KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI, GHANA

Microbiological Food Safety Standards Practiced by some Hotel Industries in Kumasi

By

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CERTIFICATION PAGE

I hereby declare that this submission is my own work toward the MPhil. degree in Food Science and Technology and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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ABSTRACT

Numerous microbiological hazards and risks are associated with the food industry and research has indicated that eating food prepared in restaurant is the main source of most foodborne illnesses globally. In Ghana, there is an increase of foodborne diseases which has led to street food studies; however, there is less microbiological information on hotel foods. This study therefore aims in determining the microbiological food safety standards practiced by some hotel industries in the Kumasi Metropolis. A total of ten hotels with regular patronage viz., 3 'three star', 3 'two-star', 2 'one-star' and 2 'budget hotels' were selected from the study area using simple random sampling technique. A total of forty structured questionnaires were distributed to the cooks, chefs and their assistants. The questionnaires covered the demographic information of respondents, knowledge of food hygiene and safety practices and kitchen sanitation. For the second part of the study, a total of forty food samples were aseptically collected from five of the hotels (three star to budget) and analyzed. Standard methods were used for the dilution, spreading, incubation, enumeration and identification. Serial dilution of each food was prepared in buffered peptone water and inoculated onto malt extract agar (MEA) for fungal (mold and yeast) colony count. For bacteria count inoculation was done onto Plate Count Agar (PCA) for total mesophilic count, MacConkey Agar (MCA) for total coliform count and Violet Red Bile Glucose Agar (VRBGA) for total enterobacteriaceae count. Results of this study indicated that, majority of the respondents 74.4% knew the causes of food poisoning and that 89.7 % were aware that microorganisms could be found in refrigerated foods. In the area of sanitation, 94.9 % respondents used fly proof doors in their kitchens and 66.7 % knew that cooking environment should be cleaned in the morning, afternoon and evening. The females (56.4%) dominated in the hotel food preparation in this study. Count of yeast and moulds on foods fromH otel-01 ranged from 5.0×10^1 cfu/g to $1.0.x\ 10^4$ cfu/g, 1.7×10^2 cfu/g to 2.3×10^4 cfu/g for Hotel-02, 1.0×10^2 cfu/g to 2.3×10^5 cfu/g for Hotel-03, 1.7×10^2 cfu/g to 1.1×10^4 cfu/g for Hotel-04 and 1.3×10^2 cfu/g to 1.3×10^6 cfu/g for Hotel-05. Fungi identified include *Aspergillus tamaric, Cladosporium herbarium* and *Penicillium commune*. On enumerated bacteria for Hotel-01, total mesophillic count (TMC) ranged from 2.0 $\log_{10}$ cfu/g to 6.7 $\log_{10}$ cfu/g, total coliform count (TCC) ranged from 2.0 $\log_{10}$ cfu/g to 7.0 $\log_{10}$ cfu/g, and total enterobacteriaceae count (TEC) was from 2.0 $\log_{10}$ cfu/g. to 6.4 $\log_{10}$ cfu/g. In Hotel-02, TMC ranged from 4.6 $\log_{10}$ cfu/g to 7.0 $\log_{10}$ cfu/g, TCC ranged between 4.6 $\log_{10}$ cfu/g to 6.8 $\log_{10}$ cfu/g, and TEC was from 4.0 $\log_{10}$ cfu/g to 5.7 $\log_{10}$ cfu/g. In Hotel-03, TMC ranged from 3.5 $\log_{10}$ cfu/g to 4.6 $\log_{10}$ cfu/g, TCC was from 2.4 $\log_{10}$ cfu/g to 4.5 $\log_{10}$ cfu/g, and TEC was between 3.0 $\log_{10}$ cfu/g and 4.2 $\log_{10}$ cfu/g. TMC from Hotel-04 was from 3.0 $\log_{10}$ cfu/g to 6.5, $\log_{10}$ cfu/g, TCC was from 6.1 $\log_{10}$ cfu/g to 6.4 $\log_{10}$ cfu /g, and TEC ranged from 3.1$\log_{10}$ cfu/g to 6.3 $\log_{10}$ cfu/g. From Hotel-05 TMC ranged from 3.2 $\log_{10}$ cfu/g to 6.9 $\log_{10}$ cfu/g , TCC was from 3.1 $\log_{10}$ cfu/g to 7.2 $\log_{10}$ cfu/g, and TEC was from 2.1 $\log_{10}$ cfu/g to 6.8 $\log_{10}$ cfu/g. Among the bacteria identified were Coagulase-negative *Staphylococcus* (27.3 %) in Hotel-01, Gram negative rods (35.3 %) in Hotel-02, Gram-positive rods (100 %) in Hotel-03, *Acinetobacter* spp. (22.2 %) from Hotel-04 and *Klebsiella pneumoniae* (27.3%). For the kitchen and restaurant environments, coagulase-negative *Staphylococcus* dominated the bacteria identified. Some of the observed safety practices were not up to the required standards and could possibly be the cause of the contamination in the foods. It can be concluded that most foods served in some of the test hotels were above the acceptable limits. Similar study should be conducted in other hotels in the Kumasi Metropolis. Again, bacteriological studies should be done on more common foods to determine their contamination levels.

