specimen that had been obtained from children who suffered diarrhoea in Northern Ghana (Djie-Maletz et al., 2008). This information confirmed Newman et al.'s publication in 2011, although the clinical isolates had been collected over a one year period (2002 to 2003) of high percentage resistance observed for tetracycline, cotrimoxazole, ampicillin and chloramphenicol (Newman et al., 2011). Quinolone resistance of between 6-10% in Ciprofloxacin, Amikacin and Ceftriaxone were observed among isolates from various clinical specimen that had been collected from several health facilities across the country in that study. This was then worrisome since the aforementioned drugs were expensive and had recently been introduced on the Ghanaian market as compared to the already existing drugs like ampicillin and chloramphenicol (Newman et al., 2011). Subsequently, when Opintan et al., (2015) performed another nationwide AMR surveillance, there was a surge of resistance to third generation cephalosporins like Ciprofloxacin and also Nalidixic acid by the gram-negative isolates as well as a >70% resistance to commonly used antimicrobials such as ampicillin, tetracycline, chloramphenicol, and trimethoprim–sulfamethoxazole (Opintan et al., 2015). This is consistent with this study which showed high level of resistance to Sulfamethoxazole-trimethoprim, Tetracycline, Azithromycin and Ampicillin among the Enterobacteriaceae organisms. Low levels of Meropenem resistance among the gram-negative bacteria was observed during Opintan et al.'s study in 2015, as compared to the gradually increasing carbapenemase-producing *Enterobacteriaceae* (CPE) in Meropenem which was observed in this study. This is

an indication of a public health alert worth paying attention to, since Carbapenems are the last line of defense against AMR Enterobacteriaceae-causing diseases. Routine surveillance to monitor the resistance patterns of foodborne bacteria in studies is much needed in this regard.

Over 30% of the *Salmonella* spp recovered in this study showed resistance to Tetracycline, Nitrofurantoin, Azithromycin and Amoxicillin-clavulanic acid. This is similar to the resistance profile observed by Akuffo et al. in 2017 when *Salmonella spp* recovered from the stool samples showed resistance to Nalidixic acid, Chloramphenicol, Tetracycline, ampicillin, ciprofloxacin and Aztreonam (Akuffo et al., 2017).

Known as an opportunistic commensal mostly found in water, sewage, vegetables and soil, *Enterobacter cloacae* has also been investigated to develop carbapenem resistance which is leading to prolonged delay in the treatment of related hospital care like bacteremia in children, septicemia, pneumonia and UTIs in both developed and developing countries (Annavajhala et al., 2019). Carbapenem Resistant *Enterobacter cloacae* (CREC) was observed in this study showing an overall 10% resistance in Meropenem occurring in Maamobi and Kaneshie health facilities. Their presence should not be taken for granted like most *E. coli* which are the normal flora in the human gut due to the possible transfer of resistance to other pathogenic organisms that could lead to diarrhoeal infections which may be difficult to treat (Bai et al., 2017; Taitt et al., 2017).

Citrobacter freundii which is also an opportunistic pathogen, normally found in stool, water and soil is of growing concern due to several infections (as *E. cloacae*) it can cause, especially among immunocompromised patients and normally occurring in hospital settings as well (Liu et al., 2017). It is one of the most common enteric pathogens that are isolated among diarrhoeal patients both in Ghana and other parts of the world (Duedu et al., 2017; Kibwana, Majigo, Kamori, & Manyahi, 2020; Liu et al., 2017). Resistance to Ceftriaxone, Tetracycline, Gentamycin and ampicillin was reported among stool samples isolated from human diarrhoea

patients in Maanshan Anhui Province, China while Duedu and colleagues also reported Chloramphenicol, ampicillin and Tetracycline resistance in *C. freundii* isolates from the stool samples of diarrhoea patients in a psychiatric hospital in Ghana (Duedu et al., 2017; Liu et al., 2017).

Another Enterobacteriaceae pathogen worth paying attention to is *Proteus mirabilis*, which frequently causes catheter-associated tract infections (CAUTI), also found in the environments (soil, water, and sewage) and known to be a commensal in the human and animal gut. Its ability to swarm based on several phenotypic and genotypic factors increase their spread and resistance as well (Armbruster, Mobley, & Pearson, 2018). Second to be the most recovered bacteria, *E. coli* among stool samples from patients in a psychiatric hospital in Ghana, it was shown to be Multidrug resistant (MDR) and resistant to ampicillin, chloramphenicol and tetracycline (Duedu et al., 2017). MDR in *P. mirabilis* was also seen in this study with > 50% resistance to Sulfamethoxazole-trimethoprim, Ticarcillin-clavulanate, Tetracycline, Nitrofurantoin, Azithromycin and Chloramphenicol as well as a 12.5% resistance to Meropenem. Its commensal ability makes it highly pathogenic and virulent, making it another probable candidate capable of transferring various genetic traits like resistant genes to other pathogenic or non-pathogenic bacteria (Armbruster et al., 2018).

Most of the AMR among the pathogens recovered in both Maamobi General hospital and Kaneshie Polyclinic showed similar resistance profiles apart from Ceftazidime and Ticarcillin-clavulanate resistance which occurred only among the *E. coli* pathogens but was susceptible among all the other Enterobacteriaceae organisms recovered from Maamobi General but not Kaneshie Polyclinic. Overall, *E. coli* showed the highest occurrence in resistance to all the antibiotics as compared to all the other Enterobacteriaceae organisms. In view of this, *E. coli* when recovered in stool and other foodborne related studies should be further investigated due

to its possession of MDR properties, making it a difficult pathogen to treat and a potential source of transfer of AMR genes to other FBPs.

P. mirabilis isolates were resistant to all the antibiotics tested apart from the aminoglycosides (Gentamicin and Amikacin). Their capability to cause antibiotic resistant infections should not be overlooked due to their hypermotility and biofilm properties which could sometimes lead to the transfer of AMR to other bacteria organisms that may be present in the gut (Luzzaro et al., 2001; Oduro-Mensah et al., 2016).

5.4.2 Antimicrobial resistance of the food isolates

Enterobacter cloacae: There was 60% resistance to Nitrofurantoin and Amoxicillin-clavulanate among the *E. cloacae* food isolates with lower resistance to Azithromycin, Tetracycline, Sulfamethoxazole-trimethoprim, Gentamicin, Nalidixic acid, Amikacin, Chloramphenicol and Ciprofloxacin shows the emerging issue of AMR in food which can pose a public health threat indicating difficult to treat enteric infections when transmitted. High Ampicillin and Ceftazidime resistance (70% each) was observed among the *Enterobacter* spp recovered in RTE food in a study at Gondar Town, Ethiopia (Eromo et al., 2016).

Due to the increasing AMR problem among most FBPs, it will be good to monitor the trend of resistance especially when their presence in humans can lead to a sequel of medical events (difficulty in treatment leading to prolonged hospitalization). Meropenem resistance was absent to show the current absence of Carbapenem- resistant *Enterobacter* (CRE) in food. This is the situation when *Enterobacter* spp related infections resists treatment to Carbapenems, making their treatment complex and requires several antibiotic combinations for treatment (Ramirez & Giron, 2020).

Citrobacter freundii: *Citrobacter freundii* known to be an MDR and has shown varying resistance to several classes of antibiotics when isolated in food and other clinical and

environmental samples. Worthy of mention is the resistance of the pathogen to Doxycycline and Amikacin among food samples that were sampled in Hawassa, Ethiopia (Eromo et al., 2016). Amare and colleagues confirmed the dual presence of *C. freundii* and *E. cloacae* like most Ghanaian and other countries with a 100% resistance to Ceftriaxone, Tetracycline, Gentamycin and Ampicillin among the *C. freundii* isolates recovered from some popular street vended foods from Gondar town, Ethiopia (Amare et al., 2019). In analyzing the microbiological quality of RTEs in Dhaka, Bangladesh, Banik and colleagues identified resistance to Ampicillin and Sulfamethoxazole among the *C. freundii* isolates recovered from the food and raised the concern of targeted public awareness and timely assessment of food safety to avoid foodborne infection risks and RTE food intoxication due to their abuse (Banik et al., 2019).

Consistent with this study were the resistance observed among almost all the antibiotics except among the B-lactams; (Ticarcillin-clavulanate, Piperacillin-tazobactam) and the fluoroquinolone; (Ciprofloxacin) although there was an occurrence of ESBL in *Citrobacter freundii*, bla_{TEM} in two food items ('kenkey' and spaghetti) from the Maamobi vicinity. This supports the popular occurrence of bla_{TEM} among foodborne Enterobacteriaceae pathogens in many foodborne RTE food studies (Liu et al., 2017; Ye et al., 2018).

Highest resistance to Sulfamethoxazole-trimethoprim, Tetracycline and Amoxicillin-clavulanate consistent with the AMR profiles from the different countries, shows the gradual emerging AMR trend occurring among food samples in Ghana due to their common use in clinical, environmental, and agricultural settings. These antibiotics showed high resistance to the various Enterobacteriaceae isolates that were tested during a six-month nationwide surveillance in Ghana (Newman & Opintan, 2015). This shows the similarities of the AMR trends among humans and the environment as well.

***Pseudomonas* spp**: Resistance have been reported to occur in aminoglycosides, quinolones and B-lactams due to several factors that have been classified into intrinsic (efflux pumps, low outer membrane permeability and antibiotic inactivating enzymes), acquired (horizontal transfer or changes in mutations) and adaptive resistance (biofilm formation) (Pang et al., 2019). Despite this growing problem, fortunately, all the *Pseudomonas* spp isolates were not resistant to any of the antibiotics but inconclusive due to the lack of genotypic testing to affirm this finding.

Proteus mirabilis: Although there is paucity of data of AMR of *P. mirabilis* in food despite its frequent isolation in RTE food, clinical studies that have been conducted in Ghana have reported resistance in the bacteria to Ampicillin, Tetracycline, Cotrimoxazole, Gentamicin, Ceftriaxone, Cefotaxime, Cefuroxime and Chloramphenicol (Duedu et al., 2017; Feglo et al., 2010). Although only Nitrofurantoin and Tetracycline resistance in *P. mirabilis* was observed in this study, due to the resistance to various classes of antibiotics, especially among the beta-lactamases and cephalosporins, there is a limit on the option of treatment, causing difficulty in the treatment of *P. mirabilis* related infections like bladder and kidney stone, nephrosis and bacteremia and therefore leads to prolonged hospitalization and further nosocomial transmissions (Anning et al., 2019).

MDR *P. mirabilis* have been recovered from money (coins and notes) of various denominations on the streets of several towns in Ghana which is an indication of vendors hands being vehicles of fecal contamination and the spread of foodborne illnesses due to frequent handling (Anning et al., 2019; Yar, 2020). Resistance to Erythromycin, Ciprofloxacin, Ampicillin, Vancomycin, Amikacin, Tetracycline and Cotrimoxazole is of public health concern due to the inclusion of some beta-lactamases and cephalosporins as well, known to pose a public health concern due to difficulty in treatment and possibility of spreading among environmental, animal, and human settings.

Escherichia coli: Similar to this study but inconsistent to the number of occurrence in RTE foods have been shown to occur in several studies with common resistance to Ampicillin, Erythromycin and Tetracycline but mostly susceptible to Ciprofloxacin (Amare et al., 2019; Banik et al., 2019; Eromo et al., 2016; Sivakumar et al., 2020). Resistance to Tetracycline and Ampicillin but susceptibility to Ciprofloxacin among *E. coli* bla_{TEM} isolates is similar to Dsani and colleagues work on raw meat in Ghana (Dsani et al., 2020).

Susceptibility to all the antibiotics except Nalidixic acid Tetracycline indicates a wide array of antibiotics that can be used to treat *E. coli*-related foodborne illnesses in certain parts of Accra, therefore showing the unlimited options of antibiotic treatment. High number of *E. coli* occurrence have been reported in various RTE food studies in Ghana and other parts of the world as well (Ababio & Lovatt, 2015; Abas et al., 2019; Mensah et al., 2002; Saba & Gonzalez-Zorn, 2012; Yeboah-Manu et al., 2010).

Klebsiella pneumoniae: Owing to growing resistance to antibiotics among several bacteria; both commensal and opportunistic, the presence of *K. pneumoniae* should not be taken lightly due to its increasing resistance to several antibiotics including 3rd and 4th generation cephalosporins in several parts of the world. On the advent of carbapenem resistant *Klebsiella pneumoniae* which leads to liver abscess and leading to the transfer of various nosocomial infections, there is the need for regular monitoring of the AMR trend when it occurs in food (Hartantyo et al., 2020; Lübbert et al., 2014).

Resistance to Nitrofurantoin, Azithromycin, Amikacin, and Amoxicillin-clavulanate among the *Klebsiella pneumoniae* isolates recovered in some of the food samples in this study is inconsistent with both clinical and a food studies that have been conducted in the past (Ayeh-Kumi et al., 2014; Duedu et al., 2017; Egyir, Nkrumah-Obeng, et al., 2020; García-Vello, González-Zorn, & Saba, 2020). This implies diarrhoea related to *K. pneumoniae* transmission may be resistant to some of these commonly used antimicrobials. It is important for vendors

to practice better hygiene to reduce the spread of these resistant enteric pathogens to avoid the difficulty in treatment.

Acinetobacter baumannii: There was resistance to several antibiotics (Nitrofurantoin, Nalidixic acid, Azithromycin, Amoxicillin-clavulanate). Albeit susceptibility to Carbapenems is an indication of the continuous reliance on this group in the case of a foodborne disease outbreak which is caused by *A. baumanii.* There remains the need for continuous surveillance of FBPs in RTEs due to the emerging resistance.

***Aeromonas* spp**: There was resistance to Nitrofurantoin, Nalidixic acid, Azithromycin and Amoxicillin-clavulanate but susceptible to all the other antibiotics is similar to several reports of *Aeromonas spp* being resistant to penicillin and ampicillin, but sensitive to aminoglycosides, tetracycline, chloramphenicol, trimethoprim-sulfamethoxazole, quinolones and 2nd and 3rd cephalosporins (Stratev & Odeyemi, 2016). Feglo et al., identified *Aeromonas* spp as one of the common pathogens in all food samples including ground pepper and salads that were tested from RTEs in Kumasi (Feglo & Sakyi, 2012).

***Staphylococcus* spp**: There was no resistance among the *Staphylococcus* spp organisms, even though resistance to several antibiotics have been reported indicating difficulty to treat staphylococcal infected individuals through food (Eromo et al., 2016).

Enterococcus faecalis: This organism is sometimes used in the fermentation of certain foods like cheese and certain doughs, excesses and the possession of AMR genes could lead to resistance to several antibiotics including ciprofloxacin, quinupristin/ dalfopristin, rifampicin or sometimes Vancomycin which could lead to difficult to treat diseases like UTIs, endocarditis, bacteremia, catheter-related infections, wound infections and pelvic infections (Rolain, 2013).

Resistance to almost all antibiotics tested (Vancomycin, Erythromycin, Tetracycline, Ciprofloxacin, Nitrofurantoin, Chloramphenicol, Rifampin, Penicillin and Linezolid) was observed among all the *E. faecalis* recovered from the RTE food in this study is consistent with the Enterococcal-related RTE in food worldwide occurrences (Dewey-Mattia et al., 2018; Pesavento et al., 2014; WHO/Europe, 2011).

Other organisms (*Serratia* spp and Vibrio *fluvialis*): Most RTE food studies do not have records of the presence of *Serratia* spp (Mensah et al., 2002; Ye et al., 2018). Therefore, it was not surprising when only two food items (jollof and stew) recorded the presence of *Serratia* spp in this study with resistance to only Tetracycline and susceptible to all other antibiotics. Resistance to Cephalothin, ampicillin and amoxicillin but susceptible to many antibiotics have been reported in Brazil (Amorim et al., 2018).

Although worldwide reports indicate the existence of resistance among *Vibrio* spp (quinolones, penicillins, ESBLs) (Ramamurthy et al., 2014). This bacterium was susceptible to all antibiotics, which means there would have been treatment response if *V. fluvialis* diarrhoea related infections had occurred.

5.4.3 Antimicrobial resistance of the water and palm isolates

5.4.3.1 Antimicrobial resistance profile of the Enterobacteriaceae pathogens isolated in water and palm samples

From the antibiotic susceptibility patterns recorded, five antibiotics (Ticarcillin-clavulanate, Gentamycin, Piperacillin-tazobactam, Meropenem and Ceftazidime) were recorded to be universally effective against all the bacterial strains isolated. Resistant patterns identified in stored drinking water from villages of South Tongu and East Dangbe districts in 2002, showed resistance of *K. pneumoniae* and *E. coli* to some Cephalosporins, fluoroquinolones, Tetracycline, and Penicillins, concurrent to this study where a similar array of antibiotics are

showing resistance by *K. pneumoniae* and *C. freundii* (Amoah, Odamtten, & Longmatey, 2006). Although several studies have been carried out to investigate the bacteriological quality of sachet/ bottled water in Ghana, there is paucity of data regarding their AMR profiles (Addo et al., 2009; Mosi et al., 2019) unlike a sachet water study that was carried out in Nigeria (Anyanwu, 2010) which showed resistance to several antibiotics including some Cephalosporins as well as fluoroquinolones. The presence of *Staphylococcus* spp in the cooking water demonstrated the lack of proper personal hygiene of the street food vendors in handling the water onsite. Recurrent fetching of water from storage containers could lead to this when care is not taken.

