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MICROBIAL ANALYSIS OF FOOD AND VEGETABLE OBTAINED FROM FOOD VENDORS IN KNUST AND ITS ENVIRONS

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ABSTRACT

Every food establishment aims to provide safe and nutritious food products. However, the hygienic standards of vendors and the foods they sell are compromised and may result in food borne diseases or intoxications when consumed. In this study, microbial load and quantity of foods such as waakye, vegetable salad and Gari and beans sold in KNUST and its environs were examined.The foods were bought from four different sites and then the total viable counts(TVC). Total coliform counts(TCC), total Staphylococcal counts(TSC),faecal coliform counts(FCC), *Escherichia coli* counts(ECC), and total fungal count(TFC) of the various foods were analysed at the laboratory. Microbial counts were expressed as means and the data exported to Microsoft Excel 2019.One-way analysis of variance(ANOVA) was used to assess statistically meaningful variations in microbial loads between all samples.All the samples analysed in this study had TVC above the recommended standard stated by the Ghana Standard board(< 5logCFU/g). The TCC of samples varied from 3.87±1.09logCFU/g to 5.38±1.53logCFU/g and they all did not fall within the acceptable limit(< 2logCFU/g). The FCC of samples was between 2.3±0.65logCFU/g and 6.21±1.46logCFU/g which were all above the acceptable level of contamination as well. *E. coli* was found in all food samples with counts ranging from 1.97±0.56logCFU/g and 5.46±1.54logCFU/g above the acceptable limit of <0.48logCFU/g. *Staphylococcus spp*. had 93.3% occurrence and ranged from 0 to 7.67±2.17logCFU/g. Two samples(waakye) out of this had counts within the recommended standards of contamination. The total fungal counts ranged from 1.96±0.56logCFU/g to 5.76±1.63logCFU/g. Some of the identified fungi isolated from samples include *Aspergillus fumigatus* (86.7%) and *Aspergillus niger*(66.7%), *Aspergillus flavus*(6.7%), and yeast(33.3%). From this study, it was evident that food sold on KNUST and its environment were contaminated where the contamination was as a result of poor hygiene practices on the part of personnel involved in food preparation. It was found that majority of food samples observed had contaminations above the acceptable levels. Both *E. coli* and *Staphylococcus spp.* identified can be responsible for food poisoning. Moulds such as *A. niger, A. fumigatus, A. flavus* may produce mycotoxins which may be harmful at certain levels when consumed. There is a need for strict and implementable measures to promote food safety activity and good hygiene at the food establishment.TheFood and Drugs Authority should organise patrol teams that will occasionally visit these food establishments and assess the sanitation and hygiene standards employed in the production of ready-to-eat or street foods.

CHAPTER ONE

1 INTRODUCTION

1.1 BACKROUND INFORMATION

Dr. Tedros Adhanom Ghebreyesus, Director-General of the World Health Organization, stated on the first UN World Food Protection Day that "food safety is the business of all"(WHO, 2019).State officials, farmers, manufacturers, and customers all have the sole liability for food safety and security(Janjic et al., 2017).Assurance that food does not harm the user unless it is cooked and/or eaten in compliance with its intended usage is known as food safety(CAC, 2003).Everyone has the right to access safe, nutritious and adequate food at all times(Fung et al., 2018; Wisner et al., 2012). Safe and nutritious food assumes an imperative job in continuing to advance great wellbeing and supporting human life.However, food safety and food securityissues tend to arise around the world and continue to be amajor concern(WHO, 2019).Food safety is absolutely critical to all since it is hard to distinguish somebody who for once has not been related to foodborne sickness.

Thefood we rely on for good and healthy life and sustainable development however, are contaminated with microbes that cause sickness and can even lead to health problems and or death(Giuseppe et al., 2010).Foodborne diseases may result from the ingestion of microbial pathogens, harmful chemicals or radioactive materials infected with food (Dun-dery & Addo, 2016). Studies show that most outbreaks of foodborne disease are the consequences of microbiological contamination(Darko, 2016).There are more than 250 foodborne diseases and public health concerns are growing worldwide(Osei-Tutu & Anto, 2016). The distribution of these diseases differs from one locality to another.According to Darko (2016), developing countries face the most significant issue owing to the prevalence of a broad variety of foodborne diseases, including those triggered by parasites.In a report given by the Ministry of Food and

Agriculture and World Bank in 2007, one out of each 40 individuals in Ghana experiences foodborne disease consistently with more than 420,000 revealed cases lasting through the year(Yeleliere et al., 2017). Out of this number, 65,000 individuals die bringing about a monetary loss of about US$ 6,900,000.00.Ghana's extrapolated food poisoning rate is 5.8 million per year (Salas, 2011). The public's concern in the food production and consumer health is on the ascent around the world (WHO/FAO, 2015).*Staphylococcus spp., Pseudomonas spp., Enterobacter spp. and Escherichia spp.*arepredominant bacteria associated with Ghanaian food (Abakari et al., 2018).

Faced with all these hurdles in Ghana and Africa as a whole, food sales services are emerging rapidly(Bobodu, 2012), especially around tertiary institution campuses(Adam et al., 2014).It is a reality that the food business has generated employment and contributes enormously to the casual part of the economies of most nations around the world, just as tending to difficult issues presenting huge social issues in developing nations on account of the job of the business in giving reasonable dinners to customers(Alimi, 2016).

Because most tertiary students, both residential and non-residential, are typically responsible for feeding themselves, they frequently have to choose between cooking on their own or buying food from vendors. However, due to time constraints, difficulties in cooking their own food and easy access to a range of healthy foods from vendors at a fairly low cost, many tertiary students prefer the latter. (Tabassum & Rahman, 2012). Most studies observed the safety and hygiene practices of street food vendors (SFVs) and the bacteriological, physical, chemical contamination of food(Amoah, 2014; Cortese et al., 2016; FAO, 2016). The hygienic aspects of vending operations are a significant wellspring of worry for food control officials.The intake of vegetables and vegetable products is crucial to the optimal health of each individual; nevertheless, microbial contamination of these vegetables has become a genuine test meriting more

prominent consideration(Abakari et al., 2018).. What, then, is the response of these vendors to the concerns raised?

1.2 PROBLEM STATEMENT

It is assumed that street food contamination is inevitable yet majority of people rely on street food for nutrition(McIntyre et al., 2013). Hygiene and sanitation are the very basic but beneficial activities that safeguard the health of individuals, prevent food contamination and the spread of food borne diseases. While hygiene promotion aims to encourage changes in behaviour regarding personal hygiene to reduce the spread of sanitation-related diseases, hygiene promotion is intended to stimulate household demand for effective regular activities to maintain a healthy environment and avoid various infections of it. In Ghana, the level of sanitation and hygiene is low-slung and reports from Ghana health service in the year 2014 states that cholera as a consequence of poor or improper food sanitation had about 5,308 events recorded and these numbers increased steadily where a total of 67 deaths were recorded. Food vending sites in KNUST and its environs are no exception where food vendors tend to overlook the threatening effects of their hygienic and sanitary actions on the quality as well as on the health of their prospective consumers. Therefore, this research aims to determine the awareness of food handlers regarding good food hygiene and sanitation activities and to examine the impact of hygiene and sanitation as well as other factors affecting the quality and health of food sold in canteens in selected areas of KNUST campus and its environs.

1.3 JUSTIFICATION

Insufficient information on food health, inadequate hygiene standards and unsanitary activity are known to be significant risk factors contributing to the growth of microbiologically contaminated street food.Various studies have identified that the main risk associated with consuming street vended food is microbial contamination. It will be surprising that after countless researches performed, street vendors still do not

have a fair on the need to provide healthy and quality food. The attention given to the sector of informal economy suggests there is still more work to be done. In an institution like KNUST, it is of the view that customers that is, students that purchase food from these vendors have at least good knowledge on food hygiene. The vendors also on the other hand have also received education upon previous encounters with agents from the ministry of Health and also researchers from this field. Therefore, analysing the microbial quality of foods sold by these vendors will determine the response or reactions towards the previous researches. In the process, ensuring that food produced through processing, distribution, and consumption is not contaminated with any possible harmful microbes and other contaminants such as chemicals and toxins(Rammutla,2016). It is therefore expedient to educate food handlers in the food sector to undergo food hygiene awareness training. This will provide information which will determine whether their reactions are positive or not. This investigation will likewise help educate and furthermore update the food merchants on food safetybyinvestigating the nature of the food vendors' hygiene practices. Feedbacks from this study to the vendors will also inform them on the progress made.

1.4 MAIN OBJECTIVE

The goal is to determine food managers 'awareness of good food hygiene and sanitation practices through the microbial examination of selected foods sold in selected areas of the KNUST campus and its surroundings

1.4.1 SPECIFIC OBJECTIVES

- To enumerate the microbial quantity/ load ofmicroorganismsassociated with foods and vegetables sold in KNUST and its environs.

- To isolate and identify microorganisms associated with foods and vegetables sold in KNUST and its environs.
- To examine the impact of hygienic practices of food vendors in KNUST and its environs on the microbial quality of vended food.