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DEDICATION

This work is dedicated to the Glory of God

CHAPTER ONE

1.0. INTRODUCTION

1.1. BACKGROUND INFORMATION

The essence of food consumption is to grow and have life. However, most diseases that affect humans come about as a result of food contamination (WHO, 2002; G T A., 2005). Studies by Egan *et al*. (2007) emphasized that the rise in food borne illness has affected individuals, food industries and the economy as a whole in terms of cost thereby raising much concern about keeping food safe. A lot of microbial contaminations are seen in the food service industry (Todd *et* al., 2009a). A lot of work has been documented on street food safety, yet not much is researched in the hotel industry in Ghana (Addo *et al*., 2007). It is well known that foodborne diseases in general and zoonotic among them are the most widespread and overwhelming public health problems in the modern world with the magnitude and consequences often underestimated (WHO, 2002).

Food borne diseases such as salmonellosis, shigellosis, and intestinal parasitosis remain a major public health concern across the globe and the problem is much severe in developing countries due to difficulties in securing optimal hygienic food handling practices (Desta, 2010). The WHO (2007) and Schlundt *et al*., (2004) reported that in industrialized countries, about 30% of the population suffers from food borne diseases annually. In the United States of America, approximately 76 million cases of foodborne diseases, resulting in 325,000 hospitalizations and 5,000 deaths, are estimated to occur each year (Salas, 2011). Developing countries bear the brunt of the problem due to the presence of a wide range of foodborne diseases, including those caused by parasites. In Ghana, the extrapolated incidence of food poisoning is 5.8 million annually (Salas, 2011). The high prevalence of diarrhoeal diseases in many developing countries suggests major underlying food safety problems (WHO, 2007). Most food borne illnesses comes about as a result of not handling the food in a hygienic manner. For instance, not handling food in the proper way resulting in illnesses after consumption makes up 97% of all food borne illnesses as this is mostly

found in the catering industries with Africa contributing 90% of cholera cases globally (Addo *et al*., 2007) with Kumasi in the Ashanti Region being the most affected (Ababio and Adi 2012). Similarly, poor food-handling practices were implicated to be the major cause of outbreaks of Infectious Intestinal Diseases (IID) in England and Wales according to Egan *et al*. (2007).

Studies show that most outbreaks of food borne diseases are associated with microbiological contamination. This is reflected in the available statistics on the etiology of food borne illness as one study estimated that people are 100,000 times more likely to become ill as a result of microorganisms in food than as a result of pesticide residue (WHO/FSF/FOS, 1998). Many developed countries have sophisticated systems for collecting data on the incidence and causes of food borne illness, yet it is known that these data represent only a fraction of the number of cases that occur. A growing body of data from foodborne disease outbreaks and studies of sporadic (non-outbreak-associated) gastrointestinal disease of various etiologies suggest that eating food prepared in restaurants is an important source of infection (Angulo and Jones, 2006). These data suggest a critical need for action that is focused on preventing disease transmission within the hotel industry.