Based on Adesoji and Ogunjobi's finding, it is possible that the storage water that food vendors and consumers in general use in Ghana could already be contaminated from source and can cause difficult-to-treat foodborne infections when consumed (Adesoji & Ogunjobi, 2016). Ecklu-Mensah et al., 2019 in exploring the bacterial diversity of drinking water samples in western Accra, Ghana isolated from waterworks, household taps and storage polytanks found various genera of bacteria, especially in the storage tanks (Ecklu-Mensah et al., 2019). This buttresses the possibility of source (waterworks) contamination and the fact that most source of water used in both domestic and commercial activities like food vending are from storage/ polytanks used to keep water from the main water works source. Several foodborne and waterborne outbreaks have been reported in the past due to microbially contaminated storage tanks (Rane, 2011). One of the key areas of water pollution that should be given attention is in water retailing, to both domestic and commercial users. Education and regular monitoring of stakeholders in this area will help to reduce the possibility of waterborne transmission through storage tanks.

This study brought to light the need for AMR surveillance to be carried out in water studies to buttress their already existing data of microbiological assessment. Good water handling

practices like acquisition from secured sources, storage conditions and use at street vending sites should be emphasized to reduce the spread of resistant bacteria.

5.4.3.2 Prevalence and antimicrobial resistance profile of the non-Enterobacteriaceae pathogens isolated in water and palm samples

Staphylococcus spp is known to cause gastroenteritis resulting in diarrhoea, fever, vomiting and abdominal pains (Bintsis, 2017; Rane, 2011). The fact that *Staphylococcus* spp is non-indicative of a possible fecal-oral transmission but skin flora predisposes the need for good hand hygiene practices to be engaged among street food vending operators. Also, the presence of *E. faecalis* in storage and washing water is indicative of fecal contamination from the street vendor operators. They are hardy and resistant to many chemicals and drying more than *E. coli* and as such very dangerous when found in water (WHO, 2011).

Although few non- Enterobacteriaceae pathogens were recovered during the study, none of the antibiotics could effectively be used in getting rid of all three pathogens. Apart from *Pseudomonas* spp which showed reduced/ no resistance to Ciprofloxacin and other antibiotics, there was a high resistance to all the antibiotics among both *E. faecalis* and *Staphylococcus* spp. This implies there could be difficulty in treating a waterborne infection in the case of transmission and infection in humans. The presence of MDR *Staphylococcus* spp in cooking and washing water is indicative of difficulty in treatment should an infection occur which is caused by the pathogen.

6 CHAPTER SIX: OCCURENCE OF EXTENDED SPECTRUM BETA-LACTAMASES (ESBL) AND DIARRHOEAGENIC E. COLI (DEC) GENES

6.1 Introduction

Extended Spectrum beta-lactamases (ESBLs) are enzymes that are produced by bacteria that confers multidrug resistance to beta-lactam antibiotics such as cephalosporins, carbapenems, monobactams and cephamycins by hydrolyzing their beta-lactam rings; therefore rendering their antibacterial properties futile (Shaikh et al., 2015).

The presence of specific AMR genes like ESBLs is known to confer resistance to various antibiotics in bacteria. In view of this, PCR was carried out on Enterobacteriaceae isolates to investigate the presence of Extended spectrum beta-lactams. Few studies in Ghana have investigated the presence of ESBLs in clinical and food samples, however, there is paucity of data regarding ESBL in RTE food and water samples (Agyekum et al., 2016; Alexander, Roland, Anthony, & Matthew, 2020; Dsani et al., 2020; Falgenhauer et al., 2019; Obeng-Nkrumah et al., 2013; Oduro-Mensah et al., 2016). This study filled that gap of investigating their presence in both stool and food from common locations.

Diarrhoeagenic *E. coli* (DEC) are strains of *E. coli* with the potential of causing disease in humans despite the fact that most strains are human gut commensals (Vidal et al., 2005).

Also, six different DEC types (EHEC, EPEC, EIEC, ETEC, STEC, EAgEC) were investigated among the *E. coli* samples that were isolated from all sample types. Various types of diarrhoea have been associated with some of these DEC types sampled from diverse sample types in from different regions of the world (Okeke, 2009). This study sought to investigate the specific DEC types occurring in stool, food and water samples in Ghana using PCR.

6.2 Materials and Methods

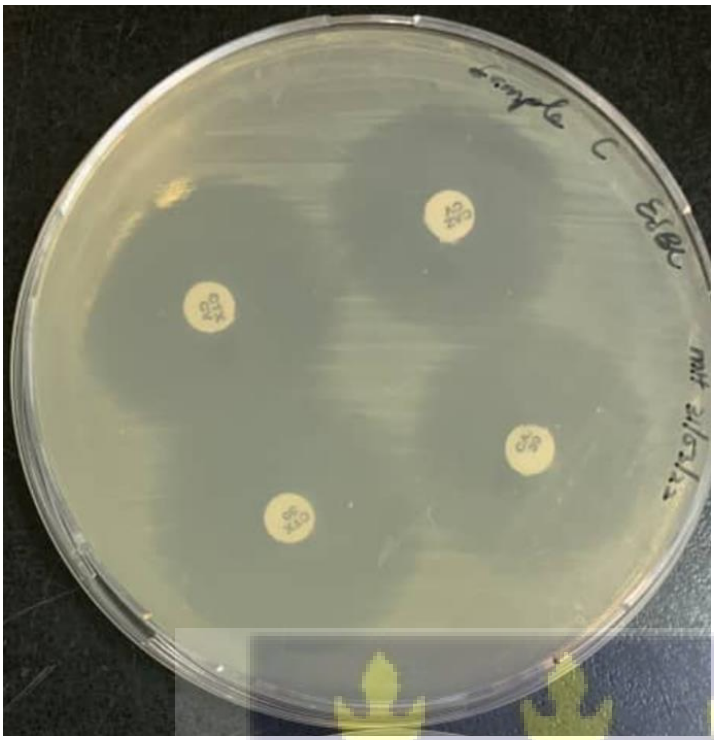


Figure 6.1: Double-disk synergy test

Key: CAZ- Ceftazidime, CAZ/CV-Ceftazidime/Clavulanic Acid, CTX-Cefotaxime, CTX/CV- Cefotaxime/Clavulanic Acid

6.2.1 Extended Spectrum Beta- Lactamase (ESBL) phenotypic testing

In the cases of MDRs, prevalence of resistant strains such as ESBLs and MRSA was detected. Briefly, ESBL phenotype was detected by the double disk diffusion test. Here, cefotaxime (30 µg) (or ceftazidime (30µg)) discs was placed on Mueller-Hinton agar plates using sterile forceps, 20 mm apart from a disc containing clavulanic acid (10µg) alone (single) and another disc containing a combination of either cefotaxime and clavulanic acid or ceftazidime and clavulanic acid (Dsani et al., 2020; Egyir, Hadjirin, et al., 2020; Egyir, Nkrumah-Obeng, et al., 2020) as illustrated in Figure 6.1. After incubation for about 37˚C for 18-24 hours, the zone of inhibition to the nearest millimeter was measured using a ruler of a digital caliper.

The difference between the zone sizes of each of the paired disks are calculated. In that, the difference between zone size for Ceftazidime and Ceftazidime/ Clavulanic acid were calculated. The same procedure applied to the difference between zone size for Cefotaxime and Cefotaxime and Cefotaxime/ Clavulanic acid. A zone size difference of ≥5mm between the single and the combination was regarded positive for ESBL production whiles negative results were indicated by zone size difference of <5mm (Figure 6.2). *Klesbsiella pneumoniae* ATCC 700603 served as a positive control for ESBL production while *Escherichia coli*, ATCC 25922 was used as a negative control with each batch of ESBL testing that was performed.

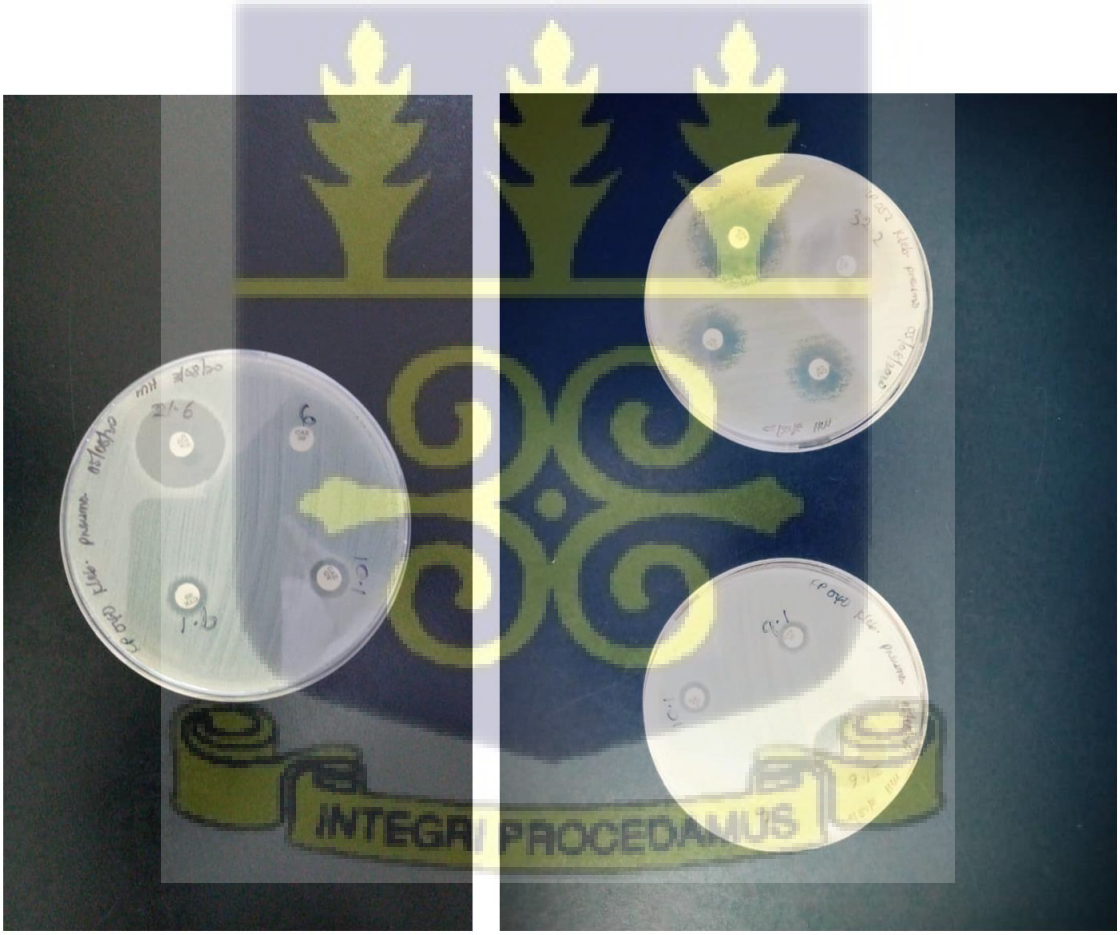


Figure 6.2: A positive ESBL phenotypic testing on three *K. pneumoniae* isolates

6.2.2 Polymerase chain reaction (PCR) of ESBL genes and diarrhoeagenic *E. coli* (DEC) genes

To have deoxyribonucleic acid (DNA) from the Enterobacteriaceae isolates, extraction is conducted using the crude method. Fresh overnight cultures of colonies from pure isolates (about 6) were transferred into 180µl of nuclease-free water. After vortexing and heating at 100℃ for 10mins, the inoculum is centrifuged for 5 minutes at 14000rpm. The supernatant was used for both genotypic ESBL and diarrhoeagenic *E. coli* PCR. Tables 6.1 and 6.2 illustrates various primer sequences for ESBL and DEC PCR testing.

Table 6.1 DEC and ESBL primer sequence details

Gene	Primer sequence (5'-3')	Target	Expected product size (bp)
Stx$_1$	CAG TTA ATG TGG TGG CGA AGG CAC CAG ACA ATG TAA CCG CTG	shiga toxin-1 gene of STEC	348
Stx$_2$	ATC CTA TTC CCG GGA GTT TAC G GCG TCA TCG TAT ACA CAG GAG C	shiga toxin-2 gene of STEC	584
eae	TCA ATG CAG TTC CGT TAT CAG TT GTA AAG TCC GTT ACC CCA ACC TG	attaching and effacing lesion of EPEC	482
lt	GCA CAC GGA GCT CCT CAG TC TCC TTC ATC CTT TCA ATG GCT TT	heat labile gene of ETEC	218
stII	AAA GGA GAG CTT CGT CAC ATT TT AAT GTC CGT CTT GCG TTA GGA C	heat stable gene of ETEC	129
VirF	AGC TCA GGC AAT GAA ACT TTG AC TGG GCT TGA TAT TCC GAT AAG TC	gene responsible for transcription of virulent factors of EIEC	618
ipaH	CTC GGC ACG TTT TAA TAG TCT GG GTG GAG AGC TGA AGT TTC TCT GC	invasion plasmid antigen H of EIEC	933
daaE	GAA CGT TGG TTA ATG TGG GGT AA TAT TCA CCG GTC GGT TAT CAG T	fimbrial adhesin gene of DAEC	542
aafII	CAC AGG CAA CTG AAA TAA GTC TGG ATT CCC ATG ATG TCA AGC ACT TC	aggregative adherence fimbrae-II genes of EAEC	378

DEC Primer sequences adopted from *Vidal et al. (2005)

Table 6.2 ESBL primer sequence details

Gene	Primer sequence (5'-3')	Target	Expected product size (bp)
bla$_{CTX-M}$	GAA GGT CAT CAA GAA GGT GCG GCA TTG CCA CGC TTT TCA TAG	cefotaximase (*CTX-M*) beta-lactamase	560
bla$_{SHV}$	GTC AGC GAA AAA CAC CTT GCC GTC TTA TCG GCG ATA AAC CAG	sulfhydryl (*SHV*) beta-lactamase	383
bla$_{TEM}$	GAG ACA ATA ACC CTG GTA AAT AGA AGT AAG TTG GCA GCA GTG	temoneira (*TEM*) beta-lactamase	459
***ESBL Primer sequences adopted from Sharma 2013**			

6.2.3 ESBL PCR

A multiplex PCR comprising a cocktail of ESBL primers was conducted to detect either one, two or three of the ESBL genes (*bla*$_{CTX-M}$, *bla*$_{SHV}$ and *bla*$_{TEM}$) in the Enterobacteriaceae isolates that were tested. A multiplex master mix consisting of 10mM of each primer, multiplex PCR master mix (13µl) (QIAGEN), PCR grade water (10µl) and 2µl DNA template, making a final volume of 25µl. Cycling conditions (Sharma, 2013) and amplification was performed in an Eppendorf Master cycler X50s. The cycling conditions were as follows; 95°C for 5 minutes, 35 cycles of 95°C for 30 seconds, 60°C for 30 seconds, 72°C for 2 minutes, and a final extension lap at 72°C for 10 minutes. DNA products were taken through agarose gel electrophoresis at 100 volts for 1 hour with a 2% agarose gel concentration. The gel was viewed on a UV platform using Gel Documentation system XR+ (Bio-Rad, USA) using Image lab version 5.0 build 18.

6.2.4 DEC PCR

The diarrhoeagenic gene of each *E. coli* isolate was determined using nine virulence genes to test for Shiga toxin-producing (*stx1* and *stx*2), enteropathogenic (*eae*), enterotoxigenic (*stII* and *lt*), enteroinvasive (*virF* and *ipaH*), enteroaggregative (*aafII*), and diffuse adherent (*daaE*). Each 25µl of reaction mixture contained 0.5µl of each primer and deoxynucleotide triphosphates, 5µl $MgCl_2$, 0.25µl of Taq DNA polymerase and 3µl of template DNA. PCR was performed based on a few modifications from Vidal et al., with the following cycling conditions; 1.5min at 94˚C for denaturation, 1.5min at 60˚C for primer annealing, amplification for 35 cycles, and 1.5 min at 72˚C for strand elongation. Agarose Gel Electrophoresis was conducted at 100 volts for 1 hour with a 1.5% agarose gel stained with SYBR Safe DNA gel stain (Invitrogen, Thermo Fisher Scientific, USA). Visualization of the gel was conducted on a UV platform using Gel Documentation system XR+ (Bio-Rad, USA) using Image lab version 5.0 build 18 (Vidal et al., 2005).

6.2.5 Data Analysis

ESBLs and DEC were described using percentages, frequencies, pie charts and bar graphs.