2 CHAPTER TWO - LITERATURE REVIEW

2.1 Food vending and Food vendors

Healthy diet is one of the most essential aspects of sustainability and wellbeing. What we consume is our fuel, and our diet has a significant part to play in deciding how healthy we are and how well our body functions. There are too many cuisines and dietary tastes, centred on cultural and ethical backgrounds, regional place and divisions of society. Food and popular meals can tell us a lot about the history and culture of nations and religions. Food is the single most important product on the list of products and services used by urban consumers. Street-selling foods are foods and beverages that are soldinpublic areas oron the streetand can be consumed readily at the point of saleor later locations without further preparation. The last preparation of food shall be made upon request by the purchaser at the distribution site. Indeveloping countries,the street food vendinghasa significant role to play, in particular with regard to urban residents' food requirements. Food transactions are part of an informal market that is part of a market that is not governed by the government(Fontannaz-Aujoulat et al., 2016)). Rheinlander (2006) has reported the number of street food vendors to be more than 10,000. Street food business operates within the informal sector of Ghana and provides job opportunities for both sexes, especially in urban and peri-urban areas of the country, with low skills and with little or no education. Many researchers around the world, for example (McIntyre et al., 2013)(Badrie et al., 2003), (Boegh-Peterson and Tostesen 2012), (Annor &

Baiden, 2011), (Rane, 2011), (Muinde & Kuria, 2005)among others have published food vending studies covering different aspects of street food. These researchers 'findings have shaped the behaviours, expectations and opinions of food vendors and their customers regarding such products. The appearance of a seller may have a significant effect on customer decisions and on their understanding of food health (Benny-Ollivierra & Badrie, 2007). The Food and Agriculture Organization (2016) has recorded a rise in street food sales and consumption in Africa over the past three and a half decades. National and local African authorities and international organizations concur on the nutritional, financial, cultural, and social significance of street food, but on the other hand are aware of important hygiene, sanitation and management issues.

2.2 Food Hygiene and Security

Food hygiene is the required condition and measure for ensuring food safety from production to consumption(WHO, 2018). Lack of proper hygiene in food can significantly contribute to foodborne illness and the death of consumers. However, this important matter is not always understood in some places, and is taken lightly. For instance, the WHOreports that unhygienic food sickens one out of three people every year globally(Buckley and Reid 2010). Local authorities, international organizations and consumer groups are becoming progressivelymindful of the societal and commercialsignificance of SFV and its accompanyingjeopardies. Cross-contamination is a significant cause of food poisoning and might move microorganisms from one food commodity to another. It is indeed worth being aware of how it travels so that you know how to stop it. The food chain commences from the farmhouse to the serving dish.

2.3 Contamination of Street foods; origin and prevention

Food contamination emergesas soon asfood housing a microbial or environmental contaminant is ingested (with microbial contaminants being more common). Bacteria, fungi, viruses, or their toxins or by-products

may be involved in microbial contamination. Although there are several possibilities that may induce food contamination, most of them come into one of the three categories: microbial, chemical or physical pollution. Biological contamination occurs as pathogens or pollutants contaminate food and is a frequent source of food poisoning and food spoilage. Physical contamination occurs when the food is contaminated by real objects.Chemical contamination occurs when chemicals such as pesticides,insecticides,kitchen cleaning agents etc. come into contact with food and can lead to chemical food poisoning. Poor personal hygiene, cross contaminating raw and cooked meats are among the major factors contributing to microbial contamination of street vended foods(Muinde & Kuria, 2005).Contamination of street foods, particularly sliced fruits and vegetables attributable to unhygienic packaging, the use of dirty utensils and the washing of such street foods with polluted water facilitates occasional fly visits and direct dust interaction(Mahfuza et al., 2016).Food can become polluted at any stage during slaughter (in the case of animals) or harvest(for plants), processing, packaging, preparation,distribution, and consumption(WHO, 2019).

2.3.1 Factors Responsible for Food Contamination During Harvesting/Slaughtering

The tools that are used for harvesting or slaughtering if not properly washed can cause contamination. The tools must be thoroughly washed and cleaned before and after used. This should be done because even with the hygiene practices, equipment used for these activities do harbour microorganisms and contaminants from cross contamination from other tools, the environment, and users. According to Matthews (2013), vegetable cross-contamination happens during harvesting by interaction with harvesting tools, scissors, workers 'hands or gloves and containers such as barrels, boxes and buckets. According to Bhunia (2008), E. coli O157:H7 can dwell in the intestines of healthy animals; hence, meat may turn out to be contaminated through slaughtering.

2.3.2 Factors responsible for food contamination during processing and production

Agricultural practices farmers adopt in producing raw materials needed for food preparation can have effect on the microbial quality of street vended food. Fruits and vegetables can be contaminated if contaminated water is used for irrigation on the field.Equipment used in food preparation or manufacturing is a threat for the product when it is rusty or poses some other contamination risk. Food equipment and working environments must be built to enable the cleaning.This can be done by using smooth and durable equipment to allow for efficient cleaning and disinfection. Following each day's operation, areas of the food preparation area where the risk of food contamination is large must be cleaned and disinfected.

2.3.3 Factors responsible for food contamination during distribution and transportation

Distribution includes providing produce to a consumer or a produce distribution system from a farm or manufacturing plant. In general, markets are far from street food stalls so raw materials and ingredients have to be transported after purchase. Transport should be hygienic in order to avoid pollution or harm to products and to safeguard their dignity, particularly their sanitary value. Efforts should be made to reduce, if not remove, the chances of contamination from ambient micro-organisms and toxins during the transport of raw materials and ingredients.In warm weather, if refrigerated food is left for a long period on a loading dock, it may hit temperatures that enable bacteria to spread. Foods may become unhealthy if they are left in the 16–52°C region for too long, the environment in which bacteria expand the quickest, so it is best not to keep food for too long at 16–52°C (WHO, 2008). Perishable goods should be transported as quickly as possible, preventing stoppages and using the shortest path(Pawsey, 2009).

2.3.4 Factors responsible for food contamination during consumption

The most effective thing that can be performed to avoid foodborne disease is to wash hands regularly with soap and warm water, particularly after using the bathroom, changing a diaper, petting an animal, and cooking or consuming food.

2.4 Common microorganisms associated with food

Since antiquity, contaminated food and water have been identified as vectors of diseases in human societies (Campbell, 2011). In developing countries, the issue of foodborne diseases is serious due to the lack of strict attention to hygienic food handling activities.Microorganisms and the toxins they generate do not have an odour or taste to help diagnose them. Microorganisms in products may be a silent killer. Some microorganisms produce spores, thick-walled, protective structures that enable microorganisms to survive cooking, freezing temperatures and some mixtures for sanitization.Even though microorganisms release toxins on food, heat-including processing can wipe out the microorganisms but not the toxin already released into the food (Purnomo, 2006; ISO, 2010). *Escherichia coli O157:H7,Salmonella spp, Listeria monocytogenes, Clostridium botulinum, Clostridium perfringens,Bacillus anthracis, Campylobacter jejeuni,* and*Staphylococcus aureus*are known to be the common causativeorganisms of food poisoning. Foods commonly included in these cases of food poisoning involve low-acid processed foods and beverages,meat and poultry products, egg and egg products,milk and dairy products, seafood and seafood products,fruit and vegetable products.

CHAPTER THREE

3 MATERIALS AND METHOD

3.1 STUDY DESIGN

This is a cross-sectional study. Food sold by these vendors were obtained and evaluated at the laboratory for their microbial quality and quantity.

3.2 STERILIZATION PROCEDURES

3.2.1 AUTOCLAVING

An autoclave is a tool used to sterilize equipment and supplies by subjecting them to a high pressurized saturated steam at a specific temperature within a predefined length of time. All aqueous solutions and culture media were sterilized with an autoclave. This equipment employs the use of steam under pressure at a temperature of 121^0C for 15 minutes to kill all microbes present.

3.2.2 HOT AIR OVEN

Petridishes and metal instruments were sterilized withan electrical hot-air oven with a dry heat at a thermostatically controlled and devised at a minimum temperature of 160^0C for two hours. The oven was then switched off and allowed to cool slowly and the petri dishes and metal instruments were removed.

3.2.3 FLAMING

By passing them through a Bunsen flame, inoculating loops as well as the tops of sample tubes were sterilized. Flaming act was used to decontaminate container mouths and test tubes.

3.2.4 WASHING

All glass wares were thoroughly washed with detergents and rinsed with water before and after use and where necessary dried with a clean towel.

3.2.5 ETHYL ALCOHOL SOLUTION

Surface of work areas such as benches were sterilized by use of 70% alcohol mixed with ethylene.

3.3 MEDIA USED AND PREPARATIONS

The agars needed for isolation and identification of the microorganisms were prepared based on the manufacturer's prescription. They were autoclaved at 121^0C for 15 minutes to sterilize themif necessary. The agars to be used include Eosin Methylene Blue agar,Nutrient agar,Mannitol salt agar, Potato Dextrose agar, peptone water,Plate count agar,MacConkey agar. Reagents used for gram staining include 0.5% crystal violet, 1% safranin, iodine and alcohol.

3.3.1 Mannitol salt agar

55.5 grams of mannitol salt agar powder were dissolved and mixed carefully in 500mls of distilled water. Todissolve the agar,the solution was heated and autoclaved at 121°C for 15 minutes to sterilize it. Forculturing of microbes,10 to 15mls were poured into the petri dish and dried under the laminar flow for solidification.

