KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

COLLEGE OF SCIENCE

FACULTY OF BIOSCIENCES

DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY

MICROBIAL SAFETY OF FOODS IN SELECTED SENIOR HIGH SCHOOLS

BY

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NOVEMBER, 2018

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(BSc. Environmental Science)

A Thesis submitted to the **Department of Food Science and Technology**, Kwame Nkrumah University of Science and Technology, Kumasi in partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE FOOD QUALITY MANAGEMENT

NOVEMBER, 2018

DECLARATION

I Shirley Gloria Davis declare that this submission is my own work towards the MSc. and that, except for the other people's investigations which have been duly acknowledged, this work is the result of my own original research undertaken under supervision, and that this thesis, to the best of my knowledge, contains no material previously published by another person, nor material which has been accepted for the award of any other degree or diploma of the University or other educational institute.

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DEDICATION

Ebenezer: ―Thus far has the Lord brought us‖!

This thesis is dedicate to my husband Mr. Wilson Kow Andoh and my lovely daughters of Zion; Daisy Wilhelmina Ayeyi Andoh, Keziah Shirley Aseda Andoh and Kayla Lyanne Nyamedzi Andoh.

ACKNOWLEDGEMENT

―To God be the glory, great things he has done, greater things will he do‖

My profound gratitude goes to my supervisor Dr. Mrs. Gloria Ankar- Brewoo for her patience, guidance and technical support throughout this work. Also to Dr. Isaac W. Ofosu (Senior lecturer) and Dr. Lutterodt (Course Coordinator) all of Department of Food Science and Technology, KNUST for their directions and encouragements.

I am also very grateful to Mr. Emmanuel Birikorang of the Laboratory Technology Department of University of Cape Coast, for his immense help with the laboratory analysis and guiding me through this research. Special thanks also goes to the Heads, Domestic bursars and Matrons of all the selected Senior High Schools in Cape Coast for their contribution towards this research.

To my brilliant and supportive husband Mr. Wilson Kow Andoh, my lovely girlsDaisy, Keziah and Nyamedzi Andoh and my one and only sister Linda P. Davis I say a big thanks for all your love. Could not have made it without you all, love you all.

I am indebted to my parent Mr. and Mrs. Davis, it so unfortunate both of them are not here to witness this day but their love and encouragement can never be forgotten.

Mum thanks for encouraging me to get the form for this MSc.

I would also want to acknowledge the support of Mrs Naa Korkoi Ewudzie, Mr. Micheal Ocansey, Mr. Rodrick Daddey-Adjei, Mr. Odai Tetteh, my FEJ sisters and all who through diverse means have made this work possible. God bless you all

ABSTRACT

Mass feeding which is carried out at most SHS to feed students stands a higher risk of microbiological contamination relative to small scale food preparation because of issues associated with handling. This study thus, was aimed at determining the microbial safety of foods served in some selected Senior High Schools. Eighteen (18) food samples including breakfast, lunch and supper were sampled from four (4) selected SHS in the Cape Coast Metropolis. Samples collected were subjected to microbial analysis for estimation of *Escherichia coli* (EC), *Salmonella* species (SS), *Staphylococcus aureus* (SA), *Bacillus cereus* (BC), total viable count (TVC) and total coliform levels (TC). Microbial counts were expressed as means and the data exported to IBM SPSS v25. One-way analysis of variance (ANOVA) was used to determine statistically significant difference of microbial loads between all samples. The schools had all their food samples falling within acceptable limits for TVC which implies that the general hygiene status of the food samples is satisfactory. However only one school had all its samples falling within acceptable limits for TC, the rest had 25% -30% contamination from pathogens hence a setback in the efficiency of sanitation programs in SHS. With the exception of two different schools with each having its food samples falling within the acceptable limits for SA and SS, the remaining had 25%-50% contamination from SA and 25% contamination from SS. For BC there was 25% contamination from all the schools. *Staphylococcus aureus* had the highest percentage occurrence in the food samples followed by *B. cereus* and *E. coli* respectively. Contaminations such as TVC, TC, SA, SS and BC are usually due to absence of consistent rigorous surveillance and weak implementation of the law in institutional kitchens thus a system to monitor and control the generally food chain in the country from farm to fork, including suppliers of raw materials to SHS is required.

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ABBREVIATIONS

SHS	Senior High School
FBD	Foodborne Disease
FAO	Food and Agriculture Organization
WHO	World Health Organization
WFP	World Food Programme
UNESCO	United Nations Educational Scientific and Cultural Organization
GH/GHC	Ghana/Ghana Cedis

TVC	Total Viable Count
TC	Total Coliform
EC	*Escherichia coli*
SA	*Staphylococcus aureus*
BC/B.cereus	*Bacillus cereus*
SS	*Salmonella* species
ANOVA	Analysis of Variance
GS	Ghana Standard
ND	Not detected
g	gram
CDC	Centre for Disease Control
CFU	Colony forming Unit
Min	minute
H	hour
°C	Degree Celsius

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CHAPTER ONE

INTRODUCTION

1.0 Background Statement

Universally, infections from ingesting food contaminated with bacteria, viruses or parasites are the major health problem associated with food (Nguyen-Viet *et al.*, 2017). Microbial contamination may occur as a result of pathogens in foods. This is usually caused by cross-contamination including unsatisfactory cleanliness of staff, the surroundings and/or by inadequate conditions such as temperature abuse or inadequate cooking that facilitate microbial growth or survival (Osimani *et al*., 2013). Contamination normally ensues during food preparation or serving however it might occur at any point from farm to fork (Ameme *et al*., 2016). Foodborne diseases are usually caused by ingesting foods contaminated with some microorganisms and this encompass a wide range of illnesses (Osimani *et al*., 2013). Although most of the cases resulting from foodborne diseases are trivial, a significant number of incidence are lethal thus a lot of money is lost as a result of medical expenses, decrease productivity and recurrent recalls due to high prevalence of severe infections and chronic sequelae (Saba and Gonzalez-Zorn, 2012). For instance, in Ghana, one out of forty persons suffer severe food borne disease annually which cost the government US $ 69,000,000.00 since 420,000 cases on foodborne diseases are stated with a yearly death rate of 65,000 (Ababio and Lovatt, 2015).

Food safety, has thus become an increasingly important public health issue (Osimani *et al.*, 2013) as it is a critical aspect in the progress of developing countries worldwide (Saba and Gonzalez-Zorn, 2012). It is even more vital in catering for mass institutions

because of the huge amount of meals served in establishments such as schools, hospitals, child cares, businesses and canteens worldwide every day

(Osimani *et al.,* 2013). Mass catering is carried out at most Senior High School (SHS) to feed boarding students and in some cases even the day student. Deliberate or accidental contamination of food during bulk catering may threaten the health of the consumers as it may leads to foodborne illness, outbreaks such as this feature prominently in national statistics thus may have negative consequences on a country (Annor and Baiden, 2016). Meals served in dining halls of SHS are mostly prepared ready-to-eat meals which requires adequate hot holding temperature and time control for safety (Ababio *et al*., 2016). Students lodging on school campuses of Senior High Schools (SHS) in the country are feed three times daily (Ababio *et al.*,2016). Thus the development, welfare and the overall healthy lifestyle of SHS student is highly dependent on the school communal feeding programmes (Ababio *et al.,* 2016). It is therefore essential to government and other stake holders to provide students with safe meals as it enhances their wellbeing, development and progress of beneficiaries and encourages sustainable education in developing countries (Ababio *et al*., 2016). In the provision of meals, it is vital that precautionary principles are applied with respect to food handling and preparation safety to ensure not just a nutritious meal but a meal free of harmful pathogens. It is only when the food is safe that the children will obtain the full nutritional benefit (Owusu, 2010).

Nevertheless the government and stakeholders are still faced with amassed reports of foodborne infection from schools (Ababio *et al.,* 2016). Saba and Gonzalez-Zorn (2012) reported the decline of microbiological food safety. Also, according to Ababio and Lovatt (2015) there is still work to be done on microbiological safety and quality

of institutional meals such as schools and hospitals. Information on microbial safety of food is scant and most especially that in Senior High Schools. In essence, the microbial safety of foods served to senior high school students need to be critically examined and this is the focus of this study**.**

1.1 Problem Statement

According to the World Health Organization (WHO) reports, food poisoning fatal cases that occur worldwide annually is approximately 2 million and this happens particularly in developing countries (Lund, 2015). Recent estimates by WHO, indicates that in Africa, 700,000 deaths annually are as a result of food and waterborne related diseases (Mama and Alemu, 2016) of which Ghana is not an exception. There is documented evidence that show that mass feed programes are often associated with microbial contaminated foods. In Ghana the School Feeding Programme has also been running, however there are no strong monitoring of food safety issues associated with the programme and this could lead to possible advert Health effect. Thus this study seek to determine the level of microbial contamination in food samples of some selected senior high schools.

1.4 **Justification of Research**

Although there are rampant reports on food poisoning cases on the media, most especially from institutional set ups particularly in schools, much research focus has been concentrated on commercial food sector in the country, with special attention given to street foods (Ababio and Lovatt, 2015). Presently, though there are no reliable estimates globally for the encumbrance of foodborne diseases, 1.9 million children are killed globally every year by diarrheal diseases alone-which form a sizeable proportion

of foodborne diseases (Ameme *et al*., 2016). Microbial contamination is cause by the incidence of pathogens in foods (Osimani et al., 2013).

In Ghana microbiological food contamination is alarming however research has shown a decline on the study of microbiological safety of foods (Saba and GonzalezZorn, 2012).

It is therefore imperative to determine the incidence and level of microbes in the foods served at some selected Senior High School. The result of this study would contribute to academic knowledge on the microbial safety of foods served to student at Senior High Schools. Furthermore, it would provide adequate information for policy-makers to apportion appropriate resources for food safety control and intervention efforts in the preparation of foods served to student at Senior High Schools. Thus resulting in the prevention of foodborne diseases and food poisoning outbreaks in schools and the nation as a whole.