Association between ESBL carriage (ESBL associated diarrhoea versus ESBL not associated with diarrhoea) with the antibiotics tested was done using Fishers' exact.

6.3 Results

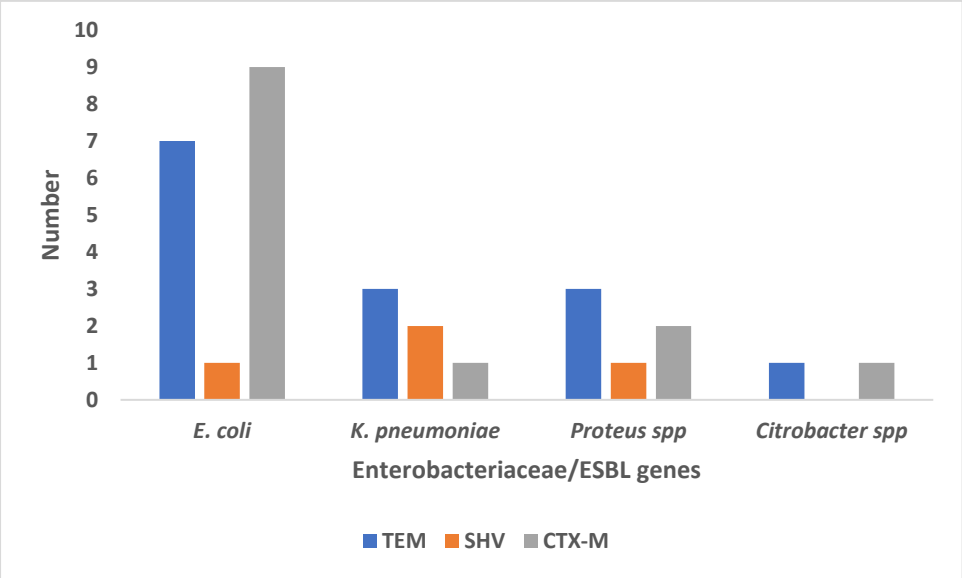


Figure 6.3: The different ESBL genes recovered among the various Enterobacteriaceae isolates

6.3.1 ESBLs in stool

Three different ESBL genes were identified during the study among some of the *E. coli*, *K. pneumoniae* and *Proteus mirabilis*, and *Citrobacter freundii* isolates (Figure 6.3). These were *TEM, SHV* and *CTX-M* genes identified among all three Enterobacteriaceae bacteria apart from *Citrobacter freundii* isolate which did not harbor the *SHV* gene. The *CTX-M* gene showed the highest occurrence among the *E. coli* isolates from stool as compared to the other ESBL genes.

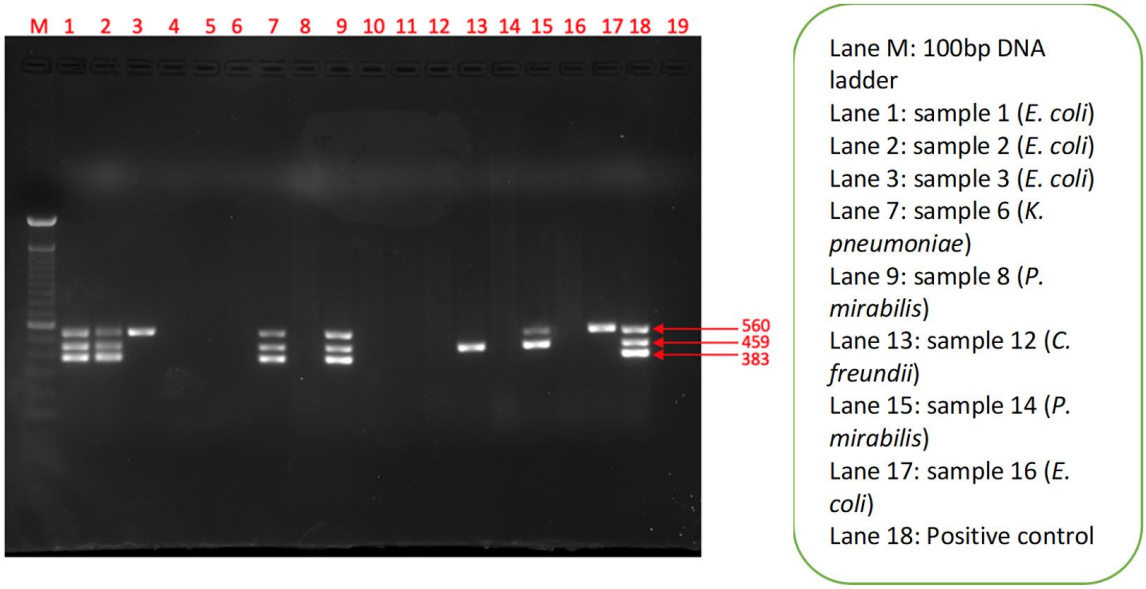


Figure 6.4: Gel red-stained agarose gel (2%) of amplified PCR products for the identification of ESBL-PE

The molecular weight marker or the DNA ladder, denoted by M (Lane M) was used to detect the appropriate sizes of the molecules that were run on the gel as illustrated in Figure 6.4. Lanes 1, 2, 7 and 9 were isolates that possessed all three ESBL genes.

6.3.2 Antibiotic resistance among ESBL Associated diarrhoea and non ESBL Associated diarrhoea

During the study, the relationship between ESBL Associated diarrhoea and non ESBL associated diarrhoea with antibiotic resistance among the stool isolates was sought to understand the existing strains recovered and Multidrug resistance (MDR). It was noticed that patients whose diarrhoea was caused by ESBLs were more likely to be MDRs as compared to patients whose diarrhoea were non ESBL associated (89.5% vs. 62.5%; p=0.027). In this light, ESBL isolates recovered had a significant association with resistance to multiple drugs such as Chloramphenicol, Ciprofloxacin, Nalidixic acid, Piperacillin-tazobactam,

Sulfamethoxazole-trimethoprim, Ticarcillin-clavulanate, Nitrofurantoin, and Gentamicin as compared with non ESBL isolates (Table 6.2).

Table 6.3 Antibiotic resistance among ESBL Associated diarrhoea and non ESBL Associated diarrhoea

Characteristics	Total	ESBL Producing diarrhea n(%)	Non ESBL Producing diarrhoea n(%)	*p*-value
SXT				
Resistant	58	17(89.5)	41(64.1)	0.046
Non-Resistant	25	2(10.5)	23(35.9)	
TIM				
Resistant	20	10(52.6)	10(15.6)	0.002
Non-Resistant	63	9(47.4)	54(84.4)	
TE				
Resistant	61	15(79.0)	46(71.9)	0.768
Non-Resistant	22	4(21.1)	18(28.1)	
F				
Resistant	29	12(63.2)	17(26.6)	0.006
Non-Resistant	54	7(63.2)	47(73.44)	
CN				
Resistant	8	5(26.3)	3(4.7)	0.014
Non-Resistant	75	14(73.7)	61(95.3)	
NA				
Resistant	37	12(63.2)	25(39.1)	0.073
Non-Resistant	46	7(36.8)	39(60.9)	
TZP				
Resistant	11	6(31.6)	5(7.8)	0.015
Non-Resistant	72	13(68.4)	59(92.2)	
AZM				
Resistant	55	14(73.7)	41(64.1)	0.583
Non-Resistant	28	5(26.3)	23(35.9)	
AK				
Resistant	4	1(5.3)	3(4.7)	1.000
Non-Resistant	79	18(94.7)	61(95.3)	
C				
Resistant	27	10(52.6)	17(26.6)	0.050
Non-Resistant	56	9(47.4)	47(73.4)	
MEM				
Resistant	4	1(5.3)	3(4.7)	1.000
Non-Resistant	79	18(94.7)	61(95.3)	
CIP				
Resistant	28	11(57.9)	17(26.6)	0.025
Non-Resistant	55	8(42.1)	47(73.4)	
CRO				
Resistant	18	14(73.7)	4(6.3)	<0.001
Non-Resistant	65	5(26.3)	60(93.8)	
CAZ				
Resistant	12	11(57.9)	1(1.6)	<0.001
Non-Resistant	71	8(42.1)	63(98.4)	
AMC				
Resistant	13	3(15.8)	10(15.6)	1.000
Non-Resistant	70	16(84.2)	54(84.4)	

Multidrug Resistance				
MDR	26	17(89.5)	40(62.5)	0.027
Not MDR	57	2(10.5)	24(37.5)	
Total	**83**	**19**	**64**	-

Key: SXT-Sulfamethoxazole-trimethoprim; TIM- Ticarcillin-clavulanate; TE-Tetracycline; F-Nitrofurantoin; CN-Gentamicin; NA- Nalidixic acid; TZP- Piperacillin-tazobactam; AZM- Azithromycin; AK-Amikacin; C- Chloramphenicol; MEM-Meropenem; CIP- Ciprofloxacin; AMC- Amoxicillin-clavulanate; MDR- Multidrug resistant

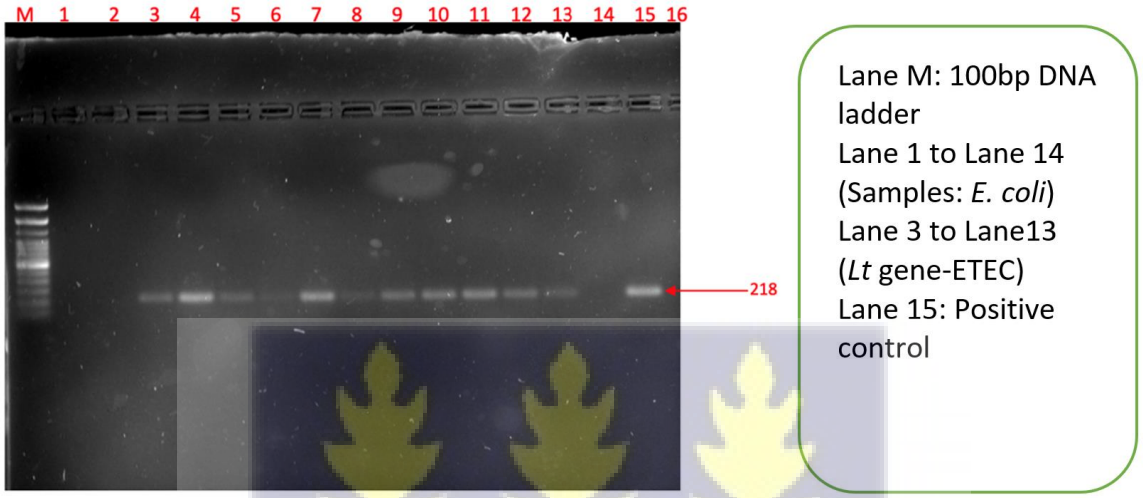


Figure 6.5: Gel red-stained agarose gel (1.5%) of amplified PCR products for the identification of DEC

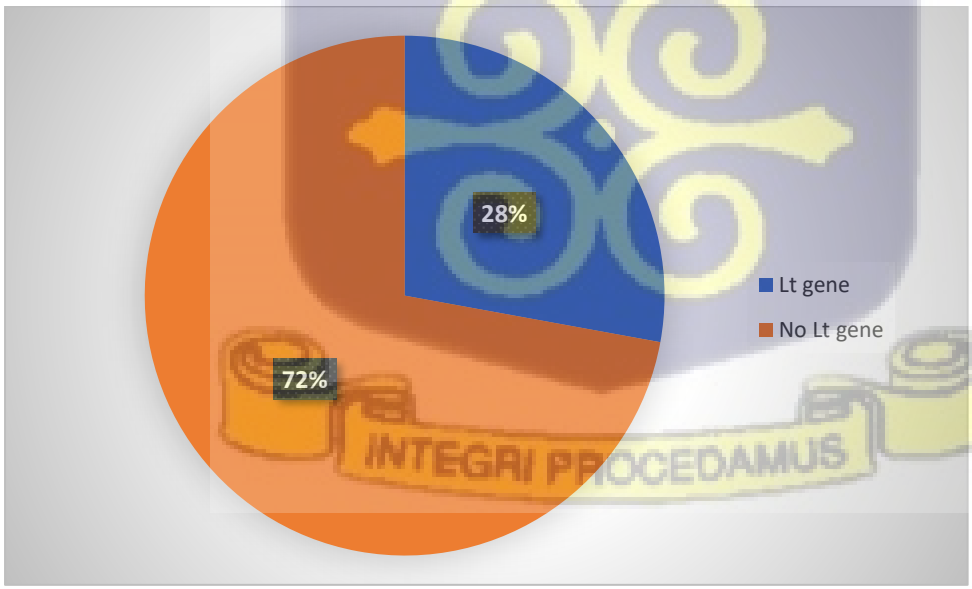


Figure 6.6: Diarrhoeagenic *E. coli* (DEC) recovered from both Maamobi General hospital and Kaneshie Polyclinic showing the presence of the *Lt* gene of Enterotoxigenic *E. coli* (ETEC)

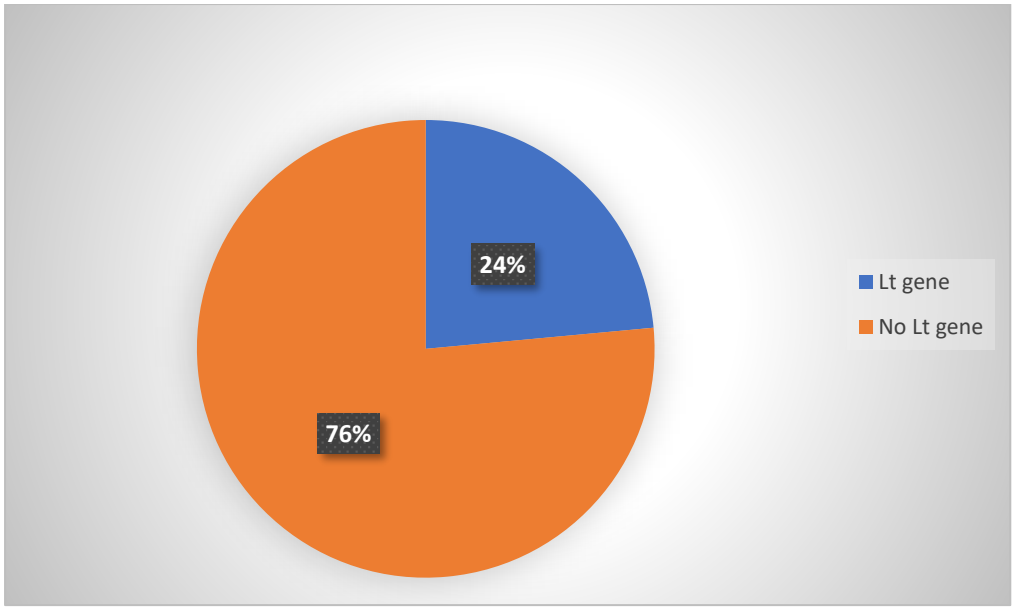


Figure 6.7: Diarrhoeagenic *E. coli* (DEC) recovered from Maamobi General hospital showing the presence of the *Lt* gene of Enterotoxigenic *E. coli* (ETEC)

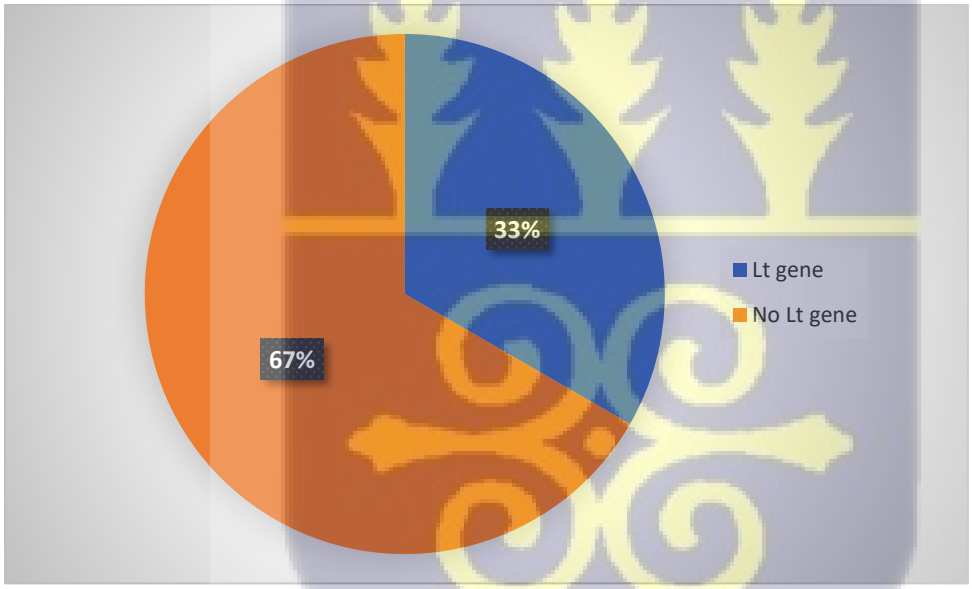


Figure 6.8: Diarrhoeagenic *E. coli* (DEC) recovered from Kaneshie polyclinic showing the presence of the *Lt* gene of Enterotoxigenic *E. coli* (ETEC)

6.3.3 Diarrhoeagenic *E. coli* (DEC) occurrence in stool

Out of the six diarrhoeagenic *E. coli* genes that was tested among the 76 *E. coli* isolates from stool, 34 tested positive for the heat labile gene (*Lt* gene) of Enterotoxigenic *E. coli* (ETEC). All *E. coli* isolates tested negative for the rest of the five diarrheagenic *E. coli* genes.