1.2. PROBLEM STATEMENT

It is estimated that about 70% of all bacterial food poisoning is caused by caterers (Wilson *et al*., 1997; Annor and Baiden, 2011). Although the public is increasingly concerned about food related risks, the rise in food poisoning cases suggests that people still make decisions on food consumption, food storage, and food preparations that are less than ideal from a health and safety perspective (O'Riordan *et al*., 2002). A recent study of campylobacteriosis in developing countries revealed that the major sources of human infection were food. Numerous food hygiene studies conducted in some parts of Ghana have revealed poor food safety knowledge and attitude of some food service personnel (Annor and Baiden, 2011). To this effect, scientists and researchers have

allotted enough of attention to street foods and street food operators in Ghana concerning various aspects of food safety; however, there is dearth of knowledge on the microbiological food safety standards as far as the hotel industry is concerned. Similarly, data is abundant on the food hygiene knowledge attitudes and practices of food handlers and consumers; however, knowledge is scanty on the food safety and microbiological standards among food handlers in the hotel industries in Kumasi. This study investigates the microbial safety standards practiced by some hotel industries in Kumasi.

1.3. JUSTIFICATION

Time scarcity, the failure of not having enough time, has been implicated in changes in food consumption. There is also a decrease in food preparation at home and an increase in the consumption of foods already prepared outside the home such as in hotels. Eating out from home has now become the lifestyle of many Ghanaians. It is common these days for majority of Ghanaian families, both middle and high income class, to patronize hotel meals especially from "three star" to "budget". Thus, the lives of these families are in the hands of the caterers in the kitchens. For these reasons many researchers are aggressively addressing food safety issues in food facilities to protect consumers from preventable biological, chemical, and physical hazards. The outcome of this study will inform food workers on the importance of practicing food safety and ensuring cleanliness in the food preparation area. This study will also help the Ghanaian populace to know the safety levels of foods prepared by these categories of hotels. By establishing the presence of microbes in cooked food, this study will help hotel managers to be more informed about the need to control hazards through proper screening of the workers in the food area and the essence of keeping the environment clean at all times.

It is again perceived that the higher the star rating of a hotel, the safer the food but studies have shown some contamination in hotel foods (Annor and Baiden, 2011; Sabbag and Hepsag, 2011).

The chances of food contamination due to improper handling during large scale cooking are very high. In theory, food safety is managed completely but practical experience shows some deviations. The microbiological tests conducted in this study will help determine whether there are organisms in the foods prepared in these hotels and also whether the organisms are those that can cause food borne infections or food poisoning. The outcome of this study will help intensify regular hand washing, use of colour coded chopping boards and cutting knives and use of thermometers inside refrigerators, time and temperature control of cooked foods to prevent microbiological contamination of foods in hotels. Feedbacks from this study to managers of the hotels will help managers to provide the required equipment and tools for efficient and safe food production as well as routine education and inspection of the kitchens. This will press managers to effectively train staff to reduce food safety problems at the work place and again, be willing to provide the resources and systems for food handlers in the hotels to implement good practices such as posting operational practices on walls in the kitchen to remind employees on what to do at different stages of production.

1.4. MAIN OBJECTIVE

The main objective of the study was to investigate the microbiological food safety standards practiced by some hotel industries in Kumasi in the Ashanti Region of Ghana.

1.4.1. SPECIFIC OBJECTIVES

To determine the knowledge of kitchen staff on food safety

To determine the level of microbial contaminations in the cooked food samples

To characterize microbiologically, medically important foodborne pathogens

To determine the microbiological safety of the cooking environment and also conduct observational studies in the hotel kitchens

1.5. CONTRIBUTION OF THE STUDY TO SCIENCE

The study will help improve the knowledge of the kitchen staff in some selected hotels in Kumasi on food hygiene and sanitation. These hotel food handlers would be advised on improving their handling of food to prevent infection. Foodborne pathogens that can contaminate food would be ascertained and these may help in stricter supervision practiced during cooking to prevent outbreaks of food borne illnesses that could result in substantial cost to individuals, the food industry and the economy. Food borne pathogens that are medically important would be known and this will be passed on to the hotel managers for prevention. Should an outbreak of food poisoning occur, the organisms would be known and appropriate measures taken.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. FOOD SAFETY

Food safety is defined as the degree of confidence that food will not cause sickness or harm to the consumer when it is prepared and eaten according to its intended use (FAO/WHO, 2003, Ali, 2004; Raspor, 2008; Lawley *et al*., 2008). On the other hand, food safety is the maximum frequency and/or concentration of a (microbiological) hazard in food at the time of consumption that provides the appropriate level of health protection (Purnomo, 2006). The practice of making food safe brings about minimum level of risk without causing serious problem to the food supply

(Alli, 2004). It is a crucial factor in the growth of developing countries worldwide (Saba and Gonzalez-Zorn, 2012) and as stated by Baston (2007), food safety is equally a priority for governments worldwide as foodborne diseases with related deaths and economic losses occur in countries worldwide. The WHO, for instance, estimates that unsafe food sickens one in three people every year worldwide (Buckley and Reid, 2010).