1.2 Objectives

To provide important basis for policy formulation for an effective food safety regulation for foods prepared for students at Senior High Schools to prevent outbreak of foodborne diseases and food poisoning in schools and to improve food safety in the school as a whole. Hence this study specifically is to determine the microbial safety of foods served in some selected Senior High Schools.

CHAPTER TWO

LITERATURE REVIEW

2.0 Catering Services in SHS

An established catering system which usually comprise of a domestic bursar (senior matron), assistant matrons (supervisors), cooks, pantry men and a procurement unit manages Senior High school‘s feeding services. The state agencies responsible for surveillance and monitoring of SHS kitchen are the Food and Drugs Authority and the Environmental Protection Units of the Metropolitan, Municipal and District Assemblies (Ababio *et al*., 2016) Codex Alimentarius hygiene requirements is the principal hygiene standard used.

Ghana Education Service also uses its unit called School Health Education Programme (SHEP) to monitor health and cleanliness activities in schools (Ghana Education Service, 2012). There has been rampant reports of inadequate cleanliness at the kitchens of senior high schools in the country though matrons mostly do not consider that their practices could be of risk to students. Student of that lodge at the campus of Senior High Schools (SHS) in the country are given meals thrice daily (Ababio *et al.*,2016). Food are served to SHS students at the dining halls. Meals served in dining halls are prepared ready-to-eat meals that needs adequate hot holding temperature and time control for safety. The development, welfare and the overall healthy lifestyle of SHS student is highly dependent on the school communal feeding programmes (Ababio *et al.*, 2016). Mass catering is carried out at most Senior High

School to feed boarding students and in some cases even the day student. Deliberate or accidental contamination of food during bulk catering may threaten the health of the consumers as it may leads to foodborne illness, outbreaks such as this feature prominently in national statistics thus may have negative consequences on a country (Annor and Baiden, 2016).

Mostly young children are susceptible to pathogenic bacteria. Consumption of food contaminated with disease causing microbes such as *Escherichia coli, Staphylococcus aureus* and other opportunistic pathogens has the risk of causing pathological conditions, such as haemolytic uremic syndrome and osteomyelitis. Illnesses from pathogenic bacteria usually last up to 3-5 days thus likely epidemic amongst student are of great concern (Nhlapo *et al.*, 2014).

Factual statistical information indicate that caterers are the cause of about 70% of all food poisoning problems resulting from bacterial infestation (Annor and Baiden, 2016). This is usually caused by cross-contamination including unsatisfactory cleanliness of staff, the surroundings and/or by inadequate conditions such as temperature abuse or inadequate cooking that facilitate microbial growth or survival (Osimani, *et al.*, 2013). Due to communal feeding at institutions, catering at establishments came out as the unit with massive number of consumers and these include students, the sick and susceptible people from hospitals who -likewise need great care when it comes food safety issues. However, among catering services at establishments, schools stance out of statistics as a hypothetical cause of food hygiene problems. For instance, in Ghana, schools are the food establishments stated to be responsible for 77% of all traceable food borne diseases (Ababio and Lovatt, 2015)

It is essential to government and other stake holders to provide students with safe meals as it enhances their wellbeing, development and progress of beneficiaries and encourages sustainable education in developing countries (Ababio *et al*., 2016).In the provision of meals, it is vital that precautionary principles are applied with respect to food handling and preparation safety to ensure not just a nutritious meal but a meal free of harmful pathogens. It is only when the food is safe that the children will obtain the full nutritional benefit (Owusu, 2010).

2.1 Report from scholarly research articles, media &online sources

Infections from ingesting food contaminated with bacteria, viruses or parasites are the major health problem associated with food worldwide(Nguyen-Viet *et al.*, 2017). According to the World Health Organization (WHO) reports, food poisoning fatal cases that occurs worldwide annually is approximately 2 million and this happens particularly in developing countries (Lund, 2015). In the United States for instance, food-borne disease epidemics that have been reported with allegations of mishandling, have been associated with 79% from foods from commercial or institutional establishments and 20% from homes. Twenty five percent of these report predictably, could have been eluded by the practice of safe food handling (Annor and Baiden, 2016). Food safety have been placed as a priority European government because of escalating occurrences of food poisoning due to *E.coli* and *Campylobacte*r (Annor and Baiden, 2016).

Recent estimates by WHO, indicates that in Africa, 700,000 deaths that occurs annually are as a result of food and water-borne related diseases (Mama and Alemu, 2016) of which Ghana is not an exception. One out of forty persons in Ghana ,suffers severe food borne disease annually which cost the government about US $ 69,000,000.00 since 420,000 cases on foodborne diseases are reported with an annual death rate of 65,000

(Ababio and Lovatt, 2015). This information could be under estimated due to low rate in reporting and also in developing countries only the cost borne by individuals through hospitalization and medication is considered whereas in developed countries even the cost to employers, established bodies including research laboratory, investigations, infirmity cost and cost of family members who take care of the sick member and untimely death are considered (Ababio and Lovatt, 2015).

Factual statistics indicate that 70% of all bacterial food poisoning is caused by caterers. This is superior to incidences reported from any other food sector. Most of these food poison outbreaks are as a result of inadequate time and temperature control of food, and the outstanding thirty percent are due to cross contamination. In Turkey, it was identified that most of the issues that impose risk on food safety are due to industrialization and mass production, fast food consumption, street vendors and growing inter- national trade and tourism. Thus a similar account could be cited for Ghana as the likely causes of food safety problems (Annor and Baiden, 2016)

The outcome of foodborne pathogens infections usually causes severe consequences in susceptible persons than in healthy adults (Lund, 2015). In sub-regions of Africa, diarrheal disease agents were the leading cause of foodborne disease burden (WHO, 2015). Though presently, there are no reliable estimates globally for the encumbrance of foodborne diseases, 1.9 million children are killed globally every year by diarrheal diseases alone-which form a sizeable proportion of foodborne diseases (Ameme *et al.*, 2016).

The headlines are continually hit with outbreaks from institutional foodborne illness. A number of outbreaks associated with food served in schools has been reported (Lund, 2015). Most illness as a result of pathogenic bacteria may last up to three to five days

thus possible epidemics amongst school children are of great concern (Nhlapo *et al.,* 2014). For instance in 2014, 43% of the total foodborne poisoning incidents in Malaysia was contributed by epidemics in academic institutions of which inappropriate food handling by food handlers attributed more than 50% of the total food poisoning cases (Lee *et al.*, 2017)**.** In Ghana, 77% of all traceable food borne diseases are from schools' food establishment (Ababio and Lovatt, 2015).

In September 2005 an outbreak of *Escherichia coli* O157 in South Wales occurred, which was epidemiologically linked to cooked, sliced meats supplied to schools across the region. It involved 157 cases and was the largest ever seen in Wales. In light of this outbreak, it was decided by the Welsh Food Microbiological Forum (WFMF) members that it was important to assess the general microbiological quality of food served in schools in Wales to identify any further hazards present (Meldrum *et al.,* 2009).

In Japan, outbreaks of *Salmonella, Campylobacter jejuni, Escherichia coli,* and *Staphylococcus aureus* have been linked to school food (Meldrum, *et al.,* 2009).

The Ghana Feeding Programme reported of food poisoning in May 2007 at the Ga East Municipality. Thousand three hundred school children suffered various degree of food poisoning according to Wednesday May 30, 2007 edition of the Daily Graphic (Owusu, 2010). The Joy FM in February 2010 stated over one hundred(100) students of Archbishop Porter Girls Senior High School in Takoradi were rushed to the Effia Nkwanta hospital after suspected food poisoning. Most of the students were reported to have complained of diarrhea, headache or both. Reports indicated contamination of grinding mill for tomatoes due to negligence of kitchen staff to clean or sanitize mill before reusing it.

In 2013, Citi FM reported of over 40 students hospitalized in Adonten Secondary School in Eastern Region over food poisoning. There is also the food poisoning incidence at Mfantsipim School, Cape Coast which was published in The General Telegraph edition on 16th March, 2016.

Joy News on November 2017 again reported that some students of Kaleo Senior High School in the Nadowli-Kaleo District of the Upper West Region in Ghana complained about the poor quality of food served them .The students claimed they repeatedly fall sick as a result of eating bad food.

With regards to the actual level of food-borne diseases ,it is difficult in establishing the exact numbers of incidents since cases are significantly underreported (Annor and Baiden, 2016).

2.2 Foodborne illnesses in SHS and its impact on academics and the country's economy.

Economic loss occurs in countries worldwide due to foodborne illness and Ghana is not an exception (Ababio *et al.*, 2016). Food safety, has thus become an increasingly important public health issue (Osimani *et al.*, 2013) since it is an essential feature in the growth of developing countries worldwide (Saba and Gonzalez-Zorn, 2012). The benefits of providing student with meals by proprietors are to ensure their growth, good health and encourage sustainable education (Ababio *et al.*, 2016) as this lead to economic growth of a country as a whole. Food-borne diseases have been estimated to cause 76 million illnesses, 323,000 hospitalizations, and over 5,000 deaths annually in the United States (Saba and Gonzalez-Zorn, 2012).

In Africa, FBD causes huge economic losses and severe danger to individuals thus it has become an unbearable public health burden. According to WHO's most recent

estimates, in Africa, food and water-borne related diseases causes 700,000 deaths per year (Mama and Alemu, 2016). Foodborne diseases due to ingestion of foods contaminated with disease causing microbes encompass a wide range of illnesses (Osimani *et al.*, 2013). Although most of the cases resulting from foodborne diseases are trivial, a significant number of incidence are lethal thus a lot of money is lost as a result of medical expenses, decrease productivity and recurrent recalls due to high prevalence of severe infections and chronic sequelae (Saba and Gonzalez-Zorn, 2012). For instance, in Ghana, one out of forty persons suffer severe food borne disease annually which cost the government US $ 69,000,000.00 since 420,000 cases on foodborne diseases are reported with an annual death rate of 65,000 (Ababio and Lovatt, 2015).