3.3.2 Tryptophan

8 grams of tryptophan powder were dissolved by vigorous shaking in 500mls of distilled water. It was thenautoclaved at 121°C for 15 minutes to sterilize it. 5mls were distributed into test tubes for indole test.

3.3.3 Nutrient agar

Eight point four grams (8.4g) of powdered nutrient agar was dissolved by thoroughly mixingin three hundred millilitres (300mls) distilled water. Heat was appliedfor complete dissolution of the agar. It was then autoclaved at 121°C for 15 minutes to sterilize it, after which 5mls were poured into test tubes for the sub culturing and dried under the laminar flow for solidification.

3.3.4 Plate count agar

Plate count agar (PCA) is a growth medium commonly used to monitor or to assess viable or "total" bacterial growth of a sample. The plate count agar composition may differ, but usually it comprises (w/v): 0.1% glucose,0.25% yeast extract,0.5% peptone, 1.5% agar with pH adjusted to neutral at 25°C. PCA is not a selective medium.

7grams of the powdered Plate count Agar was measured in 400mls in distilled water and heated for complete dissolution. It was then autoclaved at 121°C for 15 minutes tosterilize it

3.3.5 Eosin methylene blue agar

Eosin Methylene Blue (EMB, also known as "Levine's formulation") is used to differentiate distinctly between lactose fermenting colonies and nonfermenting microbes. It is used as a differential medium, which provides a colour indicator distinguishing between lactose fermenting organisms (e.g., E. Coli) and those that do not ferment lactose (e.g., Salmonella, Shigella). It is a two-strainblend; eosin and methylene blue in the ratio of 6:1. It slightly inhibits the growth of Gram-positive bacteria and promotes the growth of Gram-negative bacteria.

11.2grams were measuredinto in 300mls of distilled water and heated for complete dissolution. It was then autoclaved at 121°C for 15 minutes to sterilize it. Samples were cultured tryptophan broth before they were streaked onto the EMBA on the plates.

3.4 DATA COLLECTION AND SAMPLING

3.4.1 SAMPLING SITE

The study was conducted in KNUST and its environs. KNUST and its neighbouring towns belong to the Oforikrom sub metro of Kumasi Metropolitan Assembly in the Ashanti region.29,748 peoplewere estimated to present at the Ayeduase communityaccording to the 2012 population census and that of

KNUST is estimated to be 40,000 persons (Danso,2017). Food and vegetable samples randomly obtained from vendors within randomly selected sites identified as site 1 to site 5. These places were selected based on the rate at which students patronize food from vendors in these areas.

3.4.2 SAMPLE COLLECTION

Samples were purchased from their respective vendors under normal purchase conditions. The samples were subsequently wrapped in a sterile bag and delivered in an ice chest containing ice packs for microbial examination at the laboratory. They were analysed on the same day after two hours of collection for their microbiological properties at the KNUST Biological Sciences Microbiology laboratory.

3.5 PROCESSING OF SAMPLES

3.5.1 SAMPLEHOMOGENIZATION

Ten grams of collected samples was weighed into sterile bags. Toobtain 10^{-1} dilution stock solution,the weighed sample was filled withninety millimetres distilled water and was shaken vigorously for 2 minutes in a pulsifier. Using a sterile pipette, fifty milliliters of the liquid wastransferred to a sterile fifty millilitre centrifuge tube.

3.5.2 SERIAL DILUTIONS

Toprepare 10^{-2} dilution,one millilitre of the sample was pipetted from the 50-milliliter centrifuge tube into a separate test tube containing 9ml peptone water in duplicate. 1ml of the liquids was pipettedinto another diluting tube holding 9ml of the sterile distilled water to obtain a 10^{-3} dilution after aspirating with sterile pipette to mix them and then and new pipette was used to mix the resulting solution. Corresponding dilutions were made by repeating the above procedure to derive a final dilution of 10^{-6}.

3.5.3 POUR PLATING

Usinga sterile pipette,one millilitre(1ml) of each of the sample dilutions was taken and poured into the centre of separate sterile petri dishes. Eachpetri dish containing the inoculum was filledwithFifteen millilitersof plate count agar and uniformly mixed.Theplates,after solidification of the agar,were inverted and incubated at 37°C for 24-48 hours after which growth of bacterial colonies were observed and enumerated for the total viable count (TVC). The plates which had the best and conspicuous growth was kept in a fridge for identification of the various microbes' present using the Gram staining technique.

For molds and yeasts colonies quantification and characterization, potato dextrose agar was used and incubated for 3-5 days at room temperature.

3.5.4 MOST PROBABLE NUMBER (MPN)

Lactose (MacConkey) broth was prepared according to the formula presented by the manufacturer; 6.75grams were dissolved in 180mls of distilled water. The broth was pipetted into test tubes and then placed in an autoclave at 121 °C for 15 minutes tosterilize it. Three (3) test tubes each with 5mls of MacConkey broth were inoculated with samples from the serial dilutions according to their dilution factors for each of the sample types for faecal coliform and total coliform counts each. The samples were incubated at 37°C and 44.5^{o}C for 24hrs for growth to occur. A colour change from pink to yellow denotes the presence of coliform and hence were recorded as positive. This is as a consequence of the coliforms' metabolic activities which break down the lactose in the broth and converts it into organic acids.

3.6 CHARACTERIZATION AND ENUMERATION OF MICROORGANISMS

Aftertwenty-four hours (24hrs), the inoculated plates containing the microorganisms were then removed from incubator for microbial colony counting. The microorganisms and their representative colonies were studied based on their colonial and morphological characteristics which include shape, chain formations, colour, and margins of isolates and angles of elevations or inclinations from the Petri dishes.

Enumeration was doneusing a colony counter for plates showing between 30-300 colonies. The means of the replicates were calculated and multiplied by their dilution factor and then reported as the colony forming unit per gram(CFU/g). Counts less than 30 were recorded as "Too Few to Count" (TFTC) while colonycounts above 300 were recorded as "Too Numerous to Count" (TNTC). After3-5 days of incubation,enumerationand characterization of inoculated plates for yeasts and moldswere done. The colonies were kept in the refrigerator for further examination.

3.6.1 ISOLATION PROCEDURES

To obtain pure colonies for identification,sub-culturing was performed on nutrient agar after 24 hours. Petri dishes containing the agars were allowed to solidify after which a sterile loop was used to transfer a colony to a small area of the plate and then zig-zag streaks were. Incubation of plates were done immediately at 35-37 ^{0}C for 24 hours.

3.6.2 MAINTENANCE OF PURE CULTURES

Pure isolates from Plate count agar were kept on nutrient agar slants in kept in the refrigerator at -40°C.The test tubes which record the highest positives (the highest positive dilution) were also kept in the refrigerator for identification of the various microbes' present using the Gram staining technique after being streaked onto sterile plates containing various media solutions.

3.7 BIOCHEMICAL TESTS

The Biochemical tests were conducted as a confirmatory test for the identification of the isolated microorganisms. Biochemical tests that were considered include the gram staining, catalase test, indole test, andcoagulase test.

3.7.1 GRAM STAINING

Gram stain is used to distinguish betweengram positive and gram-negativebacteria based on their different cell wall constituents. The gram stain distinguishes between these by colouring their cells red or violet. A small volume of well isolated colony was placedin a drop of physiological saline water on a dry slide and the slide was flamed to killand fix microorganisms unto the slide. Crystal violet staining reagent was placed on the heatfixed cell and washed with distilledwater was used to gently wash the slide. Gram A mordant, gram iodine, was placed on slide and washed with alcohol used as a decolorizing agent. Safranin was used as a counter stain and placed on the side then washed with distilled water until colour disappears. A drop of immersion oil was placed on each slide to observe it under microscope.

3.7.2 CATALASE TEST

Microorganisms in the oxygenated environment produce the enzyme catalase which transforms hydrogen peroxide into oxygen and water. A small amount of bacterial colony was moved to the surface of a clean, dry slide using a flamed loop. A drop of 3% of hydrogen peroxide was placed on the colony and mixed on the slide. The rapid evolution of oxygen thus evidence of bubbles indicates a positive result within 5-10 seconds. This is used to distinguishcatalase negative *Streptococci* from*catalase* positive *Staphylococci.*

3.7.3 COAGULASE TEST

Coagulase test is used for the differentiation of Staphylococcus species. A 1-in-6 rabbit plasma dilutionis prepared in saline and 1ml of the diluted plasma is measured into small tubes. The isolated colony on the nutrient agar was picked using a flamed and cooled inoculating loop and emulsified in the tubes. The tubes were then incubated at 35^0C in ambient air for 3 hours. Clots formations were observed by tilting the tube through 90^0at 1hour interval. Negative tubes were left overnight at room temperature and re-examined.

3.7.4 INDOLE TEST

Bacterial species with the ability to convert tryptophan into indole are determined using the indole test. The indole test helps to differentiate *Enterobacteriaceae* such as *Escherichia coli* from other genera. Five milliliters (5ml) of the broth were pipetted into each test tube, capped and sterilized at 121°C for 15 minutes in anautoclave. 1ml of pure culture was inoculated from the positive faecal test results into test tubes containing tryptophan broth and incubated at 45^0C for 24-28 hours. 2 to 3 drops of Kovac's reagent was added directly to the tube after the incubation periodto test for indole production. Afterthe addition of the Kovac's reagent,a positive result for*E. coli* is indicated at the top of the solution by a red ring layer whilst a negative result without E. coli indicated an orange ring layer at the top of the solution.