Fig 6.5 shows the DEC results of PCR products after gel electrophoresis.

Figure 6.6 shows the total numbers of the *Lt* gene recovered from both sites. A greater percentage of the *Lt* gene were recovered from the Maamobi hospital (76%) as compared to Kaneshie polyclinic (67%). Pie charts of the recovery of the *Lt* genes according to the sites are shown in Figure 6.7 and Figure 6.8.

The molecular weight marker or the DNA ladder, denoted by M (Lane M) was used to detect the appropriate sizes of the molecules that were run on the gel as illustrated in Figure 6.5. Lane 3 to Lane 13 show *E. coli* isolates with the *Lt* gene of ETEC.

6.3.4 Occurrence of ESBL and DEC genes in food

Among the various food items that were collected, bla_{TEM} was found among two *Citrobacter freundii* isolates that were recovered from Indomie/ spaghetti and 'kenkey' respectively.

The *Lt* gene of ETEC was recovered from two food items: Porridge and rice (Table 6.1).

6.3.5 Occurrence of ESBL and DEC genes in water and palm

Two types of ESBL genes, bla_{SHV} and bla_{TEM} were found in the *C. freundii* and *K. pneumoniae* respectively, isolated from storage water that was to be used in cooking, washing, and cleaning at the Maamobi vicinity.

There was no *E. coli* recovery in water and palms of vendors.

6.4 Discussion

6.4.1 Extended Spectrum Beta-Lactamase producing Enterobacteriaceae (ESBL-PE) from stool

Although AMR among foodborne pathogens in stool isolates have been commonly observed all over the world and in Ghana (Akuffo et al., 2017; Bintsis, 2017; Duedu et al., 2017; Natarajan, Kumar, Mandal, Biswal, & Stephen, 2018; Ouchar Mahamat et al., 2019; Tadesse et al., 2019), there are only a few reported cases of Extended Spectrum Beta-Lactamase producing Enterobacteriaceae (ESBL-PE) that have been recovered (Bintsis, 2017; Cantón et al., 2008; Falgenhauer et al., 2019; Oduro-Mensah et al., 2016; Opintan et al., 2015; Ouchar Mahamat et al., 2019; Tham et al., 2010). As compared to other continents, there is a high prevalence of ESBL among stool isolates in $bla_{CTX\text{-}M\text{-}15}$, followed by bla_{SHV} and $bla_{CTX\text{-}M\text{-}14}$ genes chronologically (Storberg, 2014). With evidence of complicated treatment and prolonged hospitalization among diarrhoea patients, the spread of ESBL-PE has serious effect on the public health and socio-economic status of the general populace (Ouchar Mahamat et al., 2019; Tham et al., 2010).

In this study, the high occurrence of the bla_{TEM} concurs with an earlier study by Oduro-Mensah and colleagues in 2016 from the Korle-Bu Teaching Hospital in Ghana where different Enterobacteriaceae isolates were recovered from several specimen including stool samples (Oduro-Mensah et al., 2016). The coexistence of bla_{TEM} and $bla_{CTX\text{-}M}$ is inconsistent with other studies that have been reported in other African countries (Saka et al., 2020; Storberg, 2014; Tellevik et al., 2016).

However, the high numbers of the *CTX-M* gene recovered during this study is consistent with its widespread in Europe, Asia, and Africa. This phenomenon is attributed to various genetic factors including horizontal gene transfer (Agyekum et al., 2016; Rossolini et al., 2008;

Storberg, 2014) attributing to resistance to various classes of antibiotics including fluoroquinolones, cephamycins and aminoglycosides. This generally leads to difficulty in diarrhoea treatment in health care facilities leading to prolonged hospitalization. It is worth noting that the *SHV* gene had few occurrence which is inconsistent with other diarrhoeal research studies that have been carried out in Nigeria, Tanzania and China, even though there was a high similarity of the *CTX-M* and *TEM* gene occurrence (Bai et al., 2017; Saka et al., 2020; Tellevik et al., 2016).

Although few *P. mirabilis* isolates were recovered during the study, majority of them (4/7) showed high levels of ESBL phenotypic and genotypic possession as well as being MDR as well. Similar observations have been made in other studies elsewhere (Adesoji & Ogunjobi, 2016; Luzzaro et al., 2001).

6.4.2 Antibiotic resistance among ESBL Associated diarrhoea and non ESBL Associated diarrhoea

The high MDR occurrence observed in association with ESBL (89.5%) shows the impact bacteria with ESBL properties have with resistance to multiple drugs in Ghana. Although resistance to Tetracycline was high (79%) among the ESBL organisms recovered, there was no statistical significance between Tetracycline and ESBL related diarrhoeal infections. However, other enteric studies in Ghana have recorded over 50% resistance of Tetracycline (Duedu et al., 2017; Newman & Opintan, 2015). Co-resistance to Sulfamethoxazole-trimethoprim was high (89.5%) among ESBL causing diarrhoea showing how the drug has remained ineffective in the treatment of gastrointestinal infections in some parts of Ghana and Tanzania (Djie-Maletz et al., 2008; Kibwana et al., 2020). Other antibiotics such as Chloramphenicol, beta lactams (Piperacillin-tazobactam, Ticarcillin-clavulanate), the fluoroquinolones (Ciprofloxacin, Nalidixic acid), the aminoglycoside, Gentamicin and

Nitrofurantoin that showed a general highly ineffectiveness in the treatment of ESBL related diarrhoeal disease concurred with other previous Ghanaian studies that had been conducted (Akuffo et al., 2017; Newman & Opintan, 2015; Obeng-Nkrumah et al., 2013; Opintan et al., 2015). This is indicative of a gradual narrowing of the most routinely prescribed and readily available treatment options for diarrhoea in the country due to the selective pressure that may have been placed on these antibiotics over the years, as well as the over-the-counter availability of some of these antibiotics as well (Kibwana et al., 2020). Our findings in relation to MDR occurrence and the various classes of AMR was consistent with similar findings from Ethiopia (Diriba et al., 2020), Tanzania (Kibwana et al., 2020; Tellevik et al., 2016), Nigeria (Saka et al., 2020) and Chad (Ouchar Mahamat et al., 2019). Decreased resistance to meropenem was observed, albeit, a major public health concern in Ghana, since its gradual resistance shows possible abuse after its introduction on the Ghanaian market since 2002, therefore limiting treatment algorithms (Obeng-Nkrumah et al., 2013).

6.4.3 Diarrhoeagenic *E. coli* (DEC) in stool

Most diarrhoeagenic *E. coli* (DEC) (447%) identified in this study were the heat labile (*Lt*) gene of Enterotoxigenic *E. coli* (ETEC). ETEC has been the most recorded and documented cause of travelers' diarrhoea among travelers' visiting Africa and has been the most common cause of acute diarrhoea among children under the age of five years due to malnourishment and lack of hygiene from care givers (Okeke, 2009). ETEC forms part of the six main foodborne diseases which are known to cause 1.3 million DALY'S to the global burden according to the WHO (Havelaar et al., 2015). Opintan and colleagues in 2006 and 2007 identified ETEC among other strains in the stool of younger children who were referred to the pediatric unit of the Korle-bu Teaching hospital (Opintan et al., 2010) since 1988 (Agbodaze, Abrahams, & Arai, 1988). There is however a paucity of data regarding *E. coli* serotypes, epidemiology and impact in Ghana (Akuffo et al., 2017). The high percentages of ETEC

obtained in this study shows their existence and the need for more attention to be paid to further investigate *E. coli* as a common FBP that causes diarrhoea in Ghana. This is because in Bangladesh, ETEC manifests concurrently with *V. cholerae* as the common cause of diarrhoea in both children and adults and also sharing a biannual periodicity in the spring and autumn (Qadri et al., 2005). Having similar mechanisms and clinical presentations as *V. cholerae*, and its presence in animals and the environment as well, requires more focused surveillance into the investigation of ETEC as a probable foodborne disease in Ghana due to the frequent cyclical presentation of Cholera in the past (Agbodaze et al., 1988; Apanga & Kumbeni, 2021; Mireku-Gyimah et al., 2018; Qadri et al., 2005).

Currently, it has been reported in several DEC studies about the increase of AMR among *E. coli* isolates to various classes of antibiotics due to overuse and misuse of some readily available antibiotics in those regions (Mendez Arancibia et al., 2009; Natarajan et al., 2018; Vila et al., 1999). Resistance to quinolones, beta lactams, Trimethoprim and sulfonamides, Tetracyclines, Aminoglycosides and phenicols have been recorded among diarrhoeal patients due to the various AMR genes that may confer mutations among the isolates recovered (Mendez Arancibia et al., 2009; Vila et al., 1999).

6.4.4 Extended Spectrum Beta-Lactamase producing Enterobacteriaceae (ESBL-PE) in food

There was an occurrence of ESBL in *Citrobacter freundii*, bla_{TEM} in two food items ('kenkey' and spaghetti) from the Maamobi vicinity. This supports the popular occurrence of bla_{TEM} among foodborne Enterobacteriaceae pathogens in many foodborne RTE food studies (Liu et al., 2017; Ye et al., 2018). Food is an important source of the transmission of diverse AMR FBPs to humans and should however be monitored to understand their pathogenesis and virulence before the event of foodborne disease outbreaks.

6.4.5 Diarrhoeagenic *E. coli* (DEC) in food

In this study, although only two *E. coli* isolates were recovered from the food items, both were positive in possessing the *Lt* gene of the Enterotoxigenic *E. coli* (ETEC). Generally, after intense cooking, most bacteria are not able to survive due to the high levels of temperature applied in the process (Newman, 2005). As such, their presence in RTE is an indication of fecal contamination which can lead to food poisoning when consumed. ETEC which is known to cause traveler's diarrhoea found in food is of a public health concern which could be as a result of lack of good hand hygiene and non-adherence to basic sanitation requirements (Lima et al., 2017).

7 CHAPTER SEVEN: TOTAL PLATE COUNT (TPC) AND TOTAL COLIFORM COUNT (TCC) OF FOOD, WATER AND PALM SAMPLES

7.1 Introduction

These methods have helped in the determination of the microbial quality of food and water in monitoring hygiene due to handling and/ or food spoilage to measure food and water safety (Allen, Edberg, & Reasoner, 2004; WHO, 2011). Being one of the commonest methods of determining the microbial quality of food and water, their roles have helped in validating the quality of food and water in various settings (Center for Food Safety, 2014; Rompré, Servais, Baudart, De-Roubin, & Laurent, 2002). High TPC and TCC on hands/ palms is an indication of lack of proper hand washing which could result in the contamination of food (Barro et al., 2006; Lues & Van Tonder, 2007; Okareh & Erhahon, 2015).

Total plate and Total coliform counts were used in interpreting the safety and hygiene level of food and water samples collected based on the total number, types of organisms detected, and the interpretation of results based on both approved and accepted national and international guidelines of RTE food and water samples. Results derived from the food, water and palm analysis showed the hygiene and safety levels involved in food vendor operations.

7.2 Materials and Methods

Figure 7.1: Serial dilution being undertaken before inoculating unto PCA and MCA

7.2.1 Laboratory processing of food samples

Total plate count (TPC) using the plate count agar (PCA) and total coliform counts (TCC) using MacConkey agar were performed on the food samples that were transported to NMIMR.

The following outlines the procedure that was used in processing the food samples before their assessment of bacterial concentration.

For liquid samples, 10g/ml was weighed into a stomacher bag containing 90 mL of 0.1% (w/v) buffered peptone water. Samples were homogenized in a stomacher blender for 3-5 minutes to make a stock solution. For dry and solid foods, 25 g of each sample was added to 225 mL of 0.1% buffered peptone water prior to homogenization. One (1) ml of the stock solution was pipetted into fresh 9ml PBS and dilute serially 4 times (10^4) for plating on the appropriate media as shown in Figure 7.1.

The Plate count Agar (PCA) is the recommended medium for the recovery of viable organisms in food, animal feed and water samples. It consists of tryptone, yeast extract, glucose, and agar. It is the recommended medium for the plate count of micro-organisms in food incorporating the preparation of pour plates using dilute samples and counting colonies after incubation of 24-48 hours at 37 ^{o}C.

The MacConkey Agar is a selective medium for the enumeration of gram-negative enteric bacteria and to show the difference between lactose fermenting from lactose non-fermenting gram-negative bacteria (rods/bacilli). Components of the medium includes lactose monohydrate (inhibits most species of gram-positive bacteria), sodium chloride (pH balance), neutral red (pH indicator), agar (solidifying agent), and pancreatic digest of gelatin and peptones (growth promoters). It is used primarily used to detect and isolate bacteria from the *Enterobacteriaceae* spp and *Pseudomonas* spp. An added advantage of this agar is the ability to inhibit the swarming of *Proteus* spp.

Plate count agar (PCA) (for total aerobic count) and MacConkey agar (for total plate count and coliform count) were prepared according to the manufacturer's instructions and allowed to cool to 50^{o}C by placing them in a water-bath. One (1) ml of each dilution was pipetted onto

labelled duplicate sterile petri dish. An amount of 25 ml of the prepared agar was poured onto the inoculated petri dish, swirled, and allowed to set. The plates were inverted and incubated; PCA plates for 24-48 hours at 37ºC and MacConkey/Coliform selective agar plates for 24 hours at 37ºC.

Figure 7.2: Counting of coliforms using the colony counter

7.2.2 Counting colonies

After incubation all colonies on dishes containing 30- 300 colonies were counted using colony counter, model CL-570 (Sibata scientific technology Ltd./Sibata) and recorded as shown in Figure 7.2. The results per dilution were counted as follows;

For coliforms, all pink colonies on MacConkey agar dishes containing 30- 300 colonies were counted and recorded per each dilution. The numbers of colonies were multiplied by the dilution factor and the result expressed as the number of coliforms and per gram of food. Colonies exceeding 300 are recorded as "TNTC" (too numerous to count).

7.2.2.1 Calculation

In the case where dishes contained 30 – 300 colonies, the calculation was performed using an example below when a single petri dish was inoculated for the count;

CFU/g or CFU/ml = C/d x v,

Where c is the number of colonies on the counted plate, d the dilution rate of the counted plate and v the inoculated volume of this dilution.

e.g. $168/10^2$ x 1 = 1.68 x 10^4

Rounding the results to the first two digits gives 17000 CFU

Expression of results

Total aerobic plate count = 17000 CFU/g or 1.7 x 10^4 CFU/ g

7.2.3 Water coliform count

Ten milliliters of the water samples collected was transferred into 90mls of PBS to get a 1 in 10 dilution. After thorough mixing, a further threefold dilution was made aseptically by transferring 10ml into 90ml of the diluent. Further serial dilutions were performed in the same buffer solution from 1:10 to 10^3 before transferring onto various sterile petri dishes for the pour plate method of inoculation as described earlier for the food analysis (Addo et al., 2007).

Aside the Plate count agar (PCA) that was used for the total mesophilic counts of bacteria, MacConkey agar (MCA) was used for coliform and Total coliform counts.

Total plate count analysis was done based on guidelines for the detection of heterotrophic plate count in drinking water which is supposed to be acceptable and of good quality when less than 500CFU/ml. Plate count values in water for use (storage, cooking and rinsing) when greater

than 500CFU/ml was considered unsafe of low quality (Allen et al., 2004; Department of Health, Services, & for Disease Control, 2019).

However, with regards to coliform counts, water is considered safe for use in drinking, cooking and personal use when the amounts are zero (not detected) according to standard guidelines for water (GSA, 2016; MWRWH (Ministry of Water Resource works and Housing), 2015; Rompré et al., 2002; Swistock, Sharpe, & Clark, 2003; WHO, 2011).

7.2.4 Palm coliform count

Ten milliliters of each water sample were transferred into 90 mls of PBS to get the 1:10 dilution. Each 1:10 dilution was then mixed thoroughly, and further threefold dilution made by aseptically transferring 10ml of it into 90 ml of the diluent. They were serially diluted in the same buffer solution from 1:10 to $1:10^3$ as shown in Figure 7.1. After that, 1ml of the dilutions were transferred onto various sterile Petri dishes and the pour plate method used to inoculate. Total mesophilic bacteria counts were carried out using PCA (Oxoid CM453), while MCA (Oxoid CM7) was used for coliform and fecal coliform counts.