According to Ababio et al., (2016), within a term 26% of 180 school going children in Ashanti Region reported of 2e4 episodes of FBD, this implies that for each academic year, 13 student out of 50 SHS student experience FBD between 3 and 6 cases. From this, it shows that the rate of food borne disease in Senior High School is higher as compared to FBD that occurs annually in Ghana which is 1 out of 40. Figures could probably be high if private schools were considered as this report only emphasized on public schools with lodging facilities. However, if other regions are considered, probably figures could even be higher. Similar is Indonesian report from poor hygiene categorized school were 118 out of 120 students experience FBD after meals. A lot of money is spent during sickness on medication and other health related examinations. For instance out of the when 180 students were sampled within a term, 60.5% had

undisputable spent money ranging from GHC 1.0 to more than GHC 50.0 on medication resulting from foodborne illness. Hence for medication alone, between GHC 108.9 / GHC 5445.00 is spent per term by 60.5% of students in SHS in Ashanti Region(Ababio et al., 2016). According to Food and Drugs Authority in Ghana, in the year 2006 due to FBD, loss of productivity was approximately 594,279 days (19,809 months) which could be massive in terms of economic loss to the state (Ababio and Lovatt, 2015).

A significant proportion of both governments and development partners' annual budget is spent on foodborne diseases in developing countries. Thus food safety must be recognized and addressed by minimizing the consumption of unsafe food which may help to ensure the good health of the population and eventually, play a vital role in the economic progress of developing countries (Saba and Gonzalez-Zorn, 2012)

2.3 Main Factors of food borne diseases

Naturally food comprises safe levels of both harmful and safe bacteria (Owusu, 2010). The incidence of disease causing microorganisms in foods may be caused by microbial contamination thus foodborne diseases due to ingestion of foods contaminated with pathogens encompass a wide range of illnesses (Osimani *et al.*, 2013). The disease produced as a result of food contamination start in the intestinal tract when either the toxin is absorbed to do its damage or that the microorganisms grow, reproduce, and cause distress to the human. Although most of cases are mild, a significant number of cases are fatal (Owusu, 2010)**.** Furthermore, the particular pathogen ingested and the physical condition of the consumer determines the severity of the foodborne disease

(Owusu, 2010). Contamination normally ensues during food preparation or serving however it might occur at any point from farm to fork (Ameme *et al*., 2016)

According to Owusu (2010) the common causes of food borne illness include the following: Cross contamination from raw to cooked food; inadequate reheating food to temperature that destroy food poisoning bacteria; Food prepared too far in advance of service; Storing cooked food at ambient temperature; Cooling food rapidly; contaminating cooked food with food poisoning bacteria; under cooking meat and meat products; infected food handler.

2.4 Microbiological Hazards

Microbes are everywhere and enter food by endless routes (Owusu, 2010). Microbial contamination is cause by the incidence of pathogens in foods (Osimani *et al.,* 2013). Contamination normally ensues during food preparation or serving however it might occur at any point from farm to fork (Ameme *et al*., 2016).

Universally, the main health problem associated with food are infections from consuming food contaminated with viruses, bacteria or parasites.(Nguyen-Viet, *et al.*, 2017). The most serious food safety problems are foodborne diseases with microbial origin and the emergence of rise in antimicrobial resistance in bacteria causing disease is aggravating this (Owusu, 2010)**.** According to FAO/WHO (2016) the worldwide burden of foodborne disease estimated 31 microbiological and chemical agents. In 2010 it was estimated that more than 600 million persons fell ill which resulted in 420,000 deaths and 33 million Disability-Adjusted Life Years (DALYs).

Microbial hazards usually occurs as a result of cross-contamination such as insufficient hygiene of staff, surroundings and/or by inadequate circumstances that enables growth

or survival of microorganisms. Such situations include temperature abuse or inadequate cooking (Osimani, *et al.*, 2013).

Microbial safety and shelf life of foods depends on minimizing the initial level of microbial contamination, preventing or limiting the rate of microbial growth, or destroying microbial populations (McMeekin *et al.*, 1997). In ensuring microbiological safety of foods, sanitation (cleaning and disinfection) is the most effective control. Therefore it is important to ensure that cleaning is done to a point that significantly decreases cross-contamination with the assurance of food integrity (Nhlapo *et al*., 2014)

2.4.1 Pathogenicity of Salmonella species

Salmonella is a gram negative bacilli which belong to the family Enterobacteriaceae that grows aerobically and anaerobically at an optimum temperature of 37°C (98°F), readily killed by temperatures above 55°C (131°F) (Owusu, 2010). Food borne Salmonellosis is usually as a result of the ingestion of contaminated animal products such as raw meat, poultry and eggs. Other sources include: eating fresh fruits and vegetables without washing them, inadequate cleaning of contact surface at the kitchen use in the preparation of foods such as raw meat (Mama and Alemu, 2016)

Salmonella organisms of various types are spread through contamination of water and food as well as by direct contact. An infection with salmonellae most commonly is rather acute, involving nausea, vomiting and diarrhea, and may develop into an enteric fever similar to typhoid. Diarrhoea may persist for several days, however, individuals differ in the resistance to this infection but generally morbidity is high in an outbreak (Owusu, 2010)

Salmonellosis remains a major public health problem. Recently both renowned and new food vehicles have been associated in most of the outbreaks in Europe and the United States. In the United Kingdom and Ireland an outbreak occurred which was caused by the serotype. Within the period from February to July 2008, it was reported that 119 patients in the United Kingdom and Ireland including a case in Finland were affected. The source of the outbreak strain was linked to a sandwich company in Ireland (Owusu, 2010).

Salmonella typhi is the bacterial that causes typhoid fever disease. Symptoms include high fever, malaise, headache, constipation or diarrhoea, rose-coloured spots on the chest, and enlarged spleen and liver. It usually develops 1–3 weeks after exposure, and may be mild or severe. The occurrence of typhoid fever is highest in children from 5–19 years old in virtually all endemic areas. The age of SHS student (usually from 14-19) falls within the range stated earlier on the prevalence of typhoid fever. The disease is almost exclusively transmitted by food and water contaminated by the faeces and urine of patients and carriers. The most common source of typhoid transmission is polluted water. Other sources that has shown to be a source of infection are shellfish taken from sewage- contaminated beds, vegetables fertilized with night-soil and eaten raw, contaminated milk and milk products. The disease can be transmitted as long as the bacteria remains in a person‘s body; most people are infectious prior to and during the first week of convalescence, but 10% of untreated patients will discharge bacteria for up to 3 months. Typhoid fever can be treated with antibiotics. However, resistance to common antimicrobials is widespread. Healthy carriers should be excluded from handling food (Manual for Foodborne Survillance in

Ghana , 2016)

2.4.2. Pathogenicity of *Escherichia coli.*

Escherichia coli (*E. coli*) known as colon bacillus because of its habitat being the large intestine and primary of intestine origin is a member of the coliform group of bacteria. It is a gram negative bacillus that grows *E. coli* aerobically and anaerobically at an optimum temperature of 37°C (98°F), readily killed by temperatures above 55°C (131°F). Its incubation period is 12 hours to 3 days and diarrhea is the main symptom. *E. coli* is a major part of most warm blooded animals normal gut flora and the organism is usually shed in faeces. Though most strains are harmLess, a few are pathogenic to humans. (Owusu, 2010).

Escherichia coli strains categorized by their capability to produce Shiga toxins is a vital cause of foodborne disease. A wide range of symptoms have been associated with its infections such as slight intestinal discomfort, haemorrhagic colitis (HC), haemolytic uremic syndrome (HUS), end-stage renal disease (ESRD) and death. Two names; verotoxin-producing *Escherichia coli* (VTEC) and Shiga toxin-producing *E. coli* (STEC) are also use to refer to this pathogenic *E. coli* group . (FAO / WHO, 2016)

According to Owusu, (2010), pathogenic or enterovirulent *E. coli* are divided into five groups based whether they are in/ on the body. The Eteropathogenic (EPEC) is responsible for severe infantile diarrhea and Enterotoxigenic (ETEC) produces a heat labile (LT) or heat stable (ST) toxin. Both of the above mentioned, may be produced by the same organism and are agents of the following conditions: paediatric diarrhoea, severe cholera- like illness in adults in cholera areas travellers‟s diarrhea, which may be both food and water-borne.

Also Enteroinvasive (EIEC) with invasive properties for the mucosa. They give rise to a dysentery-like disease, and they are restricted to a limited number of serotypes of largely unknown occurrence.

Most severe food poisoning is cause by the *E. coli* O157:H7 strain which affects three in every 10,000 people with the majority being children under age nine. Their contamination is caused by unhygienic cooking practices. Symptoms of the disease are characterized by blood in stools, renal failure due to blood clots in the kidney tubules and internal bleeding due to lack of blood platelets resulting in brain damage, which can occur in severe cases (Owusu, 2010). It is assumed that infection may occur when one is expose to less than 100 cells. It is estimated that the probability of contamination on exposure to a single viable cell is 26% for children and 17% for adults (FAO / WHO, 2016). One million illnesses (95% uncertainty interval [UI]:
754,000 to 2.5 million), 128 deaths (UI: 55 to 374), and nearly 13,000 DALYs (UI: 5951 to 33,664) are as a result of Foodborne STEC (FAO / WHO, 2016).