Food safety which is synonymous with food hygiene embraces anything involving the processing, preparation and handling of food to ensure that food is free from contamination and safe to eat (Griffith, 2006). Food safety has become of great concern to the World Health Organisation. Thoroughly cooking food, separating raw and cooked food are among the five key practices developed by the WHO to ensure that food are safe for consumption (WHO, 2007 a). These key practices has been of great importance to food vendors especially in developing countries as this has gain significant impact on food safety.

2.2. Sources of Food Contamination in the Food Service Industry

According to Schlundt *et al*. (2004), preventing contamination requires control measures at all stages of the food chain, from agricultural production on the farm to the processing, and

preparation of foods in the commercial establishments. Food provides an ideal nutritional source for microorganisms and generally has a pH value in the range needed to contribute to microbial cell proliferation (Mariott and Gravani, 2006). Kibret and Abera (2012) in their study found that illness caused by pathogenic bacteria mostly is as a result of the food handlers disregarding hygienic measures.

According to the Centre for Disease Control and Prevention, one-third of disease outbreak in the U.S. has been related to cross contamination from the kitchen of catering or food industries (Todd *et al*., 2007). Food contamination can be attributed to five risk factors: food from unsafe sources, inadequate cooking, improper holding temperatures, contaminated equipment, and poor personal hygiene (Donkor *et al*., 2008; WHO 2007 b).

2.2.1. Food from Unsafe Sources

It is accepted that in the muscle tissues of healthy live food animals bacteria are absent, undetectable or at extremely low population levels. The procedure or processes used in slaughtering the animal and the environment can however lead to the carcass being contaminated especially through the exposed cut surfaces. This can result in a vast array of pathogens, either Gram-negative or Gram–positive bacteria including the gastrointestinal tract. *E. coli* 0157:H7 has been found in faecal samples of cattle and are the most significant source of contamination (Mariott and Gravani, 2006).

Vegetables are mostly contaminated during their production process with the use of contaminated water and poor post harvest handling such as storage (Fatica and Schneider, 2011). Staphylococcal food poisoning can be endemic in the processing environment with the causative microbe growing and producing staphylococcal enterotoxins (SE) (mainly enterotoxins A–E) resulting in the staphylococcal food poisoning (SFP). Although the bacterium can be eliminated by heat treatment of the food, the toxin is heat resistant and will therefore remain and can cause SFP (Panagiotis and

George-John, 2011). Therefore, only supplies and materials gathered in accordance with recognized good practices should be used.

2.2.2. Improper Holding Temperatures

Time and temperature play a huge role in whether food is safe to eat or needs to be thrown out. Food safety should therefore be a top concern for every commercial kitchen (Marzano, 2010). According to Foskett and Ceserani (2007), poor temperature control or prolonged holding in the danger zone can result in food poisoning bacteria multiplying to large numbers and contaminating the food. Foods must thus be out of the danger zone (temperature between 10 ^{0}C and 60 ^{0}C), the temperature range in which harmful bacteria multiply at an exponential rate. After two hours in the danger zone there will be too much bacteria and the food will have to be thrown out.

Cold foods can be displayed for a maximum of 4 hours. According to Mariott and Gravani (2007), if foods are served from a buffet, they should be presented on a steam table or ice tray, depending on temperature requirements. During food display, the food is mostly protected by a transparent shield and this shield protects the food against contamination.