7.3 Results

7.3.1 Total plate count analysis (TPC) of food

Various Ready-To-Eat (RTE) foods (179) were sampled during the study. The commonest were stew, followed by rice, porridge, 'waakye' and 'banku'. The lowest total mean plate count from fried egg was 1.6×10^4 CFU/g as compared to the highest total mean plate count of 1.4×10^5 CFU/g from stew were at borderline and unsatisfactory respectively by interpretation according to the standard guideline governing RTE food. The lowest mean plate count recorded from the Maamobi vicinity was 2.0×10^4 CFU/g as compared to 1.8×10^4 CFU/g

from Kaneshie vicinity. High mean plate count of 2.1 x 10^5 CFU/g and 1.6 x10^5 CFU/g were derived from Maamobi and Kaneshie vicinities respectively. The high mean plate counts were recorded from stews and rice respectively which are both the most popular food that are sold in both vicinities. Porridge, mostly 'hausa kooko', a popular breakfast mostly sold in the morning had high mean plate counts of unsatisfactory interpretation in both Maamobi and Kaneshie vicinities (Table 7.1).

Table 7.1 Mean Total plate count of ready to eat food from Maamobi and Kaneshie vicinities

Food Items	Total Number of food items	Total Plate count Mean CFU/g	Maamobi Vicinity Mean CFU/g	Kaneshie Vicinity Mean CFU/g
Ampesi	7	1.8 x 10^4	2.0 x 10^4	2.3 x 10^4
Banku	15	1.3 x 10^5	1.7 x 10^5	2.4 x 10^4
Beans	6	4.1 x 10^4	9.8 x 10^3	4.9 x 10^4
Bofloat/ Koose/ Pinkaso	7	5.8 x 10^4	6.1 x 10^4	-
Bread	2	4.4 x 10^4	4.4 x 10^4	NA
Fish/ meat	6	2.7 x 10^4	3.7 x 10^4	1.8 x 10^4
Fried egg	3	1.6 x 10^4	1.6 x 10^5	NA
Fried rice	1	-	NA	-
Gari	1	-	NA	-
Grounded pepper	11	3.8 x 10^4	4.1 x 10^4	-
Indomie/ spaghetti	10	3.6 x 10^4	4.8 x 10^4	2.2 x 10^4
Jollof	8	8.4 x 10^4	1.4 x 10^5	2.8 x 10^4
Kenkey	8	2.6 x 10^4	3.3 x 10^4	9.2 x 10^4
Porridge	18	1.2 x 10^5	1.3 x 10^5	1.1 x 10^5
Rice	21	1.2 x 10^5	4.3 x 10^4	1.6 x 10^5
Salad	1	-	NA	-
Fried pepper (Shitor)	2	3.1 x 10^4	NA	3.1 x 10^4
Soup	9	3.1 x 10^4	3.2 x 10^4	3.1 x 10^4
Stew	26	1.4 x 10^5	2.1 x 10^5	2.7 x 10^4
Waakye	17	8.6 x 10^4	1.3 x 10^5	2.6 x 10^4

TPC for RTE food (cfu)/g: Satisfactory <10^3; Borderline 10^3- <10^5; Unsatisfactory ≥10^5

7.3.2 Total coliform count analysis (TCC) of food

The highest mean coliform count of the various food items exceeded >10^4 of being unsatisfactory, which was seen in stew and 'waakye' (2.2 x 10^5 CFU/g and 1.7 x 10^5CFU/g).

Generally, the lowest mean coliform count of a satisfactory result was observed among the total number of food were recorded in fish/meat and Bread (1.6 x 10^3 CFU/g and 3.5 x 10^3CFU/g) respectively. Kenkey, stew, fried egg and 'Bofloat'/ 'Koose'/ 'Pinkaso' food items from Maamobi all showed food mean coliform counts which exceeded >10^4 limits which were unsatisfactory. Most of the food items from Kaneshie vicinity apart from 'waakye' (>10^4) were either at borderline or satisfactory (Table 7.2).

Table 7.2 Mean Total coliform count of ready to eat food from Maamobi and Kaneshie vicinities

Food Items	Total Number of Food items	Total Number of Food Mean CFU/g	Maamobi Vicinity Mean CFU/g	Kaneshie Vicinity Mean CFU/g)
Ampesi	7	1.5 x 10^4	1.8 x 10^4	0
Banku	15	2.9 x 10^4	3.0 x 10^4	3.0 x 10^4
Beans	6	4.1 x 10^4	0	5.8 x 10^4
Bofloat/ Koose/ Pinkaso	7	9.5 x 10^4	1.0 x 10^5	-
Bread	2	3.5 x 10^3	3.5 x 10^3	NA
Fish/ meat	6	1.6 x 10^3	2.3 x 10^3	0
Fried egg	3	1.6 x 10^4	1.6 x 10^5	NA
Fried rice	1	-	NA	-
Gari	1	-	NA	-
Grounded pepper	11	1.7 x 10^4	1.8 x 10^4	-
Indomie/ spaghetti	10	2.2 x 10^4	2.8 x 10^4	1.6 x 10^4
Jollof	8	3.0 x 10^4	0	3.6 x 10^4
Kenkey	8	2.2 x 10^4	2.8 x 10^5	5.2 x 10^3
Porridge	18	5.2 x 10^4	5.0 x 10^4	5.7 x 10^4
Rice	21	6.7 x 10^4	3.8 x 10^4	8.8 x 10^4
Salad	1	-	NA	-
Fried pepper (Shitor)	2	0	NA	0
Soup	9	1.6 x 10^4	2.1 x 10^4	3.1 x 10^3
Stew	26	2.2 x 10^5	3.0 x 10^5	7.5 x 10^4
Waakye	17	1.7 x 10^5	7.6 x 10^4	2.1 x 10^5

TCC for RTE food for Enterobacteriaceae (cfu)/g: Satisfactory <10^2 ; Borderline 10^2 - <10^4 ; Unsatisfactory >10^4 (Center for Food Safety, 2014; GSA, 2015)

7.3.3 Total Plate and Coliform Counts of food from Maamobi and Kaneshie vicinities

Generally, more than half of all the food items analysed for TPC were at borderline whiles less than 40% were of a satisfactory result according to the guidelines. About 12% of the food items from Maamobi vicinity were of unsatisfactory interpretation, which was higher than an approximate 5% unsatisfactory result outcome of food items from Kaneshie vicinity. The total coliform count which was of satisfactory standard in both vicinities were over 60% with less than 35% of the food items falling within the borderline category. Less than 10% of the food items fell under the Unsatisfactory category (Table 7.3).

Table 7.3 Total Plate and Coliform Counts of food from Maamobi and Kaneshie vicinities

	Total Plate Count			Total Coliform Count		
	Satisfactory	Borderline	Unsatisfactory	Satisfactory	Borderline	Unsatisfactory
Maamobi vicinity	39(38.2)	51(50.0)	12(11.8)	61(60.0)	35(34.3)	6(5.9)
Kaneshie vicinity	31 (40.3)	42 (54.6)	4(5.2)	50(64.94)	20(26.0)	7(9.1)

TPC for RTE food (cfu)/g: Satisfactory $<10^3$; Borderline 10^3- $<10^5$; Unsatisfactory $\geq 10^5$; TCC for RTE food for Enterobacteriaceae (cfu)/g: Satisfactory $<10^2$; Borderline 10^2- $<10^4$; Unsatisfactory $>10^4$ (Center for Food Safety, 2014)

7.3.4 Total Plate and Coliform Counts of water and palm swab of food vendors

Three different water types were sampled from the food vendors during the study at both vicinities; cooking water (water used for cooking located at the vending sites), storage water (water stored at the vending sites used for miscellaneous activities) and washing water (last water used to rinse bowls and cutlery in serving customers). Only few vendors out of the 65 food vendors who were engaged during the community sampling consented and provided water and palm samples for analysis.

Almost 70% of the cooking water by TPC that was sampled from both sites were impure according to national and international standards. More than 50% of the samples were impure

based on TCC water consideration. Majority of the water samples from Kaneshie were impure by both TCC and TPC.

Over 50% of storage water from both sites were impure by TPC while the same percentage were compliant with the national standards for TCC (WRC, 2015).

Washing water, although collected from only the Kaneshie vicinity showed more than 50% noncompliance to the national and international standards of TPC and TCC and therefore considered impure.

A total of 10 vendors' palm swab indicated a 100% compliance to national TPC and TCC guidelines and therefore showing the clean hands of the vendors (Table 7.4).

Table 7.4 Showing the TPC and TCC of water and palm swab of the food vendors

	Kaneshie vicinity		Maamobi vicinity		Totals	
	TPC	TCC	TPC	TCC	TPC	TCC
Cooking water						
Impure n(%)	3(100.00)	3(100.00)	6(60.00)	4(40.00)	9(69.23)	7(53.85)
Pure n(%)	0(0.00)	0(0.00)	4(40.00)	6(60.00)	4(30.77)	6(46.15)
Storage water						
Impure n(%)	1(33.33)	2(66.67)	14(53.85)	12(46.15)	15(51.72)	14(48.28)
Pure n(%)	2(100.00)	1(33.33)	12(46.15)	14(53.85)	14(48.28)	15(51.72)
Washing water						
Impure n(%)	17(56.67)	17(56.67)	-	-	17(56.67)	17(56.67)
Pure n(%)	13(43.33)	13(43.33)	-	-	13(43.33)	13(43.33)
Palm Swab						
Unclean n(%)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
Clean n(%)	2(100.00)	2(100.00)	8(100.00)	14(53.85)	10(100.00)	10(100.00)

*Total coliform counts (not more than 500cfu/ml). Total plate count and *E. coli* count= less than 1cfu/100ml. (Low: 1-10 cfu/ 100ml, Intermediate: 11-100cfu/100ml, High: >100cfu/100ml) WHO (2011) and GSA (2015)

7.4 Discussion

7.4.1 Total Plate and Coliform Counts of food from Maamobi and Kaneshie vicinities

Street food vendors need to be educated and monitored since more than half of the food samples from both Maamobi and Kaneshie vicinities were at borderline by TPC limits for RTEs. Personal hygiene and the implementation of WASH (access to safe water, sanitation, and hygiene) among street food vendors will help to improve the quality of RTEs sold in the vicinities. The possibility of fecal contamination in the food samples were minimal by TCC (Enterobacteriaceae analysis for RTEs) which implies that most of the food samples can be considered microbiologically safe for consumption. This is similar to the general observation made by Mensah and colleagues in 2002 (Mensah et al., 2002) but inconsistent with other street food studies that have been conducted in Ghana (Abakari et al., 2018; Marras & Agbendech, 2016; Yeboah-Manu et al., 2010; Yeleliere et al., 2017).

A critical look at the various food types in general, together with their average mean TPC and TCC values will indicate the food types to consider generally safe or unsafe for consumption.

In this study, stews from both vicinities had a high mean TPC of $\geq 10^5$ (Center for Food Safety, 2014) which was above the acceptable limits and therefore considered unsafe for consumption. High TCC of $>10^4$ was also recorded for stews from the total food mean count recorded for both vicinities which was an indication of faecal contamination at the time of sampling for analysis. This is consistent with the findings of the review of Yeleliere and colleagues who in a review of the microbial food contamination and food hygiene in selected capital cities in Ghana identified stews in general to be the most contaminated food among the various studies (Yeleliere et al., 2017). The preparation of stews in Ghana requires rigorous boiling of tomatoes, onions and other vegetables, coupled with frying in oil for prolonged periods; an activity which makes it difficult for the activity of coliforms to thrive in them (Newman, 2005). The presence of mesophilic coliforms in the stew could however be an indication of post

exposure factors after preparation such as storage/ storage conditions, handling or serving. Majority of the vending sites who exhibited prolonged storage of stew in buckets and bowls without putting them in refrigerators were prone to spoilage and contamination with microbes. More targeted education on food safety practices among food vendors are encouraged.

Rice which was the second most popular food sampled (21) after the stews also showed general TPC that were above acceptable limits (1.2 x 10^5CFU/g) and therefore considered unsafe for consumption. Rice samples from Kaneshie were more contaminated (1.6 x 10^5 CFU/g) as compared to Maamobi vicinity. Also, waakye, which is one of the most popular and favorite food in Ghana showed high levels of coliform counts in general as compared to aerobic counts among the 17 samples that were collected. Mensah and colleagues attributed the contamination of rice dishes to possible contaminated bowls/ chests and polyethene that were used in their transfer to the vending sites before serving to consumers. Low levels of coliforms were recorded in cases when food items were sold directly from cooking pots (Mensah et al., 2002). Rice dishes were among the RTE food samples that were microbiologically considered as unsafe for eating on the streets in Yeleliere and colleagues' review on microbial food contamination and food hygiene among some selected capital cities in Ghana (Yeleliere et al., 2017). Inconsistent to this study was by Yeboah-Manu and colleagues, 2010s during an RTE study on and around the university of Ghana campus that had no counts among the rice dishes that were sampled (Yeboah-Manu et al., 2010). A similar street food study conducted in roadside cafeterias and retail outlets in Alice, Eastern Cape Province, South Africa showed high levels of aerobic contamination in several food samples including rice dishes (4.3–6.7 log10 CFU g^{-1}) (Nyenje et al., 2012).

TPC analysis in porridge, popularly referred to as 'hausa kooko' revealed high levels of contamination and considered microbiologically unsafe as well. This is inconsistent with the findings from Mensah and colleagues' study that showed that it was least contaminated due to

the high temperature (50-90˚C) and time duration (2-3 hours) used in getting sold out on the breakfast market. They attributed the few contamination occurrences to the lack of personal hygiene of the vendors and unrenewal of washing/ serving water during vending (Mensah et al., 2002). Similar observation was made in this study, as vendors failed to renew the water often and failed to practice basic personal hygiene like proper hand washing, handling of money and dirty serving apparatus.

'Banku', a staple food which is prepared from maize and cassava dough, boiled into paste, rolled into balls and placed into polythene bags with the aid of the bare hands coupled with bowl/ calabash and water before serving also showed high levels of TPC similar to Mensah et al.'s study (Mensah et al., 2002). They attributed the cause of the contamination of food to the continuous exposure to bare hands and the probability of contaminated materials used in its rolling into balls after cooking.

Like several RTE research conducted in Ghana (Ababio & Lovatt, 2015; Feglo & Sakyi, 2012; Yeleliere et al., 2017), ground pepper was not considered microbiologically safe since it was at the borderline according to the guideline interpretation. This shows risks of possible contamination of this food and as such, the need for more education of vendors and operators on proper handling vegetables due to their ability to transmit FBPs (Abakari et al., 2018; Hartantyo et al., 2020; Karikari et al., 2017; McMahon & Wilson, 2001; Nyenje et al., 2012; Stratev & Odeyemi, 2016). Ground pepper is known to be prepared with fresh vegetables, ground in earthenware pots/ blenders and served to consumers as accompaniments for meals such as banku, akpler, gari and beans, kenkey and other meals. Their lack of cooking renders them prone to contamination from the various crockery used in their preparation and the raw vegetables (if not washed properly) that are used as well.

Fried meat/ fish as well as bread was the least contaminated among the food items that were tested. This could be as a result of less handling practices by the vendors in this study, unlike

high mean aerobic counts in their local breads ('kita- 6.1 X 10^5 CFU/gm and 'Ambasha'- 3.0 x 10^5 CFU/gm)that was recorded in some selected street vending areas in Hawassa, Ethiopia (Eromo et al., 2016).

The link between several RTE food and their microbiological safety in this study as compared to other studies in the past shows the need for further education and enhanced inspection of WASH, health certification and food licensing.

7.4.2 Total Plate and Coliform Counts of water and palm swab of food vendors

The importance and use of water in street food vending cannot be overlooked in all processes of food preparation and vending. As such, being a critical raw material implies being a risk when its integrity is questionable. It is with this understanding that contamination could create a public health threat when it is used for cooking, washing, drinking and other purposes. Organisms such as *Salmonella* spp, *Vibrio* spp, *Campylobacter* spp, *E. coli* and other similar pathogens have been identified in contaminated water in various studies that have been carried out (Rane, 2011). Okere and colleagues in Ouagadougou, 2006 identified mesophilic aerobic bacteria counts that were classified as impure and very impure in dish washing water, attributing the phenomenon to placement on the floor, unrenewal of washing water and the presence of small puddles , animals and insects at the vending sites (Barro et al., 2006). Similar findings occurred in this study where over 50% of dishwashing water were impure by both TPC and TCC analysis.

Water that is used in washing and rinsing plates were noted to be used frequently and unrenewed regularly. Also, vendors were fond of using the same water in washing their hands after using the bathroom which caused an increase to the possible transfer of pathogens to the washing water that was meant for the crockery instead. Running water should be encouraged onsite for safe hand washing (tap water, 'Veronica buckets') to avoid the contamination of

water that are used for vending. The unrenewal of washing water leading to prolonged use despite being dirty was also of great concern and a reason for the low bacteriological quality in food during Mensah and colleagues research in 2002 (Mensah et al., 2002).