2.4.3 Pathogenicity of *Staphylococcus aureus*

Staphylococcus aureus is an anaerobic organism which usually forms irregular clusters and a member of the genus Staphylococcus. It consists of gram- positive cocci, with a diameter of 0.5 to 1.5 µm. The organism is fairly tolerant to dissolved sugars and can grow well in high concentration of salt solution. The organism forms toxins when they are growing in food before it is consumed and not after ingesting into the body. These toxin that are produced can withstand heat and may remain active after light cooking, however they are gradually destroyed during boiling for at least 30 minutes (Owusu, 2010).

Staphylococcus aureus is a worldwide leading cause of hospital and community bacterial infections in humans (Rao *et al*, 2015) since *S. aureus* is a major cause of food poisoning and spread mainly by poor sanitary practices by food handlers. It is associated with numerous mild skin and soft tissue infections, as well as lifethreatening pneumonia, bacteraemia, osteomyelitis, endocarditis, sepsis and toxic shock syndrome. *S. aureus* may colonize the human body as a part of the normal flora. Approximately 30% of healthy people are inhabited by it (Rao *et al*,2015). *Staphylococcus aureus*. The key source is in the human body with the principal reservoir being the nose and it can also be transferred into food through the hands, expulsion from the respiratory tract and other (Owusu, 2010)

In contrast to the rest of the world in recent year, the occurrence of *S. aureus* clone ST121 seems to have been increasing in Africa. Thirty-nine 39 isolates were typed as ST121 (12.7 %, 39/308) from six healthcare institutions located across northern, central and southern Ghana (Rao *et al.*, 2015)

2.4.4 Pathogenicity of *Bacillus cereus*

Bacillus cereus is an aerobic endospore and a psychotropic microorganism, former pathogen of humans and other animals (Montanhini *et al.*, 2015). *Bacillus cereus* is a gram positive spore-forming, rod shaped bacterium that causes foodborne illness with two different clinical presentations depending on the toxin involved (Hassan, 2016).

The enterotoxin produced inside the host‘s small intestine causes diarrhoeal illness and the toxin pre-formed in food causes emetic (vomiting) illness. Three types of diarrhoea enterotoxins are produced by B. cereus: the haemolytic BL toxin (HBL), non-haemolytic enterotoxin (NHE), and the cytotoxin K (CytK) (Montanhini *et al.*, 2015).

B. cereus is prevalent in nature and readily found in soil, where it assumes a saprophytic life cycle; germinating, growing and sporulating in this environment. Due to the metabolic dormancy and tough physical nature of spores, they are more resistant to environmental stress than vegetative cells (Montanhini *et al.*, 2015). The spores of B. cereus can be resistant to heating and dehydration and therefore may survive cooking and dry storage. When foods containing B. cereus spores are in the _temperature danger zone‘ the spores may germinate, and the bacteria may grow, produce toxins, and make people sick. Such illness is frequently linked with starchy foods of plant origin such as rice, pasta, potatoes, pastry and noodles (Hassan, 2016).

The emetic syndrome is an intoxication due to ingestion of a cyclic peptide toxin called cereulide that is pre-formed in the food during growth by B. cereus. This syndrome has a short incubation period and recovery time. Symptoms include nausea, vomiting and abdominal cramping that occur within 1–5 hours of ingestion, with recovery usually within 6–24 hours (Hassan, 2016).

The diarrhoeal syndrome incubation period before onset of disease is 8–16 hours and the illness usually lasts for 12–14 hours, although it can continue for several days. Symptoms are usually mild with abdominal cramps, watery diarrhoea and nausea (Hassan, 2016). An irreversible tissue damage to the photoreceptors of the retina can be caused by *Bacillus cereus* in less than 24h thus causing blindness in the affected eye. It is serious in food industry because endospores are mostly partially resistant to the heat of pasteurization, dehydration, gamma radiation and other physical stresses

(Mckillip, 2014)

The mode of transmission of B. cereus food poisoning is either by ingesting food contaminated with pre-formed toxin (emetic type) or ingesting contaminated food with

huge numbers of bacterial cells and/or spores (diarrhoeal type).Transmission of this disease results from eating of unclean foods, inappropriate food handling/storage and inadequate cooling of cooked foodstuffs (Hassan, 2016).

2.4.5 Total Viable Count

For the calculation or assessment of the number of viable bacteria in a population, there are a lot of procedures used. In direct methods, a suspension containing a suitable number of bacteria is transferred to a solid nutrient medium which, under suitable conditions, permits each viable bacterium to grow to a colony. Direct count of the colonies and reference to the dilution of the original suspension yield the colony count (Holms, 2018). The "plate count" is at best an interpretation of an approximation of the number of cells present since microbiological data is an inherent variable (Sutton, 2011). It is thus use to enumerate aerobic and facultative bacteria in food that are capable to grow under condition employed.

The recorded number of colony forming unit (CFU) are what most microbiologists claim to be the microbiological data. The number of colonies on a plate is an interpretation by someone(skilled technician),however experience has shown that different technicians (each skilled) can and frequently do observe different counts on the same sample (Sutton, 2011).

Colony Forming Unit (CFU) is the estimated amount of cells present. It is a skewed approximation at best as the only cells able to form colonies are those that can nurture under the conditions of the test (e.g. incubation media, temperature, time, oxygen conditions). However, those do not denote a single cell, but rather those that occurred

to be well separated on the plate and thus can be distinguished after growth. A colony could arise from one cell or several thousand (Sutton, 2011).

According to Holm (2018), Colony count by pour plates and Colony count by tube dilution can be used in finding the total viable count. Large dilutions of bacterial suspensions were made at 45" in a buffered complex medium containing agar. The tubes of medium with the serial dilutions of bacteria were solidified by cooling; subsequent incubation at 37" permitted bacterial growth in discrete colonies. The density of colonial growth was enumerated by comparison with a set of prepared standards calibrated by the pour-plate technique. A large range (log 2-5 to 10.5) of colony counts was covered in only four test tubes without any prediction of the approximate count. The method has advantages of speed, economy of reagents and apparatus, as well as range and precision.

Sutton (2011) established the following facts: There is an established linear range for common bacterial counts on standard sized plates ;Standard Operating Procedure (SOP) should be used in occurrence of unusual plate count situations: .Rounding and averaging rules can play a role in the deduced bacterial count thus rules should be in agreement with the United States Pharmaopeia and described in the SOP ; Plate counting can have an important impact on compliance with product specifications and process controls.

2.4.6. Total coliforms

Coliform bacteria originate as organisms in soil or vegetation and in the intestinal tract of warm-blooded animals (fecal coli). The increase or decrease of many pathogenic bacteria can be estimated by the observation of coliform bacteria. It has long been an indicator of water contamination and possible presence of intestinal parasites and

pathogens (Treyens, 2009) .Some found in food have been lethal thus their presence should be seriously be looked at (Treyens, 2009).

Total coliforms refer to a large group of rod-shaped, gram-negative bacteria that share numerous characteristics. These groups include thermotolerant coliforms (bacteria of faecal origin), as well as some bacteria that may be isolated from environmental sources thus the presence of total coliforms may or may not indicate faecal contamination. A high count for the total coliform group may be associated with a low, or even zero, count for thermotolerant coliforms in extreme cases (Bartram and Pedley, 1996).

Total coliforms include species that may inhabit the intestines of warm-blooded animals or occur naturally in soil, vegetation, and water. Often found in fecallypolluted water and are usually associated with disease outbreaks. Though they are not usually pathogenic, their presence in drinking water indicates the possible presence of pathogens. One species of the coliform group-E. coli, is always found in faeces and is, therefore, a more direct indicator of fecal contamination and the possible presence of enteric pathogens (USEPA, 2002).

Total coliforms are indicators and are more common and easy to grow. Testing for them provides a margin of safety (Treyens, 2009). In the laboratory total coliforms are grown in or on a medium containing lactose, at a temperature of 35 or 37 °C. They are conditionally identified by the production of acid and gas from the fermentation of lactose (Bartram and Pedley, 1996)

2.5 Microbiological Tests

Microbiological tests play significant role in the identification of product. Assessments may further assist in developing and implementing preventative measures to promote food safety in school feeding schemes (Nhlapo *et al.*, 2014). Microbiological analyses are therefore essential tool to collect data to be used for the development and verification of HACCP plan as well as to assess the effectiveness of sanitation operations, to evaluate the compliance of incoming ingredients with safety criteria, and to determine the safety of end products (Osimani *et al.*, 2013). In Ghana microbiological food contamination is alarming however, studies shows that there is a decline on research on microbiological food safety (Saba and Gonzalez-Zorn, 2012).

CHAPTER THREE

MATERIALS AND METHODS

Description of the Study Area

Cape Coast Metropolis is the capital of the Central region and is traditionally known as Oguaa which ascends from ―gua‖ which means ―market‖ in the Fante language. It occupies an area of approximately 122 square kilometres. It is bounded to the north by the Twifu Heman lower Denkyira District, to the South by the Gulf of Guinea and to the East and West by Abura Asebu Kwamankese District and Komenda Edina Eguafo Abrem Municipality respectively. It is located 1° 15'W longitudinally and 5°06'N in latitude. According to the Ghana Statistical Service, (2010), the age of the population between 15-19 years in Cape Coast Metropolis stands at 22,291 and this corresponds with the age of student at Senior High School. Cape Coast is Known to be the seat of education and thus endowed with several SHS. Four schools were selected from the public SHS for this study.