3.8 IDENTIFICATION OF FUNGAL COLONIES

To preparedslides of fungal cultures, the mycelial mat on the sub cultured plate were gently lifted with a sterile inoculation loop into a drop of lactophenol blue on a slide, teased, covered with a slip. This was

observed under microscope. Different features of the isolated fungi were identified and used in characterizing them.

3.9 DATA ANALYSIS

Data gathered was presented in tables and graphs.Means of the results were convertedlogCFU/g. Microsoft Excel 2019 was used both for tabulating the results obtained and for calculating the percentages. For easy interpretation, one-way variance analysis (ANOVA) was used to determine statistically significant difference in microbial loads among all the samples under study.

CHAPTER FOUR

4 RESULTS

4.1 Enumerated microorganisms from food obtained from the vending sites

4.1.1 Microbial load of food samples collected from Vending Site-1

From Table, the total viable count (TVC) ranged from 5.36logCFU/g to 7.65logCFU/g with the lowest value recorded for waakye from vendor 1 and the highest value recorded for Gari and Beans from vender 2. The total *Staphylococcus spp.* Count (TSC) recorded also varied from one vendor to the other. The highest value of 6.08logCFU/g was recorded for waakye from vendor 2 and the lowest value of 3.11logCFU/g was recorded for vegetable salad from vendor 1. The *E. coli* enumerated ranged from 1.96logCFU/g for vegetable salad from vendor 1 to 4.63logCFU/g for Gari and Beans from vendor 1. Total Coliform Count (TCC) ranged from 3.88logCFU/g to 5.38logCFU/g while faecal coliform count (FCC) ranged from 2.30logCFU/g to 4.97logCFU/g. The fungi enumerated ranged from 2.26logCFU/g to 5.04logCFU/g. likewise, the fungi enumerated range from 2.26logCFU/g to 5.04logCFU/g

Table 1.Microbial load on foods from Site-1

Food	Vendor	**TVC/log CFU/g**	**TSC/log CFU/g**	**ECC/log CFU/g**	**TCC/log CFU/g**	**FCC/ Log CFU/g**	**TFC/ Log CFU/g**
Waakye	1	5.36±1.52	TFTC	4.04±1.15	5.18±1.46	4.04±1.15	2.26±0.64
	2	7.33±2.07	6.08±1.72	3.2±0.91	5.08±1.44	3.46±0.98	3.28±0.93
VegetableSalad	1	5.47±1.55	3.11±0.88	1.97±0.56	3.88±1.1	2.3±0.65	4.12±1.17
Gari and beans	1	6.2±1.75	3.58±1.01	4.64±1.31	3.97±1.12	4.88±1.38	3.58±1.02
	2	7.65±2.16	5.45±1.54	4.36±1.23	5.38±1.53	4.97±1.41	5.04±1.43

Values are means of duplicates ± standard deviations

4.1.2 Microbial load of food samples collected from Vending Site-2

The total viable count (table 4.2) ranged from 5.34±1.51logCFU/g to 8.57±2.42logCFU/g with the lowest value recorded for Gari and Beans from vendor 1 and the highest value recorded for Waakye from vender 2. Bacterial load for Gari and beans from vendor 2 were recorded as "too numerous to count (TNTC)". The total *Staphylococcus spp.* Count (TSC) recorded also varied from one vendor to the other. The highest value of 7.59logCFU/g was recorded for Waakye from vendor 2 and the lowest value of 4.64logCFU/g was recorded for Gari and Beans from vendor 1. On the other hand, *E. coli* enumerated ranged from 3.15logCFU/g for *v*egetable salad from vendor 1 to 3.97logCFU/g for Gari and Beans from vendor 1. Total Coliform Count (TCC) ranged from 4.45logCFU/g to 5.08logCFU/g while faecal coliform count (FCC) ranged from 3.15logCFU/g to 4.32logCFU/g. Coliform counts for Gari and Beans from vendor 1 were recorded as TNTC. The fungi counted ranged from 2.36logCFU/g to 3.60logCFU/g.

Table 2.Microbial load on foods from Site-2

Food	Vendor	**TVC/log CFU/g**	**TSC/log CFU/g**	**ECC/log CFU/g**	**TCC/log CFU/g**	**FCC/ Log CFU/g**	**TFC/ Log CFU/g**
Waakye	2	8.57±2.42	7.59±2.15	3.46±0.98	4.97±1.41	3.56±1	3.12±0.88
Salad	2	7.09±2.01	5.19±1.47	3.15±0.89	4.45±1.26	3.15±0.89	3.56±1
Gari and Beans	1	5.34±1.51	4.64±1.31	3.97±1.12	5.08±1.44	4.33±1.22	2.36±0.66
	2	TNTC	5.49±1.55	TNTC	TNTC	TNTC	3.6±1.02

Values are means of duplicates ± standard deviations

4.1.3 Microbial load of food samples collected from Vending Site-3

From Table 4.3, the total viable count (TVC) ranged from 6.56±1.85logCFU/g- to 7.91±2.24logCFU/g. The bacterial load for Gari and Beans from vendor 1 were record as "too numerous to count". The total *Staphylococcus spp.* Count (TSC) recorded also varied from one vendor to the other. The highest value of 7.67±2.17logCFU/g was recorded for Gari and Beans from vendor 1 and the lowest value of 5.58±1.58logCFU/g was recorded for vegetable salad from vendor 2. The *E. coli* enumerated ranged from 4.36±1.23logCFU/g for Waakye from vendor 1 to 5.46±1.74logCFU/g for vegetable salad from vendor 1. Total Coliform Count (TCC) ranged from 4.3±1.22logCFU/g to 5.33±1.51logCFU/g with the lowest value recorded for vegetable salad from vendor 1 and the highest value recorded for vegetable salad from vender 2 while faecal coliform count (FCC) ranged from 4.97±1.41

logCFU/g to 5.46±1.54logCFU/g. The fungi enumerated also ranged from 2.08±0.59logCFU/g to 5.76±1.63logCFU/g.

Table 3. Microbial load on foods from Site-3

Food	Vendor	**TVC/log CFU/g**	**TSC/log CFU/g**	**ECC/log CFU/g**	**TCC/log CFU/g**	**FCC/ Log CFU/g**	**TFC/ Log CFU/g**

Waakye	1	7.08±2	TFTC	4.36±1.23	4.81±1.36	4.97±1.41	2.08±0.59
Vegetable Salad	1	7.91±2.24	7.64±2.16	5.46±1.74	4.3±1.22	5.46±1.54	4.69±1.33
	2	6.56±1.85	5.58±1.58	4.58±1.3	5.33±1.51	6.21±1.46	3.77±1.07
Gari and Beans	1	TNTC	7.67±2.17	TNTC	TNTC	TNTC	5.76±1.63

Values are means of duplicates ± standard deviations

4.1.4 Microbial load of food samples collected from Vending Site-4

From Table 4.4, the total viable count (TVC) ranged from 6.44±1.82logCFU/g to 6.53±1.85logCFU/g. The total *Staphylococcus spp.* Count (TSC) recorded also varied from one vendor to the other. The highest value of 4.46±1.26logCFU/g was recorded for vegetable salad from vendor 1 and no growth was observed for vegetable salad from vendor 1. The *E. coli* enumerated ranged from 3.64±1.03logCFU/g for Waakye from vendor 1 to 4.08±1.16logCFU/gfor vegetable salad from vendor 1. Total Coliform Count (TCC) ranged from 3.87±1.09logCFU/g to 4.67±1.32logCFU/g with the lowest value recorded for Waakye and the highest value recorded for vegetable salad while Faecal coliform count (FCC) ranged from 3.64±1.03logCFU/g to 4.08±1.16logCFU/g. The fungi counted were 1.96±0.56logCFU/gand 4.36±1.23logCFU/gfor waakye and vegetable salad respectively. Gari and Beans was not analysed for microbial quantity.

Food	Vendor	**TVC/log CFU/g**	**TSC/log CFU/g**	**ECC/log CFU/g**	**TCC/log CFU/g**	**FCC/ Log CFU/g**	**TFC/ Log CFU/g**
Waakye	1	6.44±1.82	NOG	3.64±1.03	3.87±1.09	3.64±1.03	1.96±0.56
Salad	1	6.53±1.85	4.46±1.26	4.08±1.16	4.67±1.32	4.08±1.16	4.36±1.23

Table 4. Microbial load on foods from Site-4

Values are means of duplicates ± standard deviations

4.2 Morphology of identified Bacteria in the food samples and their Corresponding Biochemical Tests

4.2.1 Colonial Morphology of Identified bacteria in the food samples on various culture media

The colonies on the plates of the various selective media had margins that were indented and serrated with diversities of colours and different angles of inclinations with respect to their growths on the plates. Different chain formations were also observed along the streak lines on the plates. The table below shows the morphological characteristics of the growths on the various selective media.

Table 5. Morphological features of bacterial isolates on the culture media.