2.2.3. Inadequate Cooking

During cooking of food, bacteria can survive and therefore cooking should be done at temperatures above 75 ^{0}C. The food also has to be held at the specified temperature for a minimum of 15 sec so that all the bacteria can be killed (Foskett and Cesarani, 2007; Welker *et al*., 1997). The temperature and the time of cooking food is very important when one wants to have an acceptable result. It therefore becomes imperative to monitor the temperature when cooking food in batches to ensure uniformity. If it is necessary to reheat food it should be reheated to the centre of the food

to temperatures above 75 ^{0}C with the food kept covered where possible during cooking (Foskett and Ceserani, 2007).

2.2.4. Contaminated Equipment

Equipment with which food comes into contact should be kept clean and in good order. It must be repaired and conditioned to minimize any risk of contamination of the food. Even with the practice of hygiene, equipments do harbour microorganisms and debris from the air, users and materials. Equipments for processing foods or used in food preparation becomes a hazard to the food when it becomes rusty or poses any other risk of contamination (Foskett and Ceserani, 2007). Food equipment, work surfaces and fittings must be designed to make cleaning easy. This can be done by the use of smooth and durable materials to allow effective cleaning and disinfection. Surfaces of food preparation area where risk of food contamination is high must be clean and disinfected after each day's work.

The ideal cooking equipment should heat and cool evenly at a consistent rate and should be responsive to temperature change. In addition, it should not react with any food stuff or cleaning agent and should work equally well on any cooker type (Food Standard Agency Guidelines, 2007). Cutting boards should not splinter or leak preservatives to contaminate food. Several boards can be obtained, marked with different colours for different purpose and this has been found to reduce cross-contamination (Foskett and Ceserani, 2007).

2.2.5. Poor Personal Hygiene

Personal hygiene refers to the cleanliness of a person's body. Parts of the body that contribute to the contamination of food include the skin, hands, hair, eyes, mouth, nose, nasopharynx, respiratory tract, and excretory organs. These parts of the body act as carriers of detrimental

microorganisms and cause contamination through direct or indirect transmission (Mariott and Gravani, 2006). Bacteria can be found in and around the body and can be transferred onto anything with which the body comes into contact. For this reason, personal cleanliness is essential to prevent bacteria getting onto food and persons suffering from ill-health should not handle food (Foskett and Cesarani, 2007). Mariott and Gravani (2006) found that the first step of action against food contamination is how often and well food handlers wash their hands.

2.3. Importance of a Thermometer to Ensure Food Safety

Whether, cooling, cooking or keeping food warm, the only way to know if the food is safe is by the use of a meat thermometer. This is especially true when cooking meat, because a lot of people rely on visual cues of freshness (like the absence of pink on the inside) to determine that a piece of meat is done. Visual cues of doneness are not reliable, but a thermometer is reliable. Making food safety concerns second nature can only help catering business by providing tasty, bacteria-free meals to customers. According to the report of the Centre for Science in the Public Interest (CSPI, 2008), food safety violations such as poor employee hygiene, improper cooking and holding temperatures and poor food preparation surfaces and sanitation are among the risk to diner's safety.

2.4. Microorganisms Involved in Food Spoilage

According to Prescott *et al*. (2002), foods provide nutrient and therefore can provide an excellent environment for the growth of microorganisms. Baston (2007) adds that to reduce food spoilage, microbial proliferation must be controlled. Microorganisms occurring in foods are grouped into beneficial, pathogenic and spoilage microbes (Adam and Moss, 2008; Mariott and Gravani, 2006). Microorganisms that cause food spoilage do so through their growth behaviour and the enzymes

they release alter the food by changing the taste, flavour, texture and colour (Mariott and Gravani, 2006).

2.5. Microorganisms Common to Food

Bacteria and fungi are known to be the most common microorganisms common to food and to a lesser extent viruses and protozoa.

2.5.1. Bacteria

Bacteria are unicellular microorganisms and usually measure approximately 1µm in diameter. Mariott and Gravani (2006) reported that some bacteria possess flagella that aid in their locomotion whilst others bring forth pigments. Some bacteria have pigmentations of intermediate colours such as red, pink, orange, blue, green or purple. Jay (2002) has reported that those bacteria that produce pigments cause food discolouration, mostly in meat. Some bacteria such as the thermophilic types release toxins which can cause food borne illness.

2.5.2. Moulds

Moulds are among the microorganisms that causes spoilage to food product (Mariott and Gravani, 2006). Most moulds produce mycotoxins that can cause a health risk to human. Moulds are air-borne and spread easily causing decomposition and various degree of deterioration to food. Food processors encounter spoilage problems caused by moulds because they are difficult to control.