MATERIALS

3.1 List of Equipment and reagents with their source and country of make

Heidolph vertical autoclave model 2540ea (Tuttnauer USA Co. Ltd., NY, USA); Hanna HI 5522 ph meter (Hanna Instruments, Woonsocket, USA); Accumax micropipettes (Accumax Lab Technology, Gujarat, India);Indo Sati incubator 117

(The Indo Sati Instruments and Chemicals, Ambala Cantt, India); Adam balance CQT 202 (Adam Equipment Inc., CT, USA); Plate Count Agar (Oxoid Ltd., Hampshire, England); Eosine Methylene Blue Agar (Oxoid Ltd., Hampshire, England); Buffered

Peptone Water Broth (Oxoid Ltd., Hampshire, England); *Bacillus cereus* Agar (Oxoid Ltd., Hampshire, England); Mannitol Salt Agar (Oxoid Ltd., Hampshire, England); Triple Sugar Iron Agar (Oxoid Ltd., Hampshire, England); Salmonella Shigella Agar (Oxoid Ltd., Hampshire, England);Rappaport-Vassiliadis (RV) Enrichment Broth (Oxoid Ltd., Hampshire, England); Rabbit plasma w/EDTA (Oxoid Ltd., Hampshire, England); Egg yolk emulsion (Oxoid Ltd., Hampshire, England)

METHODS:

3.2 Sample and data collection

A stratified sampling method (US FDA, 2012) was used to collect 18 food samples after grouping secondary schools into two (2) categories (Class A and Class B schools according to GES SHS categorization of schools) for the study where consent from the heads has previously been sought. Specific food samples which include breakfast (wheat, porridge, cocoa beverage), lunch (―gari‖ and beans, ―waakye‖ and stew) and supper (rice and stew, ―kenkey‖, freshly ground red pepper and fried fish, rice and soup) were collected within a period of two (2) weeks from the four (4) selected secondary schools and sent to the laboratory for microbial assessment. The food samples were collected into sterile polythene bags, kept into a cold box containing ice packs and transported within one (1) hour to the research laboratory of the Department of Laboratory Technology, University of Cape Coast. These food samples were subjected to microbial analysis for estimation of *Escherichia coli*, *Salmonella* species, *Staphylococcus aureus*, *Bacillus cereus*, total viable count and total coliform levels.

3.3 Media preparation

3.3.1 Preparation of Plate Count Agar (PCA) (CM0463)

Approximately 23.5g of the solid agar was dissolved in 1litre sterile distilled water (Reasoner, 2004) adjusted to 7.0 and the solution boiled to dissolve completely at 100 °C. The agar was dispensed into flasks and autoclaved (Heidolph vertical autoclave model 2540ea) at 121 °C for 15 min. The agar was then left to cool and settle at 50°C.

3.3.2 Preparation of Buffered Peptone Water Broth (BPW) (CM1049)

The buffered Peptone water broth (BPW) was prepared by weighing 20 g of the solute into 1 L sterilized deionized water and brought to a pH of 7.2. The broth was then sterilized by autoclaving at 121°C for 15 min.

3.3.3 Preparation of Eosin Methylene Blue Agar (EMBA)(CM0069)

37.5g of the powder was dissolved in 1L and brought to boil at 100°C for 15 min to completely dissolve the agar particles. The pH as then adjusted to 7.4.

3.3.4 Preparation of *Bacillus cereus* Agar Base (CM0617)

This was prepared by dissolving 20.5 g of the solute in 475 mL of sterilized distilled water and pH adjusted to 7.2 . The agar was sterilized at 121 °C for 15 min. This was aseptically supplemented with 25 mL egg yolk emulsion and one vial (25 mL) of polymycin B after the agar had cooled to about 45 - 50 °C.

3.3.5 Preparation of Mannitol Salt Agar (MSA) (CM0085)

Approximately 111 g of the powder was suspended in 1 L of distilled water and the pH adjusted to 6.8 and the solution boiled to dissolve completely at 100 °C. The agar was then autoclaved at 121°C for 15 min.

3.3.6 Preparation of Rappaport-Vassiliadis (RV) Enrichment Broth (CM0669)

Fifteen grams (15g) was added to 500 mL of distilled water. It was heated gently until it dissolved completely. 10 mL volumes were dispensed into screw-capped bottles and sterilized by autoclaving at 115 °C for 15 min.

3.3.7 Preparation of Salmonella Shigella Agar (SSA) (CM0099)

Fifty seven grams (57 g) of the media was suspended in 1 L, pH adjusted to 7.3 and the solution boiled with constant shaking to dissolve completely at 100 °C.

3.3.8 Preparation of Triple Sugar Iron Agar (TSI) (CM 0277)

The Triple Sugar Iron (TSI) medium was prepared by suspending 19.5 g of solute in 250 mL distilled water, pH adjusted to 7.3 and the solution boiled to dissolve completely at 100 °C. It was then autoclaved at 121 °C for 15 min and later brought to cool at 50 °C. The agar was transferred into test tubes and slanted until it cooled.

3.4 Microbiological analyses

3.4.1 Sample preparation (homogenization and serial dilutions)

For every food sample 20 g was aseptically weighed into 180 mL of sterile peptone water with pH adjusted to 7.2 and homogenized for 30 secs. This gave 10^{-1} dilution. It was vortex for about 2 min to ensure uniform mixing. Using a sterile pipette, 1 mL of the 10^{-1} dilution was pipette into 9 mL of sterile salt peptone water to obtain 10^{-2} dilution. This procedure was repeated for 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} dilutions. Further, from appropriate tenfold serial dilution, 1 mL inoculum of each dilution (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}) was inoculated into sterile Petri dish plates and the appropriate media added for enumeration and/or isolation. After suitable incubation, dilutions with 30–300 colonies were selected and counted. The number of colonyforming units per gram (cfu/g) of food was calculated by multiplying the number of bacteria by the dilution. All analysis was done in duplicate for reliability of results.

3.4.2. Enumeration of Aerobic Mesophiles (Total Viable Count)

Aerobic mesophiles were cultured by the pour plate method using Plate Count Agar medium (OXOID CM 0325). The plates were incubated at 37 °C for 48 h.

3.4.3. Enumeration and Isolation of total coliform

Coliform bacteria were cultured by the pour plate method using Eosin Methylene Blue agar (OXOID CM 0069) and adjusted to pH 7.3 and incubated at 37 °C for 48 h. Pink, dark purple to black colonies are presumptive for coliforms. Colonies were confirmed using Triple Sugar Iron agar (OXOID CM 0277) at pH of 7.4 and

incubated at 37 °C for 24 h to observe for gas production.

3.4.4. Enumeration of *E. coli*

E. coli were enumerated by the pour plate method using Eosin Methylene Blue agar (OXOID CM0069) adjusted to the pH 7.3. Colonies (producing a metallic green sheen) were confirmed using Triple Sugar Iron agar (OXOID CM 0277) with pH adjusted to 6.9. Colonies that produced gas were confirmed for Indole production.

This was done by sub-culturing into Peptone water and incubating at 44 °C for 24 h. Indole test was done by adding a few drops of Kovac reagent into the culture. Red ring coloration at the surface of Peptone water indicated Indole positive and thus confirmed the presence of *E. coli.*

3.4.5. Enumeration of *S. aureus*

Enumeration of *S. aureus* was done by the spread plate method using Mannitol Salt agar (OXOID CM 0085). Approximately 0.1 mL of the aliquot was inoculated onto the surface of Mannitol Salt agar. A sterile glass rod was used to spread the medium onto the surface of the Mannitol Salt Agar. The plates were incubated at 37°C for 48 h. Yellow colonies with yellow halo were confirmed for coagulase positive using

Rabbit Plasma Serum. Coagulation of the serum indicated coagulate positive (confirmation test for *S. aureus*).

3.4.6. Enumeration of *B. cereus*

The enumeration of *B. cereus* was done by the spread plate method using *B. cereus* selective agar (OXOID CM 0617 and SR99). Approximately 0.1 mL of aliquot was inoculated onto surface of the *B. cereus* agar medium. Sterile rod was used to spread the inoculum on the surface of the agar medium and incubated at 30 °C for 24 h.

Suspected colonies (blue colonies with precipitate) were confirmed on Blood Agar dish (OXOID CM 0617). Haemolysis on Blood Agar indicated presence *B. cereus*.

3.4.7. Detection of *Salmonella* species

Approximately 25 g of the food sample were weighed aseptically and 225 mL buffered peptone water added to it. This was homogenized thoroughly and incubated at 37 °C for 16 to 21 h. After incubation, 0.1 mL of broth (buffered peptone water) was transferred into 10 mL of Rappaport-Vasilliadis (RV) (CM0669) broth and incubated in a water bath at 42 °C for 24 h. Following enrichment, a loopful of the Rappaport-Vasilliadis (RV) broth culture was streaked onto Salmonella Shigella Agar plates, and the plates were incubated at 37 °C for 24 h. Presumptive colonies (lightly transparent with black center) were streaked on TSI slants and incubated for 24 h. The formation of black precipitate in the slant confirmed the presence of *Salmonella spp.*

3.5. DATA ANALYSIS

Three meals comprising: breakfast, lunch, and supper were sampled from four selected secondary schools. These were also made up of 19 food items. Means of the results were converted $\log_{10}$cfu/g.