CULTURE MEDIA	MORPHOLOGY	Gram reaction	Catalase test	Indole test
Plate Count Agar	White, shiny, circular,	Negative rods	Positive	Positive
	Yellow, convex, circular	Positive cocci in grape-like clusters	Positive	Negative
MacConkey Broth	Pink coloration, air bubbles	Negative rods	Positive	Positive
		Positive cocci	Negative	Negative

Eosin Methylene Blue Agar	Green metallic sheen	Negative rods	Positive	Positive
Mannitol Salt Agar	Yellow colonies	Positive cocci in grape-like clusters	Positive	Negative
	Red colonies	Positive cocci in grape-like clusters	Positive	Negative

4.3 Identified fungi in the various food samples, Occurrence and their Colonial Morphology

Table 6.Colony morphology and occurrence of identified fungi

FOOD SAMPLE	IDENTIFIED FUNGI	COLONY MORPHOLOGY	OCCURRENCE
Gari and Beans	*Aspergillus fumigatus*	Large, Filamentous, Grey colonies with white margins.	5
	Aspergillus niger	Large, Black, filamentous, raised colony	4
	Yeast	Small, raised white colonies	2
Waakye	*Aspergillus fumigatus*	Large, Filamentous, Grey colonies with white margins.	3
	Aspergillus niger	Large, Black, filamentous, raised colony	1

Vegetable Salad	*Aspergillus niger*	Large, Black, filamentous, raised colony	5
	Yeast	Small, raised white colonies	3
	Aspergillus flavus	Greenish-yellow colonies with white borders	1
	Aspergillus fumigatus	Large, Filamentous, Grey colonies with white margins	5

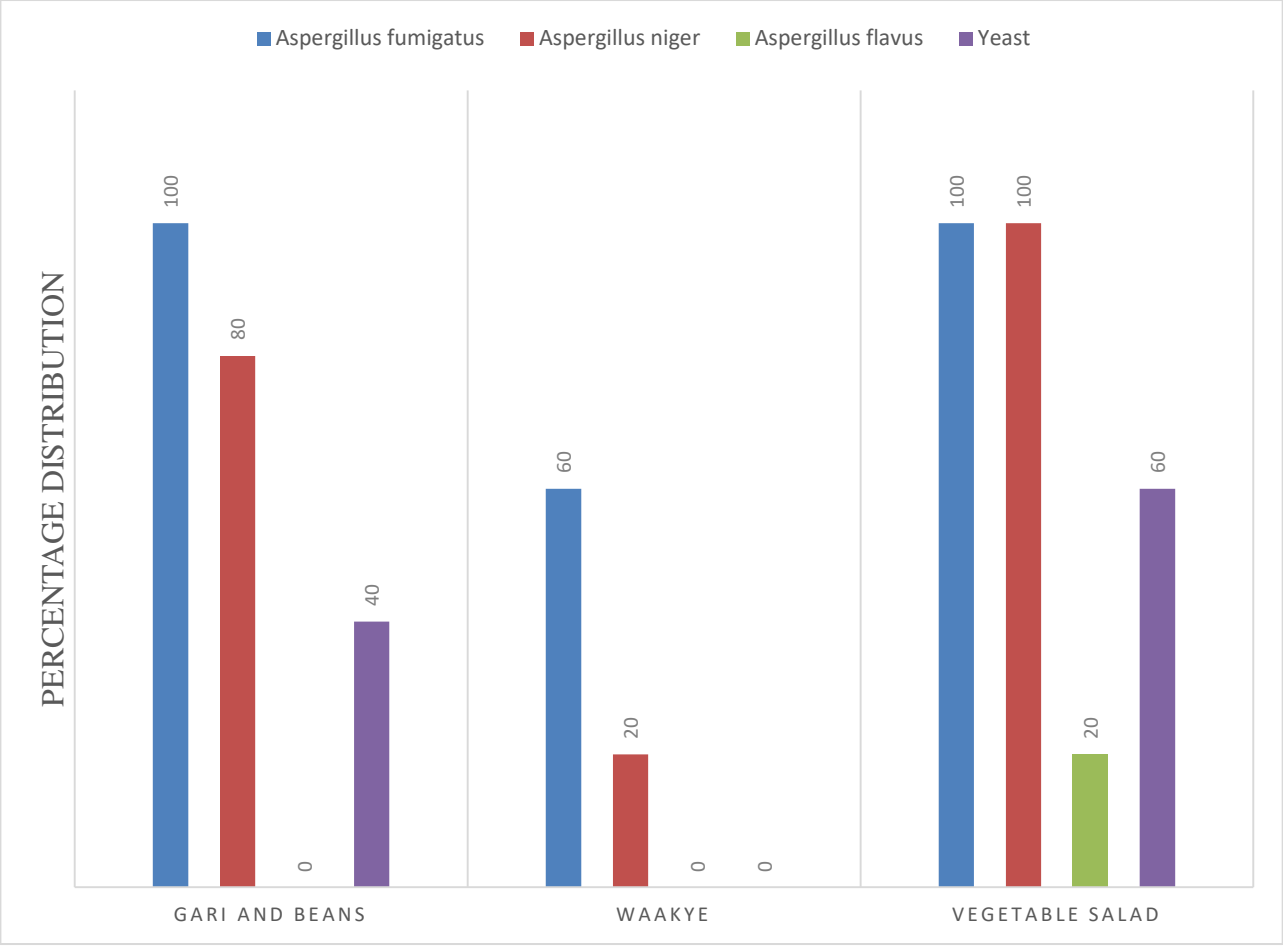

Figure 1. Percentage distribution of fungi in food samples

Figure 2 Sample of positive tubes for total coliforms in vegetable salad

Figure 3Sample of positive tubes for total coliforms in vegetable salad

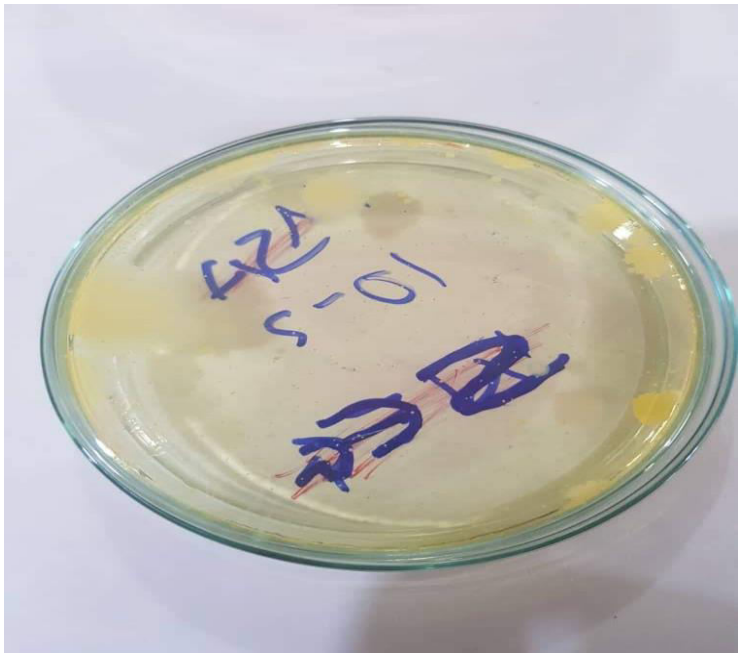

Figure 4 Sample of microbial growthon mannitol salt agar(MSA).

Figure 5 Sample showing TVC of vegetable salad on plate count agar.

Table 7 Guidance on the interpretation of results for specific food-borne pathogens in ready-to-eat food in general (colony-forming unit (CFU)/g)

Microbes	Results (CFU/g)	Results inlogCFU/g	Interpretation
Total Viable Count (TVC)	$< 10^3$	< 3	Satisfactory
	$10^3 - < 10^5$	3— < 5	Marginal
	$\geq 10^5$	≥ 5	Unsatisfactory
Staphylococcus spp.	$\geq 10^4$	≥ 4	Potentially hazardous
	$10^3 - \leq 10^4$	3 — ≤ 4	Unsatisfactory
	$10^2 - < 10^3$	2 — < 3	Marginal
	$< 10^2$	< 2	Satisfactory
Indicator organisms(Enterobacteriaceae including coliforms)	$> 10^4$	> 4	Unsatisfactory
	$10^2 - \leq 10^4$	2 — ≤ 4	Marginal
	$< 10^2$	< 2	Satisfactory
Escherichia coli	$>10^2$	> 2	Unsatisfactory
	$3 - < 10^2$	0.48 — < 2	Marginal
	< 3	< 0.48	Satisfactory

CHAPTER FIVE

5 DISCUSSION

The microbial contamination of foods(waakye, Gari and beans and vegetable salad) sold by vendors was examined with reference to total bacterial load,coliforms, *Escherichia coli*, *Staphylococcus spp*., mould, and yeast.

5.1 Total Viable Count(TVC)

From the results, the average TVC of 6.96±1.97logCFU/g of Waakye samples analysed was above the acceptable levels stated by the Ghana Standard board(< 5logCFU/g). Food from vendor 2 at site 2(table2) had the highest TVC of 8.57±2.42logCFU/g while the lowest TVC of 5.36±1.52logCFU/g was recorded for vendor 1 at site 1(Table 1). The TVCs of waakye all inlogCFU/g were 5.36±1.52 (table 1), 7.33±2.07 (table 1), 8.57±2.42 (table 2), 7.08±2 (table 3), and 6.44±1.82 (table 4).There was no statistically significant difference between the means of sample(p=0.62). This could be due to holding the food for an extended period of time at room temperature.Research have shown that the major factors causing the incidence of foodborne illness epidemics are typically cross-contamination, cooking or reheating, and inappropriate storage(Janjic et al., 2017).Oneof the most effective actions you may take is washing your hands before and after preparing food, and in between preparing multiple items to avoid contamination through cross contamination(FDA, 2017). This result complies with a study by Mensah et al.(2002) where rice was found to be highly contaminated.