Investigation of the microbiological quality of water has been carried out in several research activities internationally and nationally. Unfortunately, there is paucity of data on water used for street food vendors despite several research that have been carried out on sachet/ bottled water (Addo et al., 2009; Anyanwu, 2010; Manjaya et al., 2019; Mosi et al., 2019) and water used in irrigation for fruit and vegetable production (Abakari et al., 2018; Karikari et al., 2017) in Ghana. Data derived from this study calls for the need to perform similar studies to understand and monitor the water used among street food operators.

Based on the analysis and standards used in defining the purity of water used at the vending sites, majority of the cooking water were impure by TPC and TCC standards. The expected total coliform counts (TCC) should not be more than 500cfu/ml and the Total plate counts (TPC) should be less than 1cfu per 100ml (WHO, 2011; WRC, 2015). Several activities engaged by the food vendors were identified to be the cause for majority of the cooking water to be impure; lack of personal hygiene of food vendors involving the use of dirty hands to fetch water, inadequate/ unclean storage spaces and containers and prolonged storage of water leading to contamination with various organisms.

Although all the palm swab that were collected were pure after TPC and TCC analysis, several practices that were observed during the vending period could not go unnoticed. Practices like touching/ handling money without proper handwashing prior to serving food, washing hands in unrenewed/ dirty water and lack of potable water at the vending sites were observed. Other street food studies both nationally and internationally have identified TPC and TCC in the palms of street food vendors which implicated possible contamination of food from their palms

(Amegah et al., 2020; Barro et al., 2006; Hassan et al., 2018; Lues & Van Tonder, 2007; Tadesse et al., 2019).

8 CHAPTER EIGHT: RISK FACTOR DETERMINATION OF DIARRHOEA ASSOCIATED WITH BACTERIA FOODBORNE PATHOGEN ORIGIN AMONG PATIENTS

8.1 Introduction

Based on various demographic characteristics, general information and knowledge on sanitation, and personal hygiene, various street food studies have been able to show the risk factors involved in the transmission of bacteria FBPs in Ghana (Amegah et al., 2020; Mensah et al., 2002).

Risk factor analysis was to be established from the demographic information, behavioural, knowledge of personal hygiene and sanitation of patients. Based on the questionnaire that was administered to patients and food vendors during sample taking, the answers provided helped in determining the risk factors involved in contracting diarrhoea associated with bacteria foodborne pathogens.

8.2 Materials and Methods

8.2.1.1 Data analysis

The association between diarrhoea (with bacteria foodborne pathogen infection versus without bacteria foodborne infection) with demographic characteristics, clinical presentation, behavioural traits, and knowledge of the risks of contracting diarrhoea was done using bivariate analysis to determine risk factors. Exposure variables which had statistical association with bacterial infection status would in turn undergo a multivariable logistic regression to determine risk factors of diarrhoea with bacterial infection status.

8.3 Results

8.3.1 Risk factor analyses of demographic information

Patients who attended both facilities; Maamobi General Hospital and Kaneshie Polyclinic responded to various questions based on the questionnaire that was administered upon consent. Among the various demographic information such as gender, age, marital status, education and religion, education had an association with a patient having diarrhoea of bacteria foodborne pathogen origin (*p*-value=0.021). Gender, age, marital status, and religion had no association with diarrhoea of bacteria foodborne pathogen origin or without bacteria foodborne pathogen origin (Table 8.1).

Table 8.1 Risk factor analyses of demographic information

Characteristics	Diarrhoea with bacteria foodborne pathogen origin n(%)	Diarrhoea without bacteria foodborne pathogen origin n(%)	*p*-value
Gender			0.294
Male	34(85.0)	6(15.0)	
Female	63(76.8)	19(23.2)	
Age			
Infant	11(91.7)	1(8.3)	0.549
Children	8(88.9)	1(11.1)	
Adult	78(77.2)	23(22.8)	
Marital Status			
Single	59(82.0)	13(18.06)	0.098
Married	36(80.0)	9(20.0)	
Divorced	0(0.0)	1(100.0)	
Widowed	1(33.3)	2(66.7)	
Separated	1(100.0)	0(0.0)	
Education			
No School	19(79.2)	5(20.8)	**0.021**
Primary	22(95.7)	1(4.4)	
JHS	15(57.7)	11(42.3)	
SHS	21(80.8)	5(19.2)	
Tertiary	20(87.0)	3(13.0)	
Religion			
Christian	61(77.2)	18(22.8)	0.636
Muslim	32(82.1)	7(18.0)	
Traditionalist	2(100.0)	0(0.0)	

8.3.2 Risk factor analyses of the clinical presentations

Although there was no significant association between the various clinical presentations and a patient having diarrhoea with or without bacteria foodborne pathogen origin. Over 80% of patients who showed temperatures that were ≥38°C had diarrhoea infection with bacteria foodborne pathogen as the causative agent. Also, patients who reported to passing loose stool more than three times within 24 hours showed almost 80% of diarrhoea with bacteria foodborne pathogen origin. Almost all stools that were liquid/ loose or soft/ greasy had bacteria pathogens being the cause of diarrhoea (Table 8.2).

Table 8.2 Risk factor analyses of clinical presentations

Characteristics	Diarrhoea with bacteria foodborne pathogen origin n(%)	Diarrhoea without bacteria foodborne pathogen origin n(%)	*p*-value
Clinical Presentation			
Temperature			
<38ºC	84(79.3)	22(20.8)	0.853
≥38ºC	13(81.3)	3(18.8)	
Times passed stool within 24hours			
2-3 times	19(79.2)	5(20.8)	0.963
More than 3 times	78(79.6)	20(20.4)	
Stool form			
Liquid/Loose	95(79.2)	25(20.8)	
Soft/greasy	2(100.0)	0(0.0)	0.469
Traces of Blood/mucous			
Yes	43(82.7)	9(17.3)	0.453
No	54(77.1)	16(22.9)	

8.3.3 Risk factor analyses based on presenting symptoms

Over 50% of patients who responded positively to the following presenting symptoms; fever during illness, urgency to go to toilet, headache, flatulence, abdominal pains/ cramps, bloating, nausea, vomiting, and fatigue had diarrhoea with bacteria foodborne pathogen origin as compared to less than 30% of the presenting symptoms having diarrhoea without bacteria foodborne pathogen infection. Over 80% of patients having diarrhoea for more than 24 hours had bacteria foodborne pathogens as the cause of their disease. Generally, between 15-35% of the presenting symptoms that were presented during the study were diarrhoea without bacteria as a result of their infection (Table 8.3).

Table 8.3 Risk factor analyses of presenting symptoms

Characteristics	Diarrhoea with bacteria foodborne pathogen origin n(%)	Diarrhoea without bacteria foodborne pathogen origin n(%)	*p*-value
Presenting Symptoms			
Fever during illness			
Yes	21(84.0)	4(16.0)	0.533
No	76(78.4)	21(21.7)	
Urgency to go to toilet			
Yes	85(81.0)	20(19.1)	0.326
No	12(70.6)	5(29.4)	
Headache			
Yes	25(69.4)	11(30.6)	0.075
No	72(83.7)	14(16.3)	
Flatulence			
Yes	9(90.0)	1(100.0)	0.391
No	88(78.6)	24(21.4)	
Abdominal pain/cramps			
Yes	43(79.6)	11(20.4)	0.976
No	54(79.4)	14(20.6)	
Bloating			
Yes	12(75.0)	4(25.0)	0.632
No	85(80.2)	21(19.8)	
Nausea			
Yes	38(77.6)	11(22.45)	0.661
No	59(80.8)	14(19.2)	
Vomiting			
Yes	43(76.8)	13(23.2)	0.493
No	54(81.8)	12(18.2)	
Fatigue			
Yes	26(76.5)	8(23.53)	0.605
No	71(80.7)	17(19.3)	
Days had symptoms			
<1day	13(65.0)	7(35.0)	0.425
1day	37(82.2)	8(17.8)	
2days	14(82.4)	3(17.7)	
≥3days	33(82.5)	7(17.50)	

8.3.4 Risk factor analyses based on patients' behaviour

Majority of patients responded in the affirmative of having eaten or drank prior to the onset of illness and exhibited diarrhoea that was caused by bacteria foodborne pathogen as illustrated in Table 8.4. Over 50% of patients who had diarrhoea with bacteria cause reported drinking sachet water, took medication upon the onset of illness and washed hands with soap and water.

Table 8.4 Risk factor analyses of patients' behaviour

Characteristics	Diarrhoea with bacteria foodborne pathogen origin n(%)	Diarrhoea without bacteria foodborne pathogen origin n(%)	*p*-value
Patient Behaviour			
Ate or drank before illness			
Yes	93(80.2)	23(19.8)	0.275
No	3(60.0)	2(40.0)	
Source of drinking water			
Sachet water	94(79.0)	25(21.0)	0.373
Tap	3(100.0)	0(0.0)	
Taking medication upon onset of illness			
Yes	50(76.92)	15(23.1)	0.45
No	47(82.5)	10(17.5)	
Wash hands with soap and water			
Yes	88(80.0)	22(20.0)	0.684
No	9(75.0)	3(25.0)	
Destination of waste			
Vehicle			
Yes	6(60.0)	4(40.0)	0.111
No	91(81.3)	21(18.8)	
Pit			
Yes	2(66.7)	1(33.3)	0.577
No	95(79.8)	24(20.2)	
Lagoon or river			
Yes	1(100.0)	0(0.0)	0.61
No	96(79.3)	25(20.7)	
Burning			
Yes	19(70.4)	8(29.6)	0.183
No	78(82.1)	17(17.9)	

8.3.5 Risk factor analyses based on patients' behaviour and knowledge about the cause of diarrhoea

Patients' responses on the sanitation about where food was sold before consumption indicated about 80% of each of the descriptions, that is not tidy, tidy, and very tidy showing diarrhoea with bacteria foodborne infection. There was no significant association between the patients' knowledge about causes of diarrhoea (dirty food, water, hands, and germs) and having diarrhoea with or without bacteria as the cause of foodborne pathogen infection. However, over 75% of the patients' knowledge about causes of diarrhoea which were responded in the affirmative had diarrhoea outcomes associated with bacteria foodborne pathogen infection (Table 8.5).

Table 8.5 Risk factor analyses of patients' behaviour and knowledge on causes of diarrhoea

Characteristics	Diarrhoea with bacteria foodborne pathogen origin n(%)	Diarrhoea without bacteria foodborne pathogen origin n(%)	*p*-value
Dustbin			
Yes	80(81.6)	18(18.4)	0.24
No	17(70.8)	7(29.2)	
Sanitation of where food is sold			
Very tidy	15(79.0))	4(21.1)	0.997
Tidy	70(80.0)	18(20.5)	
Not tidy	12(80.0)	3(20.0)	
Patients' Knowledge about causes of diarrhoea			
Dirty food			
Yes	34(85.0)	6(15.0)	0.294
No	63(76.8)	19(23.2)	
Dirty water			
Yes	21(87.5)	3(12.5)	0.279
No	76(77.6)	22(22.5)	
Germs			
Yes	61(78.2)	17(21.8)	0.635
No	36(81.8)	8(18.2)	
Dirty hands			
Yes	17(94.4)	1(5.6)	0.089
No	80(77.0)	24(23.1)	

8.4 Discussion

8.4.1.1 Risk factor analyses of demographic information

This study showed the role education plays in determining the cause of diarrhoea infection in Ghana. In several studies conducted in Ghana and other African countries, it was noted that higher education among mothers influences the reduction of childhood diarrhoea transmission (Apanga & Kumbeni, 2021; Owusu & Markku, 2005; Sahiledengle et al., 2021; Tetteh et al., 2018). Higher education imparts formal knowledge on basic hygiene and sanitation requirements that helps to alleviate common mistakes people normally make in these areas. In this study, there was minimal illiteracy rate (19.7%) among the patients enrolled. Although the knowledge on the causes of diarrhoea were not reflective of this outcome, it should be noted that there could be respondents' bias in giving true answers to the questions asked. Education (formal and informal) on the need the nature, causes, effects, and the preventive measures of diarrhoea should be advocated to reduce their transmission rate.

Since none of the responses from the food vendors had an association with contracting diarrhoea, no risk factor analysis was carried out on the information derived.

9 CHAPTER NINE: CONCLUSION

This study has shown the various FBPs that exist in stool of diarrhoea patients, as well as food, water, and the palms of street food vendors in the Maamobi and Kaneshie health facilities and vicinities. Although a wide array of bacterial pathogens existed, common occurrences in both clinical and environmental samples pointed out the possibility of transmission that exist from food preparation to consumption. TPC and TCC results of the RTE food items and water recovered showed the unacceptable levels of contamination that exist during street vending activities due to noncompliance to WASH and the non-adherence to food safety policies and guidelines like Hazard analysis and critical control point (HACCP). Despite the low numbers of clinical and environmental samples, this study is a representation of most street food vending activities occurring in most towns and cities in Ghana.

The use of the MALDI-TOF in identifying FBPs in stool, food, water, and palm is the first study in Ghana to adopt a current diagnostic tool in identifying a one health problem. Although expensive, the MALDI-TOF is accurate, rapid, with a reduced turn-around time, making it an effective diagnostic tool for foodborne disease outbreaks and routine surveillance and research.

AMR, currently one of the world's greatest problems has been confirmed to influence diarrhoea which makes it difficult to treat and in the RTE food, water, and the palms of vendors. This phenomenon shows a possible community transfer of resistance, occurring between clinical and environmental samples as well as the result of the frequent abuse and misuse of antibiotics. Antibiotics frequently used in Ghana; Tetracycline, Azithromycin, trimethoprim/sulfamethoxazole, chloramphenicol, and Amoxicillin-clavulanate were common to be resistant among most of the pathogens in the samples taken. This reflection depicts the overdependency of these antibiotics, leading to the AMR occurrence. Based on this study and other similar studies on this growing resistance to commonly used antibiotics in Ghana, a

periodic amendment of the Ghana Standard Treatment guidelines is recommended to update treatment options for gastroenteritis and diarrhoea. This data supports existing data about presence of AMR in some gastrointestinal normal flora and some popular street vended food, an alert for good personal hygiene and proper sanitation.

The study demonstrated a moderate prevalence of ESBL genes among Enterobacteriaceae and non-Enterobacteriaceae organisms recovered from diarrheic patients and RTE food. These observations indicate a possible transmission and could pose a public health threat to the country as we may encounter too many difficult to treat infections leading to prolonged hospitalizations in the event of transmission of these AMR FBPs.

Diarrhoeagenic *E. coli* (DEC), *Lt* gene in most of the *E. coli* samples confirmed their relatedness to being the cause of gastrointestinal infections leading to diarrhoea and shows the possibility of causing infection due to their contamination in the RTE food that possessed them. Also, being the only DEC type detected in this study and a previous study has provided information on DEC epidemiology which is scarce in Ghana. The organism, *E. coli* and other gastrointestinal commensals should not be ignored anymore due to the possibility of harboring AMR genes and virulent factors which could be transferred to less virulent pathogens. It is in this light that routine AMR surveillance should be carried out to investigate their patterns before such outbreaks occur.

Apart from waste disposal and knowledge about the cause of diarrhoea, none of the risk factors had any association with getting infected with diarrhoea, although several symptoms known to be associated with diarrhoea infections were confirmed during this study. Also, the responses from patients and food vendors shows the lack of basic personal hygiene and sanitation practices that could pose a risk in diarrhoea transmission.

9.1.1 Limitations

The unspecific directions given by patients made it difficult for the surveillance team to locate the exact RTE food outlets food was purchased.

Upon locating the food vending sites, specific food items which had been implicated to have caused the food poisoning had run out and therefore, could not be sampled for laboratory testing.

Unwillingness of some street food vendors to consent due to several reasons like mistrust and fear of unfavourable outcome of results, business closure/sanction and customer stigmatization led to the reduction in sample numbers.

Inability of perform genomic tests such as sequencing to determine the relationship among sample types.

9.1.2 Recommendations

The major problem identified among the food vendors was adherence to basic personal hygiene like proper hand washing with soap and water as well as keeping good sanitation. Targeted street food education through mobile education or announcements, social media, and local media (television and radio) on AMR, good personal hygiene and sanitation could help improve the attitudes mentioned.

Also, the public through current advertisement platforms should be informed about the emergence and spread of AMR FBP through food. This will help improve the behavioral traits and practices that could lead to FBP transmission.

There should be regular review of standard treatment guidelines for gastroenteritis and diarrhoea due to emerging increasing AMR patterns.

General AMR patterns occurring in all the sectors showed the importance of integrated one health surveillance and its importance in monitoring the trends of resistance in the pathogens. Studies should adopt the One health approach in depicting a holistic view of emerging FBPs.