Data obtained were presented in tables and graphs. Microsoft Excel 2013 was used in the tabulation of the results obtained and also to calculate the percentages. The data was exported to IBM SPSS v25 (IBM Corp, Armonk, NJ, USA). One—way analysis of variance (ANOVA) was used to determine statistically significant difference of microbial loads between all samples under study for easy interpretation. Microbial counts were expressed as means and the significant differences among them were also compared using (Tukey‘s post hoc test). Statistical significance was set at $p < 0.05$. The

significance of any perceived differences was determined by analysis of independent samples.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Microbiological Analysis Results

4.1.1. Total Viable Counts (TVC) of Food Samples

Total Viable Count (TVC) is an estimation of the total amount of microorganism present in a particular sample. The mean counts of bacteria in the food samples were expressed as $\log_{10}$cfu/mL or $\log_{10}$cfu/g as shown in Table 2(refer to Appendix B). There was a statistically significant difference between the four schools as determined by one-way ANOVA ($p < 0.0001$). A Tukey's post hoc test revealed that the mean TVC for MF (4.15 ±3.15 cfu/mL) was significantly higher than that of HC (2.04 ± 1.23 cfu/mL, $p < 0.0001$) and UP (2.11 ± 0.00 cfu/mL, $p < 0.0001$) but there was no statistically significant difference between MF and NA (4.30 ± 3.15 cfu/mL, $p = 0.074$). Statistically, NA was also significantly different from HC ($p < 0.0001$) and UP ($p < 0.0001$). Also, HC and UP showed no significant difference in the mean TVC values ($p = 0.379$). The average TVC for the schools was 3.14 ± 1.15 cfu/mL. MF and NA showed above average mean TVC whereas HC and UP showed counts below the average. For the lunch samples, the average TVC was 3.78 ± 1.73 cfu/g. MF had a mean TVC count lower than the average whiles HS, NA, and UP all had values above the average mean TVC. A statistically significant difference in TVC between the schools was observed ($p < 0.0001$). A Tukey post hoc test revealed that the mean TVC for MF (1.04 ±0.15 cfu/g) was statistically significantly different from HC (4.10 ± 3.00 cfu/g), NA (5.05 ± 4.23 cfu/g) and UP (4.93 ± 4.03 cfu/g). Likewise, HC was significantly lower than both

NA ($p < 0.0001$) and UP ($p < 0.0001$). Statistically, there was no significant difference in the mean TVC between NA and UP. The average mean TVC for the supper food samples was 2.95 ± 1.29 cfu/g. it was observed that MF (2.14 ± 0.80 cfu/g), NA (2.76 ± 1.55 cfu/g) and UP (2.44 ± 2.03) had below average Mean TVC. Whereas only HC had a TVC value greater than the average. The TVC for HC was significantly higher than that of MF ($p < 0.0001$), NA ($p < 0.0001$) and UP ($p < 0.0001$). Besides that, there was no statistically significant difference between MF and NA ($p = 0.989$) and between NA and UP ($p = 0.970$). It can hereby be observed that, the average TVC for supper was the lowest in the day followed by breakfast with lunch registering the highest average TVC.

4.1.2. Total Coliform Count (TCC) of Food Samples

Coliforms are bacteria that can serve as indicators of unhygienic processing environments and fecal contamination (Martin, 2016). The mean counts of coliforms in the food samples are expressed as $\log_{10}$cfu/mL or $\log_{10}$cfu/g as shown in Table 2 (in the appendix). The HC (1.35 ± 0.55 cfu/mL) and UP (1.77 ± 0.75 cfu/mL) reported TCC values lower than the average (2.58 ± 1.12 cfu/mL). whereas MF (3.33 ± 1.85 cfu/mL) and NA (3.89 ± 2.33 cfu/mL) recorded above average TCC values. The mean TCC of MF was statistically significantly different HC ($p < 0.0001$), NA (p 0.001), and UP ($p < 0.0001$). Also, statistically significant differences were observed between HC and NA ($p < 0.0001$) and between HC and UP ($p < 0.002$). Lastly, the mean TCC of NA and UP were observed to be statistically significantly different ($p < 0.0001$). The MF registered a TCC of 0.00 cfu/g which is lower than the average TCC of lunch food samples of 4.04 cfu/g. conversely, NA (4.69 ± 3.69 cfu/g) and UP (4.17

± 2.93 cfu/g) had higher than average TCC values. Also, HC (3.31 ± 2.55 cfu/g) had a TCC value which was lower than the average. A one – way ANOVA reve aled that there was statistically significant difference of TCC among the four schools. A Tukey post hoc showed that HC had statistically significantly different TCC values from NA ($p < 0.0001$) and UP ($p = 0.001$). Also, NA and UP are significantly different ($p = 0.005$). The average TCC values for the supper food samples was 2.32 ± 0.94 cfu/g. TCC values for MF and NA were 0.00. There was a statistically significant difference between the four schools as established by one-way ANOVA ($p < 0.0001$). Tukey post hoc test showed that the difference in TCC between HC and UP was statistically different ($p < 0.001$). The information above shows that again the supper food samples contain the lowest average TCC value followed by morning with lunch recording the highest average TCC value.

4.1.3. *Escherichia coli* counts (ECC) in Food Samples

Escherichia coli is the preferred indicator of fecal contamination of food due to its high prevalence in the gut. Therefore, the severity of fecal contamination is determined by the concentration of E. coli in a sample. The schools, HC and UP had lower than average (2.25 ± 1.27 cfu/g) ECC values of 1.00 ± 0.00 cfu/g and 1.16 ± 0.33 cfu/g respectively. MF (3.23 ± 2.15 cfu/g) and NA (3.67 ± 2.75 cfu/g) had above average ECC values. One—way anova indicated that the difference in ECC values between the four schools was statistically significant ($p < 0.0001$). A Tukey‘s post hoc test showed that the ECC value for MF was significantly different from HC ($p < 0.0001$), NA ($p < 0.001$), and UP ($p < 0.0001$). The NA was also statistically significantly different from UP ($p < 0.0001$) and HC ($p < 0.0001$). The difference in ECC values between HC and UP was

not statistically significant ($p < 0.284$). For the lunch samples, E. coli was present in only the food samples from NA which registered a count of 4.53 ± 3.33 cfu/g. samples from MF, HC and UP did not show any growth of E. coli. MF and NA had mean ECC value of 0.00 each. HC and UP had 2.43 ± 1.15 cfu/g and 0.93 ± 0.33 cfu/g respectively. The average ECC was 1.67 ± 0.87 cfu/g. the difference between the mean ECC values of HC and UP was statistically significant ($p = 0.001$).

It can be deduced that the prevalence of *E. coli* was higher in the breakfast samples where all the food items had *E. coli* contamination compared to the supper samples which had only two food items with *E. coli* contamination out of four. In addition, the lunch samples had the least number of food items contaminated by *E. coli*, which was only one.

4.1.4. *Staphylococcus aureus* count (SAC) in Food Samples

The *staphylococcal* loads for HC and UP are 1.42 ± 0.80 cfu/g and 1.39 ± 0.69 cfu/g respectively which were lower than the average, 2.41 ± 1.10 cfu/g. By contrast, MF and NA had above average *staphylococcal* loads of 3.13 ± 2.33 cfu/g and 3.72 ± 2.45 cfu/g respectively. One -way anova indicated that the difference in ECC values between the four schools was statistically significant ($p < 0.0001$). A Tukey's post hoc test showed that the *staphylococcal* load MF was statistically significantly different between HC ($p < 0.0001$), NA ($p < 0.006$) and UP ($p < 0.0001$). It was also observed that NA had *staphylococcal* counts which were significantly different from HC ($p < 0.0001$) and UP ($p < 0.0001$). The difference in *staphylococcal* load between HC and UP was not statistically significant ($p = 0.973$). The lunch samples had an average *staphylococca*l

load of 3.16 ± 1.62 cfu/g. This was higher than the *staphylococcal* load of MF, which was 0.60 ± 0.15 cfu/g. all the remaining schools had above average *staphylococcal* loads. HC, NA, and UP had staphylococcal loads of 3.66 ± 2.93 cfu/g, 4.46 ± 3.15 cfu/g, and 3.94 ± 2.55 cfu/g respectively. The difference in *staphylococcal* load was statistically significant as was indicated by a One-way anova that was performed. A Tukey's post hoc test also revealed that the *staphylococcal* load of MF was significantly different from HC ($p < 0.0001$), NA ($p < 0.0001$) and UP ($p < 0.0001$). Again, NA had *staphylococcal* loads which were statistically significant from HC ($p < 0.003$) and UP ($p < 0.015$). But there was no significant difference in *staphylococcal* load between HC and UP ($p = 0.107$). The school- NA did not register any *staphylococcal* load in its food samples. The average *staphylococcal* load for supper was 2.57 ± 1.49 cfu/g. Both MF and HC had values lower than the average whereas the highest *staphylococcal* load of 4.48 ± 3.15 was found in the HC food sample. The difference in *staphylococcal* load between the four schools was statistically significant ($p < 0.0001$) as was shown by a one-way anova. Tukey's post hoc test provided evidence that HC had a significantly higher *staphylococcal* load than MF ($p < 0.0001$) and UP ($p < 0.0001$). It is also observed that UP had a significantly higher *staphylococcal* load than MF ($p < 0.015$).

It can be spotted from above information that the *staphylococcal* load of the lunch samples was the highest followed by supper. Breakfast had the least *staphylococcal* load.

4.1.5. *Bacillus cereus* (BC) levels in Food Samples

The average BC count for breakfast samples was 3.29 ± 0.36 cfu/mL. The UP had no BC in its breakfast sample. The MF had the highest BC count of 3.57 ± 2.63 cfu/mL followed by NA with 3.48 ± 0.00 cfu/mL. The HC had a below average BC count of 2.85 ± 2.15 cfu/mL. A one—way ANOVA that was conducted revealed a statistically significant difference in BC counts between the four schools ($p = 0.002$). It was shown in the Tukey's post hoc test that HC had a significantly lower BC count than MF ($p < 0.002$) and NA ($p < 0.003$). There was no statistically significant difference between MF and NA ($p = 0.395$). A one—way ANOVA showed that there was statistically significant difference in BC counts between the four schools ($p = 0.004$). A Tukey's post hoc test showed that the BC count of MF was significantly lower than that of HC ($p = 0.047$), NA ($p = 0.005$) and UP (0.006). There was no statistically significant difference in BC count between NA and HC ($p = 0.063$) NA and UP ($p = 0.925$) and HC and UP (0.106). The average BC count was 3.09 ± 0.50 cfu/g. The average BC count for supper was 2.39 ± 0.87 cfu/g. The NA registered a BC count of 0.00. A one—way ANOVA showed that there was statistically significant difference in BC counts between the four schools ($p = 0.002$). A Tukey post hoc showed that HC had statistically significantly different BC count from MF ($p = 0.019$) and UP ($p = 0.002$). Also, MF and UP are significantly different ($p = 0.011$).