For the vegetable salad, the mean TVC was 6.71±1.9 and ranging from 5.47±1.55logCFU/g.to 7.91±2.24logCFU/g were recorded which were all above the acceptable limit as well as other related study. There was no statistically significant difference between the means of samples from the different sites(p=0.78). In a similar study in Kumasi (Feglo & Sakyi, 2012) mean APC of 5.13logCFU/g were recorded in salad samples. However, in a recent study conducted by Amoah(2014), TVC of 3.1logCFU/g to 4.83logCFU/g were recorded which were all within the acceptable limit of the Ghana Standards Board (< 5logCFU/g) (GSB, 2003) and the UK Public Health Laboratory Services (6 to < 7logCFU/g). LowTVC may indicateproper hygienic and handling practices, indicating that the food vendors and employees involved in this study probably employed some level of poor handling, inappropriate processing or a

general lack of hygiene. It is therefore necessary for vendors to adhere to hygienic standards when preparing and serving food.

From this study, TVC of Gari and Beans ranged from 5.34±1.51logCFU/g to 7.65±2.16logCFU/gand had an average of 6.40±1.81log CFU/ml which were all above the acceptable limit of contamination that is unsatisfactory. The means of the Gari and beans were significantly similar(p=0.52) statistically.Samples analysed from Gari and Beans(table 3 and 4) were too numerous to count (TNTC) indicating high level of contamination. All food samples analysed had high TVC which is in line with a previous study conducted by Darko(2016) where she suggested that the microorganism may have originated from the natural microflora of the ingredients used in food preparation. She went on further to state that a high TVC indicate poor handling, a general lack of hygiene of food handlers, or inappropriate processing of food indicating that improper handling and processing of food were employed by the vendors.Gari is not processed further after production so it has to be stored before using in food preparation. Therefore, contamination may result from bad handling before and after storage.

5.2 Coliform Counts

Coliforms are a group of bacteria that are used as markers of the possible existence ofviruses, pathogens or parasitesin the sample. These microbes are classified as a group of bacteria with specific properties, including the capacity to grow at 35^{o}C in the presence of bile salts and the ability to cause lactose fermentation. The coliform bacteria are classified into total and faecal coliforms.

In this study, the TCC and FCC on the average did not fall within the acceptable limit(< 2logCFU/g) for waakye. The TCC and FCC of waakye on the average were 4.68±1.33logCFU/g and 3.91±1.11logCFU/g respectively. There was no statistically significant difference between the means TCC and FCC of samples

from the different sites(p=0.87 and 0.68 respectively).The average FCC of waakye was however within the expected level of 2logCFU/gto ≤ 4logCFU/g. Measures must be put in place to ensure that proper food handling to maintain the values at the acceptable levels. Ingredients used in waakye preparation may be a cause for contamination if proper washing is not implemented. This is because untreated water is used most often for irrigation on farm land. Untreated water used for irrigation may be a reservoir for coliforms.

The average TCC and FCC for vegetable salad(4.53±1.28logCFU/g and 4.04±1.14logCFU/g respectively) did not fall within the acceptable level. Statistically, there was not enough evidence to prove that the means of TCC and FCC were significantly different(p=0.83 and 0.17 respectively).Coliform associated with vegetable salad may originate from the water used for irrigation on the field and failure to properly wash organisms that stick to the surface of vegetables can lead to contamination. It could also be as a consequence of cross contamination from utensils used during processing of vegetables(Rane, 2011). In a similar study in India by Ghosh et al.(2007) where ready-to-eat food(Shawarma), coliforms were present in 20% of examined samples with anaverage of 4.3 x 10^3/g(3.63log/g). A high coliform count may be due to operation under poor hygiene conditions on the part of preparers and lack of access to toilet facilities and potable water. Spices used in the preparation of vegetable salad to impact flavour may be a cause of coliform contamination. Coliforms were identified in all spices analysed in a study by Bakobie et al.(2017).

The average TCC and FCC for Gari and Beans were 4.81±1.36logCFU/g and 4.73±1.34logCFU/g respectively. The TCC and FCC values were not within the acceptable limit. The TCC varied between 3.97±1.12logCFU/g (table 1) and 5.38±1.53logCFU/g (table 1) whereas the FCC ranged from 4.33±1.22log CFU/g to 4.97±1.41log CFU/g. There was no statistically significant difference between the means of the TCC and FCC(p=0.61 and 0.88)There is not much research on this particular food.However, a high coliform count from this study could be a consequence of contamination from human origin. Foods

such as Gari which require no further cooking had high coliform count with the reason being that heating could kill coliforms and other intestinal pathogens(Nkere et al., 2011). They found Gari and Beans to be associated with high coliforms in their study as well.

5.3 *Escherichia coli* Count

Escherichia coli (*E. coli*) is a common bacterium located in the guts of warm-blooded organisms. Some *E. coli*cause serious illness when they contaminate food, although majority of them are harmless(FDA, 2012). Some types, however, may cause human illness, includingfever, abdominal pain, vomiting, and diarrhoea. Sources of E. coli include faeces of infected people and contaminated water, contaminated food especially ground beef, unpasteurised milk and raw fruits and vegetables.*E. coli* quantification was also determined in the various food samples. Counts which were above <0.48logCFU/g were considered unacceptable.

The average *E. coli* count observed on waakye was 3.67±1.04logCFU/g and ranged from 3.20±0.91logCFU/g at site 1(table 1) to 4.36±1.23logCFU/g at site 3(table 3). All count was above the acceptable limit indicating a high level of contamination. Statistically, the means of samples were significantly similar(p=0.82).In a similar study by Canini *et* al(2013) samples had unacceptable level of contamination. 69(39.9 %) of samples that were analysed contained *E. coli*.According to Darko(2016), food preparation process where inadequate hand washing and dish washing could be a major cause of contamination. Jollof rice was found to contain *E. coli*(5.9%) in her study.She also suggested that cross contamination where utensils are shared for preparation of different kinds of food if the utensils are not properly washed, could be a cause for contamination. *E. coli* occurred in 37.5% of rice samples analysed(Wogu et al., 2011). Presence of *E.coli* is a clear indication of faecal contamination. Lack of access to washrooms and readilyavailable watermay result in faecal contamination.

From the results, all counts on vegetable salad from the various sites were above the acceptable limit. The average ECC on vegetable salad was 3.85±1.09logCFU/g. The highest (5.46±1.54logCFU/g) was recorded at site 3 from table 3 while site 3 had the least count (1.97±0.56logCFU/g). There was statistically significant similarity between the means of the counts(p=0.15). This could be attributed to the source of salad ingredients used in food preparation. Poor supervision can also result in high levels of contamination as employees may not employ hygienic methods of handling food. In a study conducted by Abakari, Cobbina and Yeleliere (2018), they discovered that 96.7% of salad vended in the central business district of Tamale contained *E. coli* within the unsatisfactory limit.In a study conducted byMugampoza et al.,(2013), E. coli levels were found to be 60%and 100%in Naguru and Nakawa Parishes respectively.Contamination of salad with such bacterial pathogens was consistent with previous observations in Ghana(Mensah et al., 2002). Approximately 6.4%of the vendors obtain their raw vegetables directly from the farms which they claim to be the cheapest way to buy their raw materials for salad food preparation and 90.4% buy them from the metropolitan retail market (A et al., 2015).

The average ECC on Gari and beans was also above the acceptable standard of contamination. Statistically, there no significant difference between the means of ECC(p=0.87). The average ECC was 4.33±1.22logCFU/g with the highest (4.64±1.31logCFU/g) recorded at site 1(table 1) and the least count (3.97±1.12logCFU/g) recorded at site 3 from table 3. Counts for samples from site 3(table 3) and site 4(table 4) were recorded as “TNTC” indicating a high level of faecal-oral contamination. The quality of the raw materials used in street food preparation is very critical because their contamination can continue through processing and cooking(Rane, 2011). Proper handling should be employed during the production of Gari to minimize the risk of contamination.

5.4 *Staphylococcus spp.* Count

Staphylococcus spp. especially *S. aureus* is liable for poisoning our food. It is capable of developing contaminants that induce food poisoning in the human body. It is also located on the skin andin the upper respiratory tract(FDA, 2012). Although*S. aureus* typically serves as a complement to human microbiota, may also become an opportunistic pathogen, a major source of skin infections. Counts less than 2logCFU/g are within the acceptable limit of contamination.

From this study, mean TSC of 6.84±1.93logCFU/g on waakye was above the minimal requirement for microbial contamination. It was found that the differences between mean counts were not statistically significant(p=0.52) However, *Staphylococcus spp.* was not found on sample observed at site 4(table 4) indicating the vendor and employees comply with hygiene protocols used in food preparation and service. Also counts on samples from site 1(vendor 1) and site 2 (vendor 2) were recorded as too few to count (TFTC) indicating contamination within acceptable limit. In contrast, vendor 2 from site 1(table 1) and vendor 1 at site 2(table 2) had count above the acceptable limit of contamination (6.08±1.72logCFU/g and 7.59±2.15logCFU/g respectively). The presence of high *Staphylococcus spp.* contamination may be as a consequence of unhygienic handling of food by merchants during preparation or service. The nose, throat, skin, and hair of vendors and those involved in cooking are the popularorigins of *S. aureus* food contamination. A minimum population of 100, 000 CFU of *Staphylococcus spp.* is required to cause infection (FDA, 2012).The findings of Mensah *et al.*(2002)where rice samples obtained from hotels were within acceptable levels with *E. coli,Salmonella,*and *Staphylococcus aureus* species not detected was in contrast to this study.The location of preparation is not necessarily safe, well-lit and is not far from the source of contamination. Preparation surfaces used by some merchants contain leftovers of pre-prepared foods that that facilitate cross-contamination.