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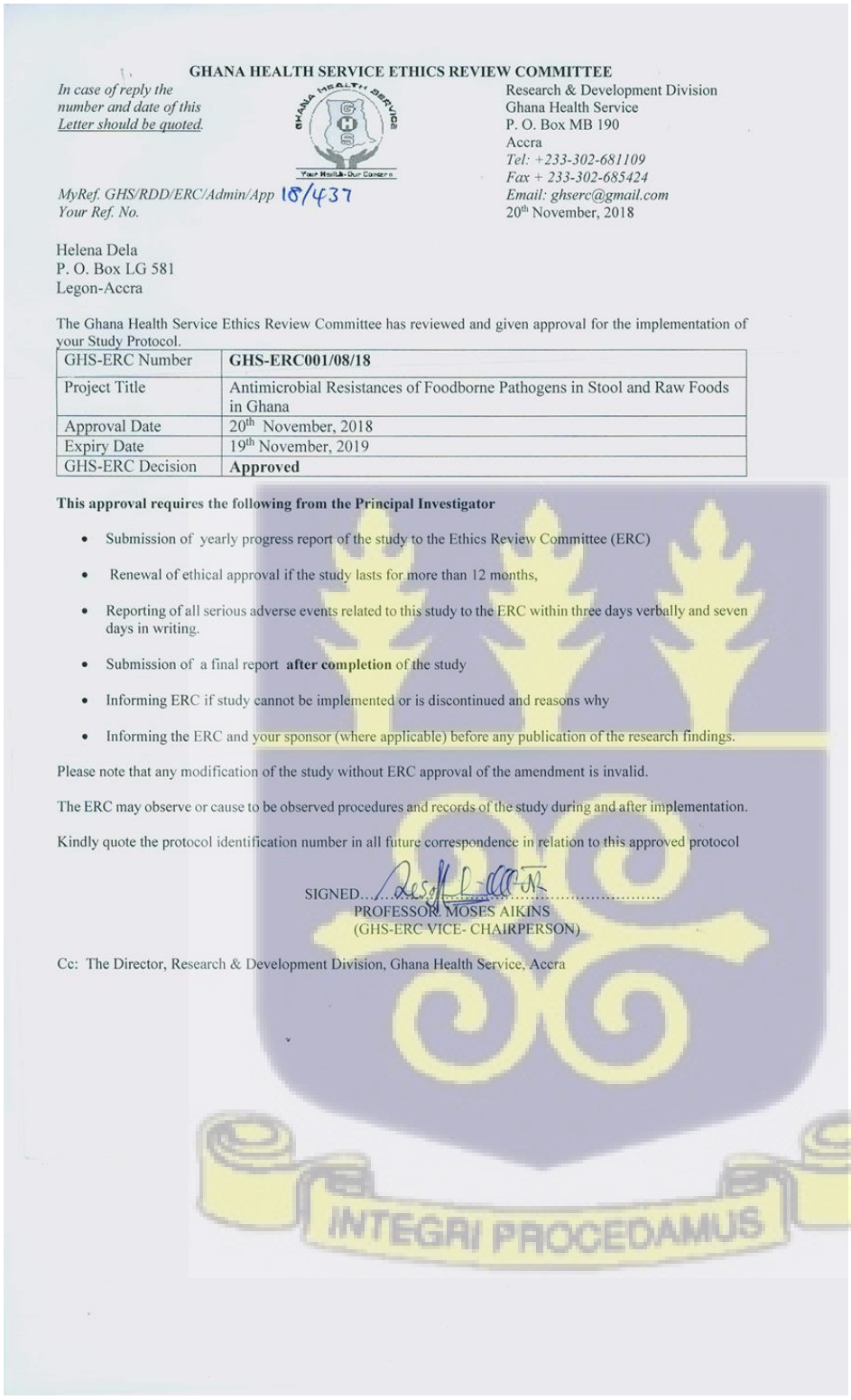
11 APPENDICES

11.1 APPENDIX A: ETHICAL CLEARANCE FROM INSTITUTIONS



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

MyRef. GHS/RDD/ERC/Admin/App 18/437
Your Ref. No.

20th November, 2018

Helena Dela
P. O. Box LG 581
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-ERC001/08/18**
Project Title	Antimicrobial Resistances of Foodborne Pathogens in Stool and Raw Foods in Ghana
Approval Date	20th November, 2018
Expiry Date	19th November, 2019
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.....
PROFESSOR MOSES AIKINS
(GHS-ERC VICE- CHAIRPERSON)

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

Appendix A1: Ethical clearance from the Ghana Health Service Institutional Review committee

Appendix A2: Ethical clearance from the Noguchi Memorial Institute for Medical Research Institutional Review Board

NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH
Established 1979
A Constituent of the College of Health Sciences
University of Ghana

INSTITUTIONAL REVIEW BOARD

Phone: +233-302-916438 (Direct)
+233-289-522574
Fax: +233-302-502182/513202
E-mail: nirb@noguchi.mimcom.org
Telex No: 2556 UGL GH

Post Office Box LG 581
Legon, Accra
Ghana

My Ref. No: DF.22
Your Ref. No:

4th July, 2018

ETHICAL CLEARANCE

FEDERALWIDE ASSURANCE FWA 00001824 **IRB 00001276**

NMIMR-IRB CPN 097/17-18 **IORG 0000908**

On 4th July 2018, the Noguchi Memorial Institute for Medical Research (NMIMR) Institutional Review Board (IRB) at a full board meeting reviewed and approved your protocol titled:

TITLE OF PROTOCOL : **Antimicrobial Resistance of Foodborne Pathogens in stool and raw foods in ghana**

PRINCIPAL INVESTIGATOR : **Helena Dela, PhD Cand.**

Please note that a final review report must be submitted to the Board at the completion of the study. Your research records may be audited at any time during or after the implementation.

Any modification of this research project must be submitted to the IRB for review and approval prior to implementation.

Please report all serious adverse events related to this study to NMIMR-IRB within seven days verbally and fourteen days in writing.

This certificate is valid till 3rd July, 2019. You are to submit annual reports for continuing review.

Signature of Chair: .....
Mrs. Chris Dadzie
(NMIMR – IRB, Chair)

INTEGRI PROCEDAMUS

11.2 APPENDIX B: RESEARCH INSTRUMENTS

VOLUNTARY CONSENT FORM

Title: Antimicrobial Resistance profiles of Foodborne pathogens of stool and food in some selected health facilities in the Greater Accra Region of Ghana
Principal Investigator: Helena Dela

Address: Department of Animal Biology and Conservation Science, Legon
Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana. P. O. Box LG 581, Legon, Ghana.

General Information about Research
Foodborne diseases are caused by germs that contaminate our food and water when consumed. They normally cause diarrhea and other stomach disorders. Treatment becomes difficult when the antibiotics that are given to the patient do not work properly. Due to the current abuse of antibiotics, there is a gradual problem of antibiotics that are showing failure to treat certain bacteria that used to work in the past. You are being asked to participate in this research because you have diarrheal disease (stools loose/more frequently than usual)
If you agree to participate, your stool samples will be collected. These samples will be coded and taken to the laboratory for analysis. Your age, sex, antimicrobial therapy and other vital information will be asked through a written questionnaire.

Possible Risks and Discomforts
There will be no risks or discomforts in giving your sample.

Possible Benefits
The results of the tests will be reported back to your doctor who will inform you about the cause of your current illness and the right treatment (antibiotics) to be given to you. These results will also be used to help doctors and the Ghana Ministry of Health on the choice of antibiotics concerning diarrhea.

Confidentiality
Your sample will be coded. Any information you give us will only be used for this study and will remain confidential.

Compensation
No compensation will be paid to you for the testing of your sample.

Voluntary Participation and Right to Leave the Research
Participation in this study is voluntary. You can decide not to participate if you are not comfortable with the procedure described above. If you do not participate, there will be no penalty to you and you will still receive treatment and care s per the standard of care at this hospital.

Contacts for Additional Information
Any questions concerning this study may be addressed to: The Principal Investigator: Helena Dela. Noguchi Memorial Institute for Medical Research, Legon. Tel: 233-242355916; email: hogum@noguchi.ug.edu.gh). For questions about the ethical aspects of this study, your rights as a volunteer, or any problem related to protection of research volunteers, contact the NMIMR IRB at 0302916438 and the Ghana Health Service Ethical Review Committee administrator, Hannah Frimpong on mobile number 020 2920651 or landline 030 2681109.

Your rights as a Participant
This research has been reviewed and approved by the Institutional Review Boards of Noguchi Memorial Institute for Medical Research (NMIMR-IRB) and Ghana Health Service (GHS-IRB). If you have any questions about your rights as a research participant, ethical aspects of this study or any problem related to

protection of research volunteers, you can contact the IRB Office between the hours of 8am-5pm through the landline 0302916438 or email addresses: nirb@noguchi.ug.edu.gh. You may also contact the Human Protection Office in NMIMR, Accra, Ghana at 0302916438, or contact Hannah Frimpong at the Ghana Health Service Ethical Review Committee on mobile number 020 2920651 or landline 030 2681109.

VOLUNTEER AGREEMENT

The above document describing the benefits, risks and procedures for the research title (**Antimicrobial Resistance profiles of Foodborne pathogens of stool and food in some selected health facilities in the Greater Accra Region of Ghana*)*** has been read and explained to me. I have been given an opportunity to have any questions about the research answered to my satisfaction. I agree to participate as a volunteer.

_____ _____

Date Name and signature or mark of volunteer

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

I was present while the benefits, risks and procedures were read to the volunteer **in a language (name the local language) the participant understands**. All questions were answered and the volunteer has agreed to take part in the research.

_____ _____

Date Name and 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 Name Signature of Person Who Obtained Consent

SAMPLE STORAGE AND FUTURE USAGE

Statement regarding the future use of biological specimen

If you agree to participate in this study, you will be required to provide a stool sample. The specimen will be tested in Ghana for different causes of foodborne diseases. Any leftover specimen will be stored for 10 years after the end of the study at the Noguchi Memorial Institute for Medical Research. Some of the testing that will be done would be to find out why some germs are not killed by the medicines that we use to treat them. **Approval will be sought from the NMIMR-IRB and GHS-ERC for future use of samples for anything beyond that initially approved**. You can still participate in the study if you do not wish your specimens to be used for further testing.

You, **authorize the use of** your stool sample collected from you as part of this research study to NMIMR. You understand that these samples may be used for other testing as determined **by GHS** and NMIMR but that your name or any information that could link your identity to these samples will be removed.

☐ Yes ☐ No

_____ _____

Signature /Thumbprint of participant Date

CHILD ASSENT FORM

Introduction

My name is Helena Dela and I am from the Department of Animal Biology and Conservation Science and the Noguchi Memorial Institute for Medical Research, Legon. I am conducting a research study entitled **Antimicrobial Resistance of Foodborne Pathogens in stool and foods in some selected health facilities in Ghana**. I am asking you to take part in this research study because I am trying to learn more about antibiotic resistant bacteria (germs) that are found in stools of patients who have diarrhea. This sometimes occurs as a result of eating or drinking contaminated food or water.
This will take 10 minutes.

General Information
If you agree to be in this study, you will be asked to provide your stool samples. These samples will be coded and taken to the laboratory for analysis. Your age, sex, antimicrobial therapy and other vital information will be asked through a written questionnaire.

Possible Benefits
Your participation in this study will result your sample tested for you without collecting any money from you. The results of your tests will be reported back to your doctor who will inform you about the cause of your current illness and the right treatment (antibiotics) to be given to you. These results will also be used to help doctors and the Ghana Ministry of Health on the choice of antibiotics concerning diarrhea.

Possible Risks and Discomforts
However, there will be no risks or discomforts in giving your sample.

Voluntary Participation and Right to Leave the Research
You can stop participating at any time if you feel uncomfortable. No one will be angry with you if you do not want to participate and you will still receive treatment by the standard of care at this hospital.

Confidentiality
Your information will be kept confidential. No one will be able to know how you responded to the questions and your information will be anonymous.

Contacts for Additional Information
You may ask me any questions about this study. You can call me at any time (Tel: 233-242355916) or talk to me the next time you see me.

Please talk about this study with your parents before you decide whether or not to participate. I will also ask permission from your parents before you are enrolled into the study. Even if your parents say "yes" you can still decide not to participate.

Your rights as a Participant
This research has been reviewed and approved by the Institutional Review Board of Noguchi Memorial Institute for Medical Research (NMIMR-IRB), Ghana Health Service (GHS-IRB) and Korle-Bu Teaching Hospital. If you have any questions about your rights as a research participant, ethical aspects of this study or any problem related to protection of research

volunteers, you can contact the IRB Office between the hours of 8am-5pm through the landline 0302916438 or email addresses: nirb@noguchi.ug.edu.gh. You may also contact the Human Protection Office in NMIMR, Accra, Ghana at 0302916438, or contact Hannah Frimpong at the Ghana Health Service Ethical Review Committee on mobile number 020 2920651 or landline 030 2681109.

VOLUNTARY AGREEMENT

By making a mark or thumb printing below, it means that you understand and know the issues concerning this research study. If you do not want to participate in this study, please do not sign this assent form. You and your parents will be given a copy of this form after you have signed it.

This assent form which describes the benefits, risks and procedures for the research titled **Antimicrobial Resistance of Foodborne Pathogens in stool and foods in some selected health facilities in Ghana** has been read and or explained to me. I have been given an opportunity to have any questions about the research answered to my satisfaction. I agree to participate.

Child's Name: ……Researcher's Name:……

Child's Mark/Thumbprint……Researcher's Signature: ……

Date: ..... **Date:** .....

Study number	Insert label here

ANTIMICROBIAL RESISTANCE PROFILES OF FOODBORNE PATHOGENS OF STOOL AND FOOD IN SOME SELECTED HEALTH FACILITIES IN THE GREATER ACCRA REGION OF GHANA

"Patient Questionnaire" Date:…../…../……

We want to ask you a few questions about Foodborne diseases, your participation is voluntary and you may stop at any time while answering the questions. Many questions will assess your knowledge, behaviors, and the environment you live in that may have contributed to cause the symptoms you have. This kind of information will be useful in planning of public health programs in Ghana and countries within the sub region.

Demographics

1. Age in years __ __	2. Gender: ☐ Male ☐ Female
3. Marital Status: ☐ Single ☐ Married ☐ Divorced ☐ Widowed ☐ Separated	
4. Education: ☐ No school ☐ Primary ☐ JHS ☐ SHS ☐ Tertiary ☐ Other_____	
5. Occupation:	
6. Ethnicity: Akan ☐Ewe ☐Ga/ Dangme ☐Nzema ☐Northner	
7. Religion: : ☐Christian ☐Muslim ☐Traditionalist ☐No religion ☐ Other_____	
8. Where do you live? _____	

Present History, Behavior, Risk factors

9. Temperature: _____

10. Date of onset of illness: ____/___/_____

11. How many times have you passed stool within the past 24 hour period? ☐ four ☐ five ☐ six ☐other

12. What was the form? ☐ Formed ☐ Semi-formed ☐ Liquid/ Loose ☐ soft/ greasy

13. Did it have traces of blood/ mucous? ☐ Yes ☐ No

14. Did you eat or drink before this illness? ☐Yes ☐No

15. Was the food prepared at home or bought? ☐ Home ☐ Bought IF Q15 IS HOME, SKIP TO 18

16. Please provide the name and location that food was bought. _____

17. What is the source of your drinking water? ☐ sachet water ☐ tap ☐ bore hole/ well

18. What symptoms do you have today? **CHECK ALL THAT APPLY** :

☐ Abdominal pain/ cramps ☐ Bloating ☐ Nausea ☐ Vomiting ☐ Fever during illness ☐ Urgency to go to the toilet ☐ Headache ☐ Fatigue/ Malaise ☐ Gas/ flatulence ☐ Other describe:

_____

19. How many days have you had the symptoms ☐ Less than one day ☐ One day ☐Two days ☐Three days ☐More than three days

20. Have you taken any medication upon onset? ☐ Yes ☐ No IF Q20 IS NO, SKIP TO 25

21. Which medication did you take?
☐ Antibiotics _____ ☐ Painkillers _____ ☐ Traditional Medicine _____ ☐ Other _____

22. Did you complete your dosage? ☐ Yes ☐ No IF ANSWER IS YES SKIP TO Q22

23. What was your reason for non- compliance? ☐ I got better ☐ Antibiotics did not work ☐ I was experiencing adverse side effects ☐ Other_____

24. Who gave you the medication? ☐ Doctor ☐ Pharmacist ☐ Friend/Family ☐ Traditional healer ☐ Other Describe: ……

25. Do you wash your hands with soap and water? ☐ Yes ☐ No

26. If Yes, when do you wash them? ☐ After bathroom use ☐ After sneezing ☐ After touching dirty things

27. What are the causes of diarrhea? ☐ Dirty food ☐ Dirty water ☐ Germs ☐ Dirty hands ☐ Others

28. Where is waste disposed of? ☐ vehicle ☐ pit ☐ lagoon or river ☐ burning ☐ dustbin ☐ Other _____

29. How would you describe general sanitation conditions at where you get food for consumption? ☐ Very tidy ☐ tidy ☐ Not tidy ☐ Other_____

(END INTERVIEW)

Comments:

_____

_____

_____

_____

_____

Study number	Insert label here

ANTIMICROBIAL RESISTANCE PROFILES OF FOODBORNE PATHOGENS OF STOOL AND FOOD IN SOME SELECTED HEALTH FACILITIES IN THE GREATER ACCRA REGION OF GHANA

"Food Vendors Questionnaire" Date:…../…../……

We want to ask you a few questions about Foodborne diseases, your participation is voluntary and you may stop at any time while answering the questions. Many questions will assess your knowledge, behaviors, environment you live in and the food you sell. This kind of information will be useful in planning of public health programs in Ghana and countries within the sub region.