4.1.6. *Salmonella* Prevalence in Food Samples

Salmonella was not detected in any of the breakfast and supper samples. But in the lunch samples, only MF did not register any *Salmonella. Salmonella* was detected in the HC, NA, and UP samples.

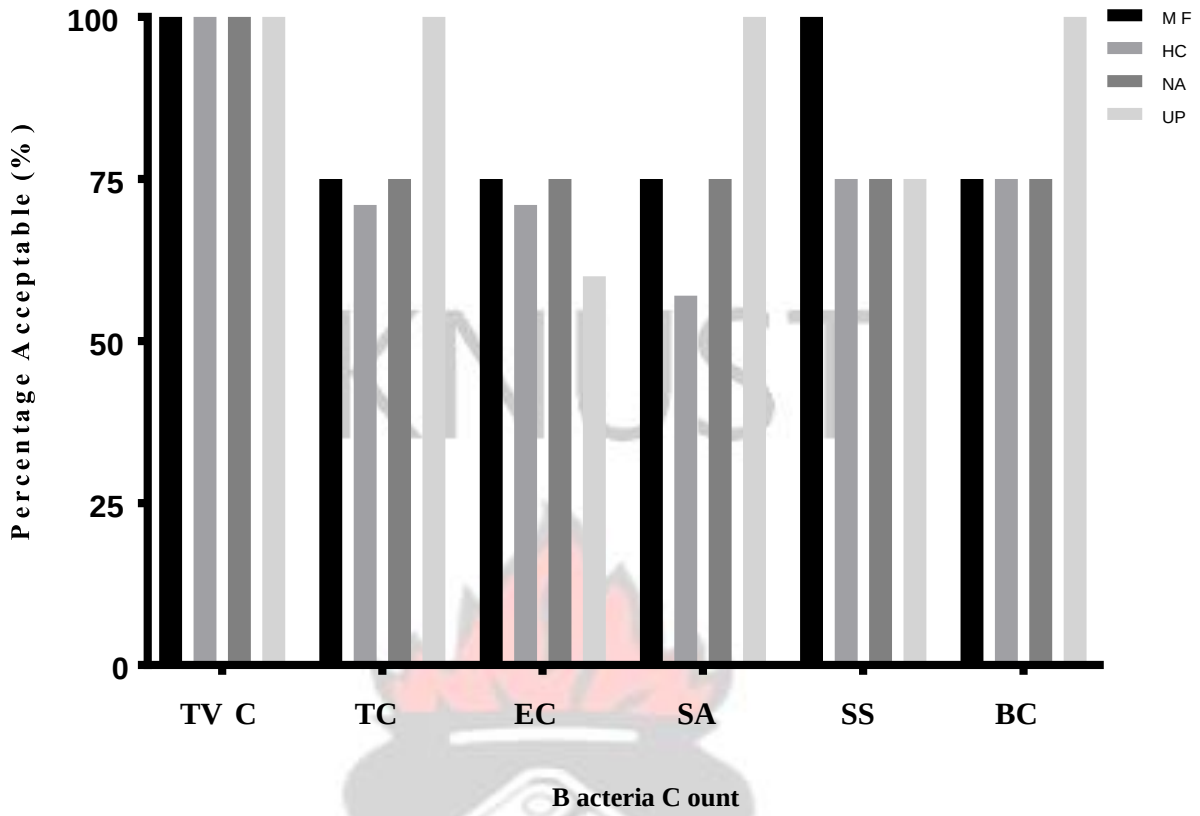

Fig.4.1 Summary of Type of microbes analyzed in Food Samples within Acceptable Limits

4.2 Discussion

All the schools (both category A and B schools) had all their food samples falling within the acceptable limits for Total Viable Count (TVC). When it comes to Total Coliforms (TC), only UP (a category B school) had all its samples falling within the acceptable limits of $<10^3$ as shown in Table 4.1 as set by the Ghana Standard Authority. Both MF and NA had 75% of their samples falling within acceptable limits and for HC it was 70%. This implies unacceptable level of total coliforms in some of the food samples thus an analytic of possible unsanitary practices which contests the microbial safety of the foods served. According to Owusu, (2010), food that is wholesome for ingestion should not have any coliforms (0 CFU/g).

With *Escherichia coli* (EC), none of the schools had 100% of their samples being acceptable that means none had (0 CFU/g) as set by GSA. Food samples from MF and NA had 75% being acceptable whereas HC and UP has 70% and 60% respectively. *Escherichia coli* (EC) whose main source is the large intestine of mammals, presence in foods is indicative of insufficient hygiene practices (Owusu, 2010). The author also stated that their presence however does not essentially implies the association of the food with faecal material as the organism is found is soil naturally however it has a food borne disease implication as most often contaminations is due to unsanitary food handling .

Only one school-UP had 100% of its food samples falling within the acceptable limits for *Staphylococcus aureus* (SA) of $\leq 10^3$ as set by GSA (refer to Table 4.1). Again, MF and NA had 75% of their food samples falling within the limits and HC having 55%. *Staphylococcus aureus* which is part of human normal microflora is a major cause of food poisoning and spread mainly by poor sanitary practices by food handlers (Rao *et al*, 2015). *Staphylococcus aureus*. According to Owusu, (2010), predictablely 30 – 50% of the human populations are nasal, throat and skin carriers that usually would transfer by cross contamination if proper hand washing is not carried out. Only one school-MF had all its food samples falling within acceptable limits for *Salmonella* (SS) as 0CFU/g as set by GSA, whiles the rest; HC, NA, and UP all had 75% of their food samples falling within the acceptable limits. Salmonella organisms of various types are spread through contamination of water and food as well as by direct contact (Owusu, 2010). According to Mama and Alemu, (2016) the major risk factors associated with salmonella infections include finger nail status, hand washing practice after toilet, and transfer of food with bare hands.

Also MF, HC, and NA all had 75% of their food samples falling within the acceptable limits whiles all the food samples from UP had acceptable levels of *Bacillus cereus* (BC) of $\leq 10^3$ as set by GSA(as shown in Table 4.1). Transmission of this disease results from eating of unclean foods, inappropriate food handling/storage and inadequate cooling of cooked foodstuffs (Hassan, 2016).

Table 4.1. Acceptable Levels of Microorganisms in Food

SN	Microorganism	Acceptable Limits
1 2	Total Viable Count (TVC)	$\leq 10^3$
	Total Coliform Count (TC)	$\leq 10^2$
3 4	*Escherichia coli* (EC)	Absent
5	*Staphylococcus aureus* (SA)	$\leq 10^3$
	Bacillus cereus (BC)	$\leq 10^3$
6	*Salmonella* (SS)	Absent

Source: GSA, 2013

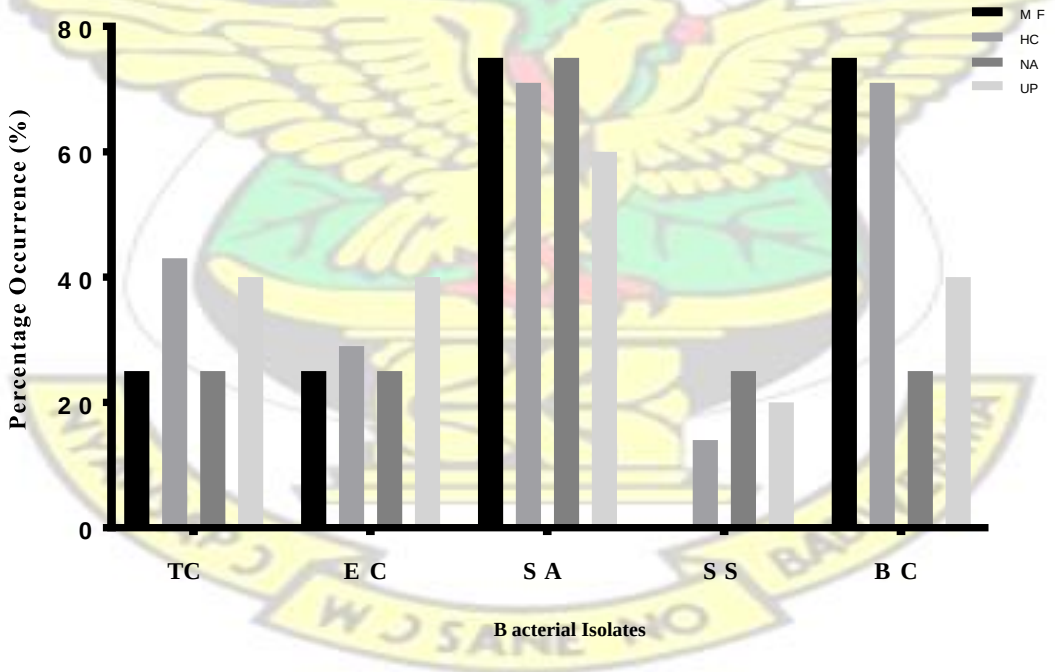

Fig 4.2 Percentage Occurrence of Isolated Bacteria

From Fig 4.2 it can be seen that for MF and NA, TC occurred in 25% of the food samples. Total coliform (TC) occurred in 40% of the samples of UP and was highest

in HC with an occurrence in 45% of food samples. *Escherichia coli* (EC) occurred in 40% of the food samples of UP and 30% of those of HC. However, it occurred in 25% of the samples of MF and NA. *Staphylococcus aureus* (SA) occurred in 75% of the food samples of MF and NA. It also occurred in 70% and 60% in HC and UP respectively. *Salmonella* species (SS) was not detected in any of the food samples from MF. It occurred in 25% of the food sampled from NA and 20% from those sampled from UP. It also occurred in 15% of the samples in HC. *Bacillus cereus* (BC) occurrence was highest in MF where it occurred in 75% of the food samples. Followed by 70% occurrence in foods sampled from HC. The least occurrence of BC was observed in the samples from NA with 25% followed by 40% in UP.