The natural microflora and excessive handling during the cutting of vegetables may be the origin of microorganisms in the tossed vegetables(Adams & Moss, 2008). The mean TSC on Vegetable salad was 5.20±1.47logCFU/g from the results. Statistically significant difference was not found between the means of TSC for vegetable salad(p=0.19). All values from the various sites were above the standard or acceptable level of contamination with the highest(7.642.16logCFU/g) recorded at site 3(table 3) and the least count of 3.11±0.88logCFU/g recorded at site 1(table 1). The elevated *Staphylococcus aureus* rates in most canteens suggest bad handling activities before and/or after salad preparation(Amoah, 2014). According to Ghosh et al.(2007), Staphylococcus aureus was found to be present in 129 (86%) of ready-to-eat salads samples. It was also found that consuming contaminated ready-to-eat salad could result in foodborne diseases. Thepreparation of raw vegetables for salads provides an atmosphere and conditions for the dissemination of pathogenic microorganisms through unhygienic handling by food vendors according to the results.

For Gari and beans, the TSC variedbetween 3.58±1.01logCFU/g(table 1) to 7.67±2.17logCFU/g (table 3) and a mean count of 5.37±1.52logCFU/g above the acceptable limit. The means of TSC for Gari and Beans were not significantly different(p=0.26). Presence of Staphylococcus spp. above the acceptable level of contamination in Gari and beans indicates high level of contamination and a potential cause of foodborne intoxication. Toxins produced by Staphylococcal species may not be destroyed by heating processes during food preparation, although the bacterium is sus. Intake of food from these vendors may be hazardous to consumers. FDA(2012)has suggested that staphylococci are likely to occur in any and all foods treated directly by humans, unless heat processes are implemented. Frequent contact with Gari for instance during preparation could be a major cause for Staphylococcal contamination. Regular hand washing by cook can help minimize the risk to Staphylococcal contamination. Ingestion of food contaminated with

Staphylococci could lead to gastroenteritis(Le Loir et al., 2003).Adequate training should be given to vendors to help improve upon their hygienic standards(Addo-Tham et al., 2020).

5.5 Total Fungal Count

Yeasts and molds pose a problem in foods by decolouring food surfaces, causing off-odours and off-flavours, causing varying degrees of spoilage, changing substrates that allow pathogenic bacteria to grow, and in some cases being able to produce mycotoxins.

From the result TFC on waakye sample were 2.61±0.74logCFU/g on the average which were within acceptable limit(< 3.0logCFU/g).there was no significant difference between the means(p=0.36). The TFC varied between 1.96±0.56logCFU/g (table 4) and 3.28logCFU/g (table 1). This result was is in contrast with a study made by Darko(2016), where fungal counts on rice was above the acceptable limit. Samples from site 1(table 1), site 3(table 3) and site4(table 4) had counts within the acceptable limit (2.26±0.64logCFU/g, 2.08±0.59logCFU/g, 1.95logCFU/g respectively). Waakye may be susceptible to contamination because of the ingredients used in its preparation. The ingredients used in preparing waakye are beans, millet stalk leaves and rice(*Homemade waakye - biscuits and ladles*). Any of these can contaminated the food if not properly washed. .*Aspergillus fumigatus*(60%) and *Aspergillus niger*(20%) were identified. In a similar study by Wogu et al.(2011) where ready-to-eat rice was analysed for their microbial load, counts above 4logCFU/g were recorded for fungi and *Aspergillus niger*(12.5%)was also identified.

Vegetable salad on the other hand, had TFC above the acceptable limit of contamination. The TFC ranged from 3.56±1.00logCFU/g (table 2) to 4.69±1.33logCFU/g (table 3) and had a mean of

4.10±1.16logCFU/gwhich is above the acceptable level of contamination. Statistically, there was no significant difference between the means(p=0.87). *Aspergillus fumigatus*(100%) and *Aspergillus niger*(100%), *Aspergillus flavus*(20%), and yeast(60%) were found on vegetable salad. This finding conformed to a study made by Feglo and Sakyi(2012) where the salad sample observed had high contamination level. *Aspergillus niger*for instance adhere to plant surfaces, so contamination can occur if ingredients are not properly washed. *Aspergillus flavus* can also produce aflatoxin which is capable of causing food poisoning(Adams & Moss, 2008). According to Aberfo(2019), lack of knowledge on food safety practices, poor personal and poor environmental hygiene are primary challengesfaced by the school food vendors.

The mean TFC of 4.07±`1.15logCFU/gon Gari and beans did not fall within the acceptable limit of contamination. There was no statistically significant difference between the mean counts(p=0.17). All but one(2.36±0.66logCFU/g from table 2) of samples analysed were above the acceptable limit. The high TFC suggests that bad management methods were engaged in the majority of the selling sites before and/or after salad preparation. The fungi identified in Gari and Beans per the results were *Aspergillus fumigatus*(100%), *Aspergillus niger*(80%),and yeast(40%).In a study at Pakistan by Shanakht et al.(2012), fungal counts above 5logCFU/g were recorded on rice samples and the identified fungi included*Aspergillus niger*, *Aspergillus fumigates*, *Penicillium spp.*,*Alternaria* spp., *Aspergillus flavus,* and *Fusarium spp*. which is similar to the findings of this study. This indicates that foods from these vendors were unsafe for consumption.

CHAPTER SIX

6 CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

Every food establishment aims to provide safe and nutritious food products. It was evident from the study that hygiene and sanitation practices have a significant role to play in the provision of safe and quality food products hence, food handlers should make a conscious effort to exercise high standards of hygiene and sanitation since no further processing is required for ready-to-eat foods.

From this study, it was evident that food sold on KNUST and its environment were contaminated where the contamination was as a result of poor hygiene practices on the part of personnel involved in food preparation. It was found that majority of food samples observed had contaminations above the acceptable levels. Both *E. coli* and *Staphylococcus spp.* identified can be responsible for food poisoning. Molds such as *A. niger, A. fumigatus, A. flavus* may produce mycotoxins which may be harmful at certain levels when consumed. Yeast was also identified.

Conclusively, sanitation conditions as well as personal hygiene practices in the studied sites were generally poor and were not as safe to guarantee the provision of harmless and quality food products to its very numerous consumers in and around the KNUST environs. Unsanitary conditions appear to be a significant environmental problem and a leading factor to food waste in Africa as food vendors work under a number of environmental challenges. For certain instances, the officials appointed to monitor their operations are not in a position to do so due to a number of factors, ranging from shortage of personnel to operate with machinery.

6.2 RECOMMENDATIONS

From the outcome of the research, the succeeding recommendations are made;

i. The need for strict and implementable measures to promote food safety activity and good hygiene at the food establishment.

ii. Public health authorities and institutions responsible for ensuring food safety should ensure all street food producers and vendors are licensed and should issue a valid card for food handlers.

iii. There should also be a periodic inspection and monitoring team from the Food and Drugs Authority that will occasionally visit these food establishments and assess the sanitation and hygiene standards employed in the production of ready-to-eat or street foods.

iv. Water should be obtained from a safe source or must be well treated if obtained from other sources before used to prepare ready-to-eat foods sold especially in bush canteens.

v. Food handlers should also be given intermittent education of personal hygiene and good sanitation practices and be enlightened on the adverse effects of their practices and activities on the safety, quality and health of their consumers.