Demographics

1. Age in years __ __	2. Gender: ☐ Male ☐ Female
3. Marital Status: ☐ Single ☐ Married ☐ Divorced ☐ Widowed ☐ Separated	
4. Education: ☐ No school ☐ Primary ☐ JHS ☐ SHS ☐ Tertiary ☐ Other_____	
5. Occupation: ☐ Vendor ☐ Assistant vendor 6. Ethnicity: ☐Akan ☐Ewe ☐Ga/ Dangme ☐Nzema ☐Northner	
7. Religion: : ☐Christian ☐Muslim ☐Traditionalist ☐No religion ☐ Other_____ 8. Where do you live _____	
9.Where is the location of your business _____	

General information on Sanitation, Personal Hygiene, Risk factors

9. What food do you sell? _____

10. Who prepares the food you sell? ☐ Self ☐ Another

11. What is the source of water for preparation? ☐ Pipe ☐ Well ☐ Bore hole ☐ Roof collection ☐ Others

12. Do you have access to sanitation facilities at the business location? ☐Yes ☐No

13. If Yes, which facilities? ☐ Bucket ☐ Flush toilet ☐ Pit latrine ☐Others (specify) _____

14. Do you wash your hands with soap and water? ☐ Yes ☐ No

15. If Yes, when do you wash them? ☐ After toilet use ☐ After sneezing ☐ After touching dirty things

*16. How will you describe the immediate environment where food is sold? ☐ heavily littered ☐ some litter ☐ no litter ☐flies present ☐ stagnant water ☐ Others (specify) _____

17. Do you cook with vegetables? ☐ Yes ☐ No

18. Do you wash them before cooking? ☐ Yes ☐ No

19. Where do you purchase ingredients for cooking? ☐ Market ☐ slaughter house ☐ street hawking ☐ supermarket ☐ Other

20. Which type of meat/fish do you use in cooking? ☐ Goat ☐ Cow ☐ Chicken ☐ Fish ☐ Other

21. Is it bought fresh or frozen? ☐ Fresh ☐ Frozen

22. What are the storage facilities you use in preserving ingredients for cooking? ☐ Refrigerator ☐ Open

23. What causes diarrhea? ☐ Dirty food ☐ Dirty water ☐ Germs ☐ Dirty hands ☐ Others

24. Where is waste disposed of at your business center? ☐ vehicle ☐ pit ☐ lagoon or river ☐ burning ☐ dustbin ☐ Other _____

25. Have you experienced any of these symptoms recently? ☐ Nausea ☐ Vomiting ☐ Abdominal pain ☐ Fever ☐ Diarrhea

☐ Fatigue ☐ Malaise ☐ Other _____

26. Have you had any of these diseases recently? ☐ Typhoid ☐ Cholera ☐ Influenza ☐ Gastrointestinal disorders ☐ Others, specify_____

(END INTERVIEW)

Comments:

_____

_____

_____

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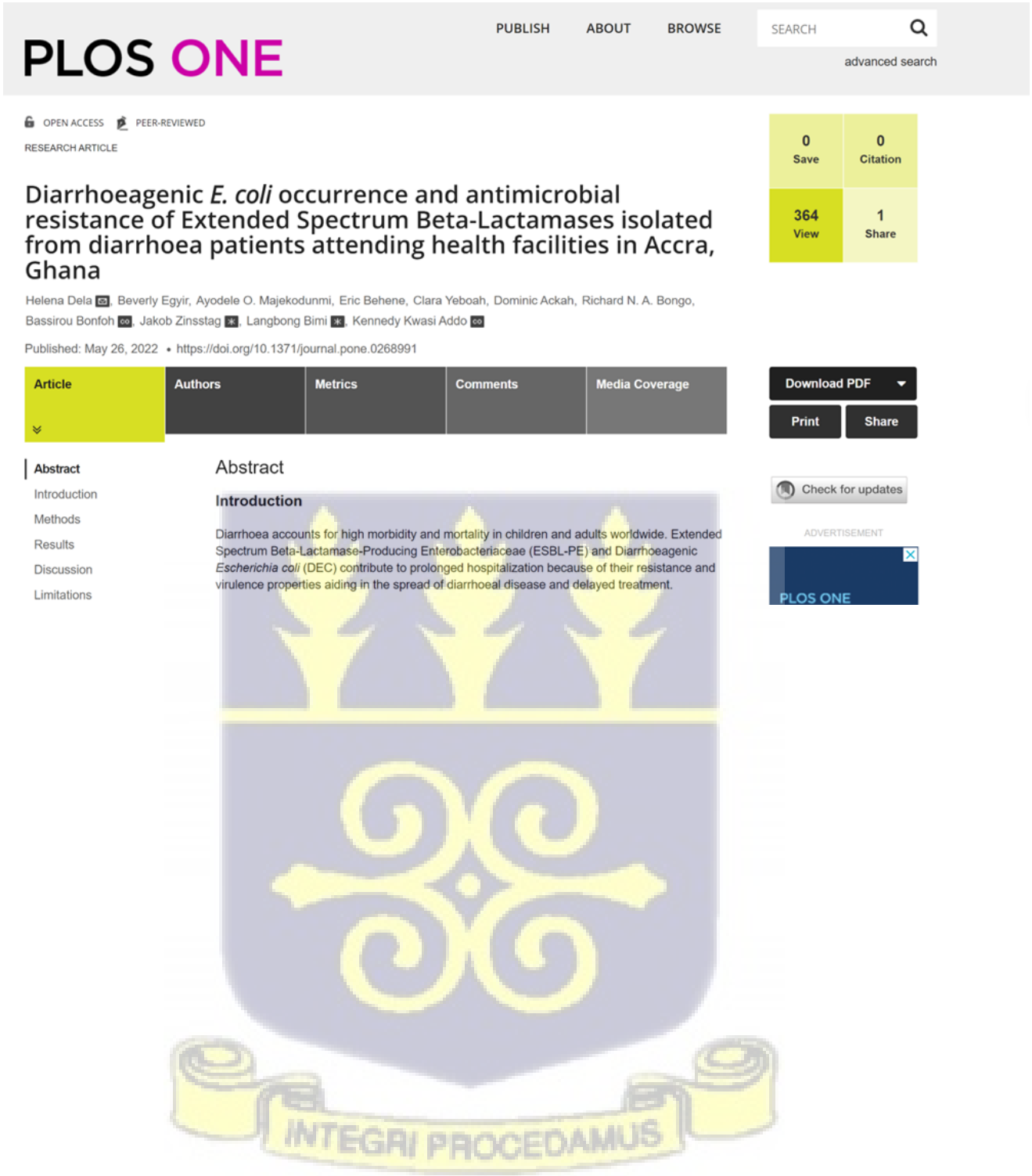
11.3 APPENDIX C: PUBLICATIONS



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RESEARCH ARTICLE

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Diarrhoeagenic *E. coli* occurrence and antimicrobial resistance of Extended Spectrum Beta-Lactamases isolated from diarrhoea patients attending health facilities in Accra, Ghana

Helena Dela, Beverly Egyir, Ayodele O. Majekodunmi, Eric Behene, Clara Yeboah, Dominic Ackah, Richard N. A. Bongo, Bassirou Bonfoh, Jakob Zinsstag, Langbong Bimi, Kennedy Kwasi Addo

Published: May 26, 2022 • https://doi.org/10.1371/journal.pone.0268991

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Abstract
Introduction
Methods
Results
Discussion
Limitations

Abstract

Introduction

Diarrhoea accounts for high morbidity and mortality in children and adults worldwide. Extended Spectrum Beta-Lactamase-Producing Enterobacteriaceae (ESBL-PE) and Diarrhoeagenic *Escherichia coli* (DEC) contribute to prolonged hospitalization because of their resistance and virulence properties aiding in the spread of diarrhoeal disease and delayed treatment.

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UNDER REVIEW

International Journal of Food Microbiology

Microbiological quality and Antimicrobial Resistance of Bacteria species recovered from Ready-to-Eat food, water samples, and palms of food vendors in Accra, Ghana

--Manuscript Draft--

Manuscript Number:	
Article Type:	Full Length Article
Keywords:	Ready-to-Eat foods; Antimicrobial resistance; Foodborne pathogens; Diarrheagenic E. coli; Extended spectrum beta-lactamase; Accra; Ghana
Corresponding Author:	Helena Dela GHANA
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	Beverly Egyir
	Eric Behene
	Hamdiya Sulemana
	Rodalyn Tagoe
	Ronald Bentil
	Richard Bongo
	Langbong Bimi
	Kennedy Addo
Abstract:	Highlights • Foodborne bacteria were found in Ready-To-Eat food, water, and palms of vendors • Antimicrobial resistance showed among some foodborne bacteria • Most of the RTE food and water were considered unsafe for consumption • Beta-Lactamase and Diarrheagenic Escherichia coli (DEC) genes occurred in some isolates

11.4 APPENDIX D: ABSTRACTS PRESENTED AT INTERNATIONAL CONFERENCES

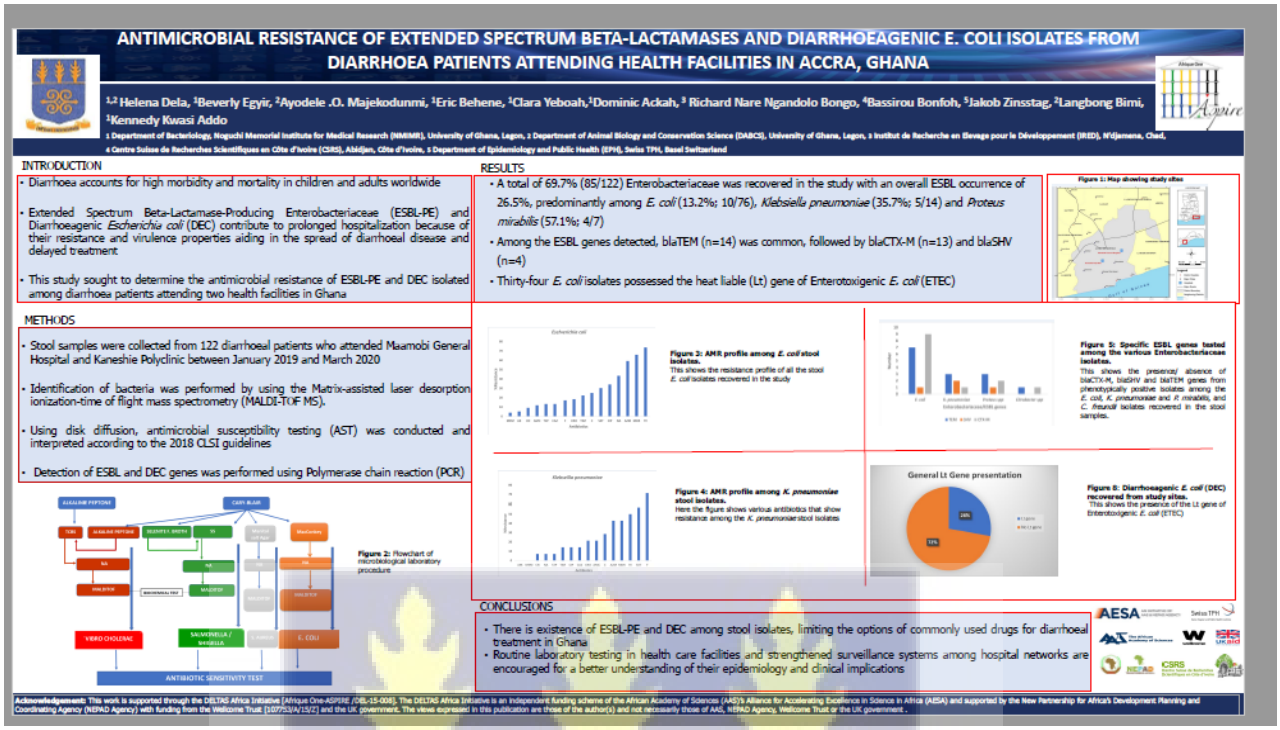

Figure 11.1: Poster presented at the Virtual American Society of Tropical Medicine and Hygiene (2021)

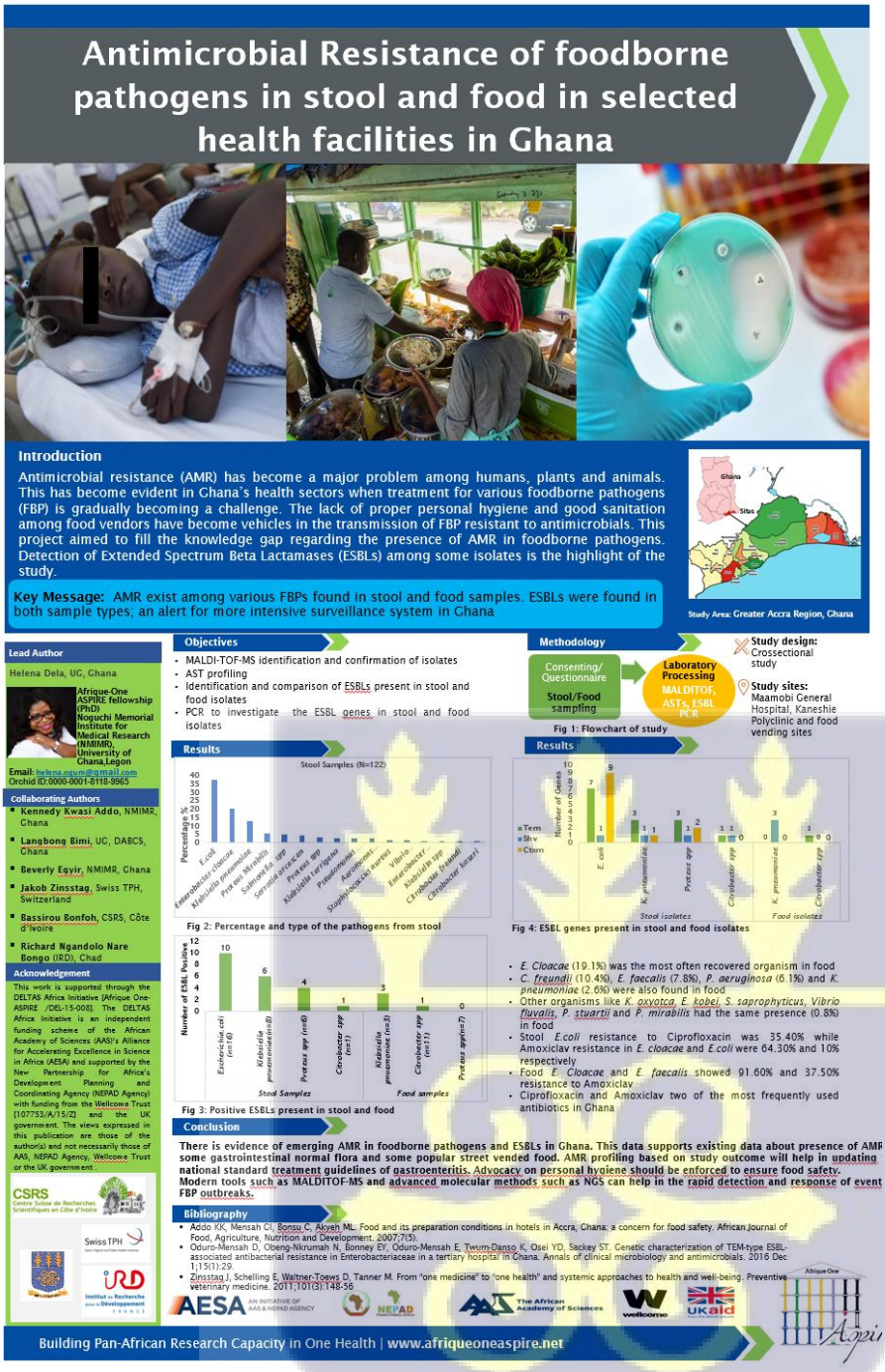

Figure 11.2: Poster presented at the Virtual World One Health congress 2020

11.5 APPENDIX E: IMPACT

Figure 11.3: Group photograph after hosting the first AMR conference in NMIMR, 2020

Figure AE2*:* Poster of the invitation to the 1st AMR seminar that was organized at NMIMR

Figure AE3: AMR campaign during World Antimicrobial Awareness Week, (WAAW) 2019

Figure AE4: Interview at Utv morning show on the effect of AMR during WAAW 2020

Figure AE5: Community engagement with market women and food vendors on the need to exercise personal hygiene and good sanitation to reduce AMR during WAAW 2019

Figure AE 6: Presentation of final results to the health workers at Maamobi General hospital during WAAW 2020