According to Nhlapo *et al.*, (2014),the purpose of measuring TVC is to give an overall indication of the presence of the number of organisms in the sample, hence representing the overall hygiene status of the sample while the incidence of coliforms shows a risk of occurrence of pathogens and thus a measure of the efficiency of cleanliness programs. Thus since all the schools (both category A and B schools) had all their food samples falling within the acceptable limits for TVC it implies that the general hygiene status of the food samples is satisfactory, however with the exception of only one school (a category B school) which had all its samples falling within the acceptable limits as set by the Ghana Standard Authority for TC, all the rest had 70% - 75% of their samples falling within acceptable limits. This implies 25% -30% contamination from pathogens and thus is a setback in the efficiency of sanitation programs in Senior High Schools). This buttress the fact in the Senior High Schools food safety report from the Ashanti Region of Ghana which stated the necessity for consistent monitoring and surveillance of school food services by appropriate agencies of the country with intensified implementation of the law and consequence, obligatory

and consistent cleanliness and appropriate training on food safety for matrons, kitchen staff and also developed available standard operating procedures with enhanced hygienic practice(Ababio *et al*, 2016).

Additionally, coliforms, comprising of *E. coli*, naturally is part of the microbiota in warm-blooded humans and other animal's intestinal tracts. Their occurrence usually specifies faecal contamination. With EC, none of the schools had 100% of their samples being acceptable which also implies faecal contamination of the sample.

Most severe food poisoning is cause by the E. coli O157:H7 strain which affects three Fin every 10,000 people and their contamination is caused by unhygienic cooking practices (Owusu, 2010). With the exception of one school (Category B school) which had 100% of its food samples falling within the acceptable limits for SA, the remaining had only 50%-75% of their food samples falling within the limits. Again with the exception of one school (category A school) which food samples falls within acceptable limits for SS, the rest all had 75% of their food samples falling within the acceptable limits thus 25% contamination of SS. *Salmonella* species is the bacterial that causes typhoid fever disease. The incidence of typhoid fever is highest in children from 5–19 years old in virtually all endemic areas. The disease is almost exclusively transmitted by food and water contaminated by the faeces and urine of patients and carriers (FAO and WHO, 2016). School going children in the secondary schools are usually teenagers (14 -18 years) that could be exposed to food hazards that has both instant and prolonged health consequences in their growing lives hence there is the need for consistent monitoring and surveillance of school food services (Ababio et al.,

2016). With BC all schools had 75% of their food samples falling within the acceptable limits except one which had all the food samples falling within acceptable levels implying 25% contamination. Transmission of disease caused by BC is due to ingestion

of contaminated foods, inappropriate food handling/storage and inadequate cooling of cooked foodstuffs (Hassan, 2016).

According to Ababio *et al*., (2016) such contaminations such as TVC, TC, SA, SS and BC are caused usually due to absence of consistent rigorous surveillance with weak law enforcement in institutional kitchens thus there is the need to implement a system to monitor and regulate the overall food chain in the country from farm to fork, including suppliers of raw materials to SHS.

Also as recommended by Ababio *et al.,* (2016) from a report from Benin which stated the risk posed on student due to extraordinary levels of microbiological contaminants on the hands of kitchen staff in schools. It was thus commended that Hazard Analysis at Critical Control Point (HACCP) should be implemented since it implementation at Italian schools enhanced cleanliness and staff sanitation. However, HACCP was absent in all schools visited in Ghana. To improve cleanliness in Ghana, adequate training, supervision, and appropriate standard cleaning procedure with time schedules for entire kitchens and ancillary rooms was required. Absence of appropriately designed kitchens is also a basis for loss of access control to both unauthorized personnel and animals with their associated risks to food. It was therefore suggested that there is the need for more improvement systems to be put in place in most of the schools. The results from this studies also support the necessity for a better food safety management systems in our senior high schools and further studies on microbial safety of foods served in SHS is a step in the right direction for policy makers.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

This study investigated the microbial safety of food served in some selected Senior High Schools in the Cape Coast metropolis. The study particularly sought to determine the level of bacterial contamination of food of the schools.

Staphylococcus aureus had the highest percentage occurrence in the food samples than all other bacteria isolated followed by *B. cereus* and *E. coli*. Conversely, *Salmonella* sp. was the least occurring bacteria isolated from the food samples. It was only detected in lunch samples including *waakye* and stew, and *gari* and beans. The presence of coliforms, *Salmonella* sp., *B. cereus* and *E. coli* in the food samples implies that there is a huge risk of the students being exposed to contaminated food that is served from the school kitchens.

All schools showed acceptable levels of total viable counts. Total coliform counts in all the samples from all the schools were not acceptable except UP. Likewise, the *staphylococcal* count and *Bacillus* count was only acceptable in the samples from UP whereas the other schools had varying percentages of acceptability. No school had a hundred percent acceptable levels of *E.coli* count. And also, only MF had a hundred percent acceptable level of *Salmonella.* This implies that most of the foods served to student in SHS are not safe since some of the food sampled did not meet the safety acceptable limit set by Ghana Standard Authority. It was further observed from the study that there was significant difference in the mean bacterial contamination of breakfast, lunch and supper served in the schools under study. The lunch foods

generally had higher levels of contamination than both breakfast and supper and also NA consistently had higher levels of bacterial contamination than all the other schools.

5.2. Recommendations

The following recommendations are made based on the results of the study:

- The study should be expanded to cover senior high schools in rural areas to see if there is any difference in hygienic practices between rural and urban schools.
- It is also recommended that physicochemical parameters should be considered in future studies to assess the physical and chemical safety of the foods in the schools.
- Future studies should also look into the level of knowledge of kitchen staff about food safety and food – borne diseases.
- A rigorous monitoring regimen should be instituted by the government regulatory bodies such as the Food and Drugs Authority and the Metropolitan assemblies to ensure that the food being served to students in senior high schools meets the acceptable limits of safety.

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APPENDICES

Appendix A:

The equation used to calculate the bacterial load is as follows:

$$N = \frac{\sum C}{[(1\times n_1)+(0.1\times n_2)\times(d)}$$ (Maturin L., Peeler J., 2001)

where:

N = Number of colonies per mL or g of product

∑C=Sum of all colonies on all plates counted

n_1 = Number of plates in first dilution counted

n_2 = Number of plates in second dilution counted

d = Dilution from which the first counts were obtained

Appendix B:

Table of Results

Table B1: Mean Level of Bacterial Contaminants in Food Samples from selected Senior High Schools in Cape Coast

MEAL	FOOD ITEM	SCHOOL	TVC	TC	EC	SA	SAL	BC
Breakfast	Wheat Porridge + Bread	MF	$4.15^{a} \pm 3.15$	$3.33^{a} \pm 1.85$	$3.23^{a} \pm 2.15$	$3.13^{a} \pm 2.33$	ND	$3.57^{a} \pm 2.63$
		HC	$2.04^{b} \pm 1.23$	$1.35^{b} \pm 0.55$	$1.00^{b} \pm 0.00$	$1.42^{b} \pm 0.80$	ND	$2.85^{b} \pm 2.15$
	Cocoa Bev	NA	$4.30^{a} \pm 3.15$	$3.89^{c} \pm 2.33$	$3.67^{c} \pm 2.75$	$3.72^{c} \pm 2.45$	ND	$3.48^{a} \pm 0.00$
	Porridge	UP	$2.11^{b} \pm 0.00$	$1.77^{d} \pm 0.75$	$1.16^{b} \pm 0.33$	$1.39^{b} \pm 0.69$	ND	0.00
		Average	3.14 ± 1.15	2.58 ± 1.12	2.25 ± 1.27	2.41 ± 1.10		3.29 ± 0.36
	P value		<0.001	<0.001	<0.001	<0.001		0.002
Lunch	Gari + Beans	MF	$1.04^{a} \pm 0.15$	0.00	0.00	$0.60^{a} \pm 0.15$	ND	$2.40^{a} \pm 1.85$
	Waakye + Stew	HC	$4.10^{b} \pm 3.00$	$3.31^{a} \pm 2.55$	0.00	$3.66^{b} \pm 2.93$	Detected	$3.00^{b} \pm 2.45$
	Gari + Beans	NA	$5.05^{c} \pm 4.23$	$4.69^{b} \pm 3.69$	4.53 ± 3.33	$4.46^{c} \pm 3.15$	Detected	$3.54^{b} \pm 2.85$
	Waakye + Stew	UP	$4.93^{c} \pm 4.03$	$4.17^{c} \pm 2.93$	0.00	$3.94^{b} \pm 2.55$	Detected	$3.48^{b} \pm 3.15$
		Average	3.78 ± 1.73	4.04 ± 0.62		3.16 ± 1.62		3.09 ± 0.50
	P value		<0.0001	<0.0001		<0.0001		0.004

Supper	Plain Rice + Stew	MF	2.14[a] ± 0.80	0.00	0.00	1.46[a] ± 0.45	ND	2.48[a] ± 2.15
	Kenkey + Freshly Ground Pepper + Fried Fish	HC	4.98[b] ± 3.93	3.14[a] ± 2.23	2.43[a] ± 1.15	4.48[b] ± 3.15	ND	3.32[b] ± 2.45
	Rice + Groundnut Soup	NA	2.76[a] ± 1.55	0.00	0.00	0.00	ND	0.00
	Gari + Beans	UP	2.44[a] ± 2.03	1.51[b] ±0 .55	0.93[b] ± 0.33	1.76[c] ± 0.55	ND	1.40[c] ± 0.85
		Average	2.95 ± 1.29	2.32 ± 0.94	1.67 ± 0.87	2.57 ± 1.49		2.39 ± 0.87
	P value		0.004	0.001	0.003	<0.0001		0.002

Values are means of duplicates ± standard deviation *p- values < 0.05 means there is significance statistically. *Different letters in the same column for each parameter indicate significant differences between meals