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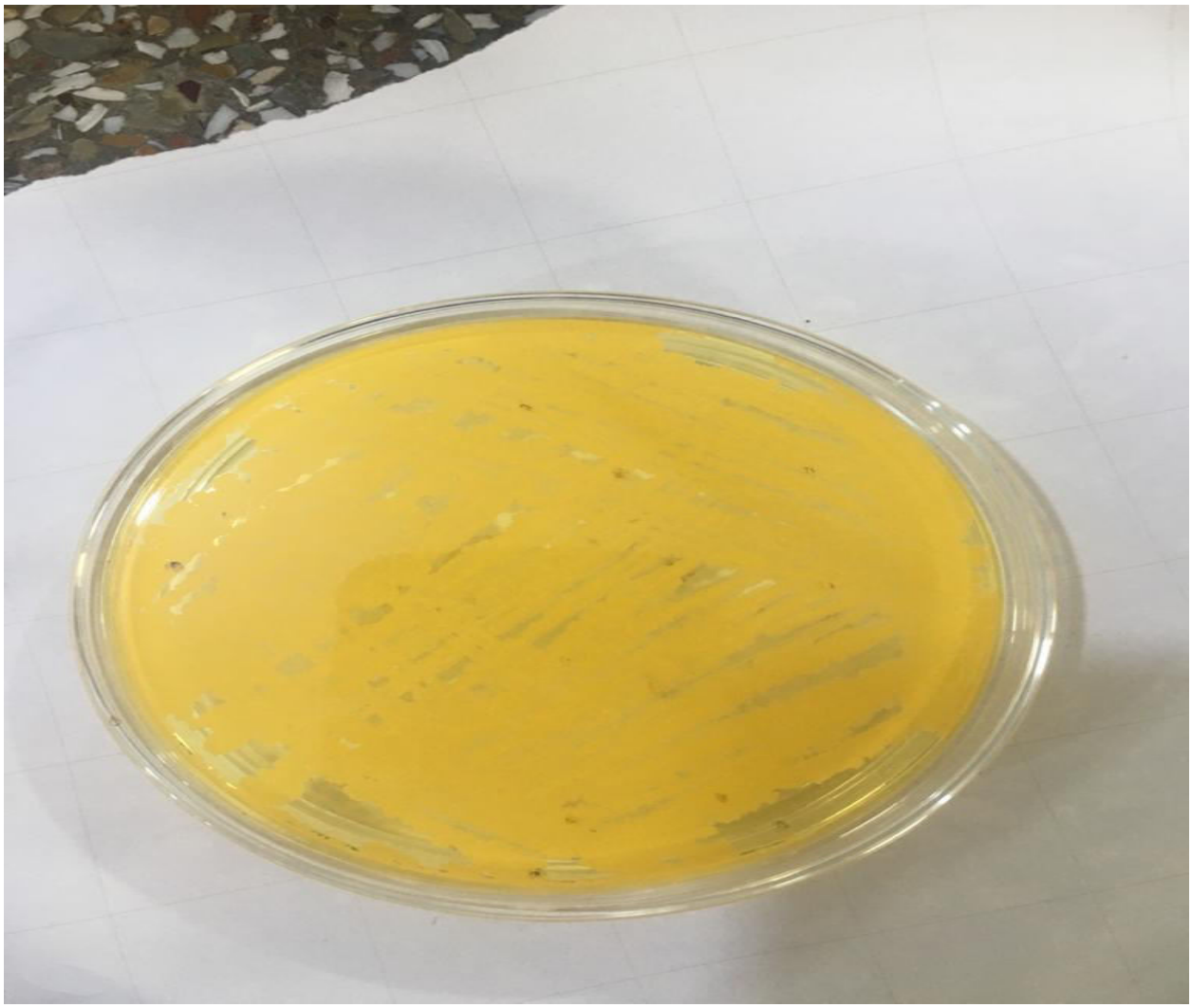
APPENDIX



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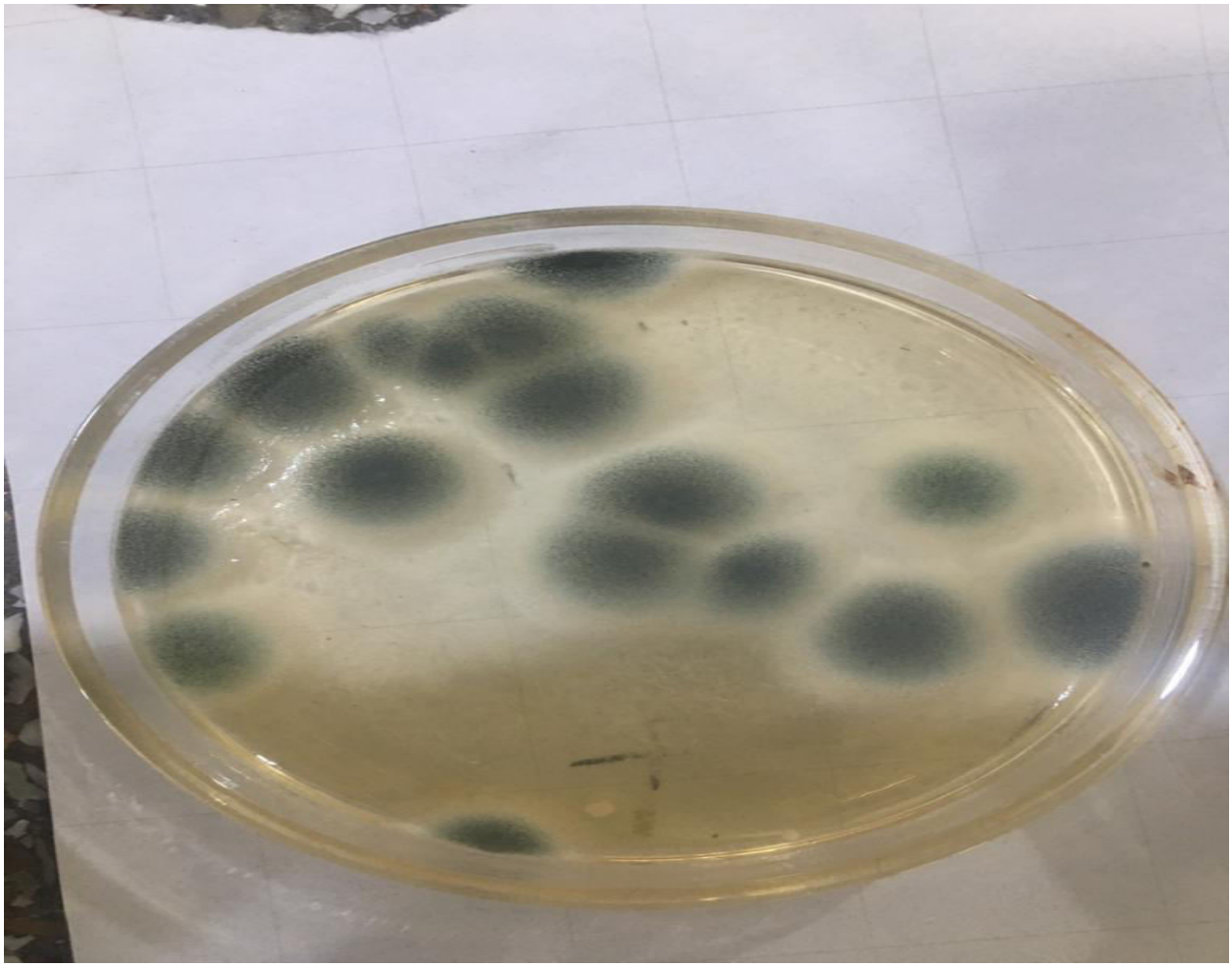
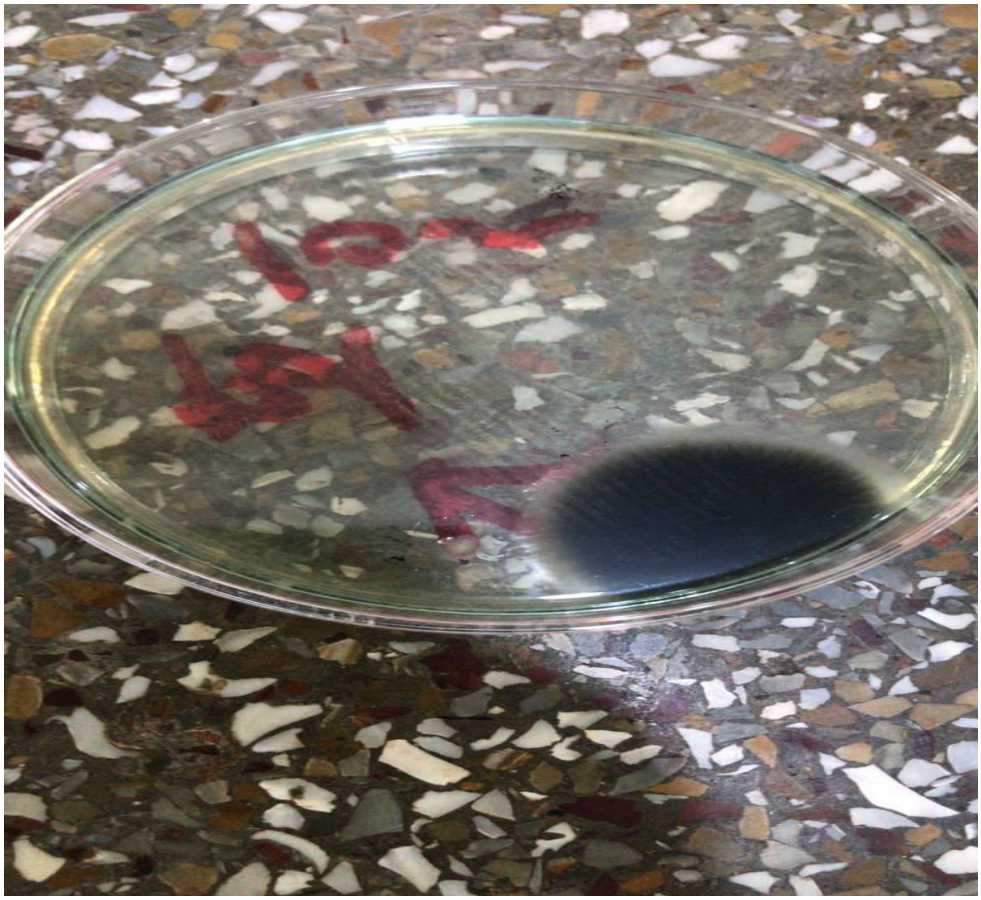


Fungal colonies on PDA

Fungal isolate on PDA



Fungal isolate on PDA