2.5.3. Viruses

Viruses mostly need another organism to aid its production and multiplication and are obligate parasite of all living organisms. Viruses mostly detected in foods are often transmitted by employees who may serve as carriers (Prescott, 2002). Food handlers with viral infection who cough or sneeze especially in the food preparation area, without covering their mouth with

handkerchief or nose mask, are most likely to food with virus. Improper washing of hands with sanitizer after using the toilet can also contaminate food with virus. One of the most common viruses noted in the food service industry is hepatitis A and this gets into foods through employees or people who have the virus.

2.6. Factors that Promote the Growth of Microorganisms

Factors that influence the growth of microorganisms can be group as extrinsic and intrinsic factors.

2.6.1. Extrinsic factors

The growths of microorganisms are influence by certain environmental factors. These include temperature, humidity and oxygen availability.

2.6.1.1. Temperature

Microbes, according to Huang *et al*. (2011) are capable of surviving in various temperatures and ranges. Production of most microorganisms occurs at the optimal temperature of between 14 °C to 40 °C. However, a few microorganisms can flourish below 0 °C and other genera can produce at temperatures up to and passing 100 °C. Microorganisms that thrive at a temperature exceeding 45 °C include *Lactobacillus thermophiles*, *Bacillus congulans* and *Bacillus stearothermophilus.* Fewer microorganisms are capable according to Prescott *et al*. (2002) to survive at a temperature approaching 0 °C and below 5 °C, spoilage microorganism is retarded and growth of most pathogens stops.

2.6.1.2. Oxygen availability

Microorganisms are classified as aerobic and anaerobic based on their oxygen requirement for survival (Jay, 2002). Those microorganisms that thrive in the presence of oxygen form the aerobic

group and include the *Pseudomonas* species (Adams and Moss, 2008). Anaerobic microorganisms are those that can survive without oxygen (that is *Clostridium* species). Those groups of microorganisms that can still thrive on food with or without oxygen present, form the facultative microorganisms and are of the *Lactobacillus* species.

2.6.1.3. Relative humidity

Microorganisms require high amount of water to aid their growth and survival. Foods with high amount of moisture contents are mostly prone to microbial attach when the medium of storage is poor. Moisture condensation of foods do occur when the relative humidity is high and this create a favourable environment for microbial growth and food spoilage as the surface of the food becomes moist for microorganisms to act. Mariott and Gravani (2006) in their study established that microorganisms that require high humidity of about 92% is bacteria followed by yeast (90%) and mould (85-90%).

2.6.2. Intrinsic factors

The substrate (foodstuff) on which microorganisms thrive on has some characteristics that support their growth and survival (Prescott *et al*., 2002).

2.6.2.1. Water activity

Reducing the amount of water present in food hinders the proliferation of microbial activity (Adams and Moss, 2008); however, it is the water available for metabolic activity that determines the degree of microbial growth and not the total amount of moisture present. The water activity optimum for microbial growth is 0.99. A water activity higher than 0.91 is favourable for bacteria and this makes it easy for them to infest food products since most foods have water activity of approximately 0.99. Although most microorganisms can grow and multiple in areas of high water

activity, where there is partial dehydration of surfaces, mould and yeast are known to thrive (Adams and Moss, 2008).

2.6.2.2. pH

Most microorganisms thrive well at pH near neutral (7.0). Jay (2002), found that the growth of some microorganisms reduces dramatically when the pH fall below 5.2. However, yeast are capable of growing in an acidic medium of pH around 4.0-4.5. Although moulds thrive well at pH range of 2.0 to 8.0, their production is much higher in an acid medium. The growth of bacteria on the other hand is more favoured at pH near neutral although acid loving bacteria (acidophilic) can survive on food at pH of 5.2.

2.7. Effects of Microorganisms on Spoilage

Growth of microorganisms on food cause detrimental changes in colour, odour, flavour and texture (Davidson, 2003). This makes the food unhealthy for human consumption due to the decomposition and deterioration that result from microbial activities.

2.7.1. Physical changes

Changes that do occur when microorganisms act on food substances are more physical than chemical (Doyle, 2007). Food spoilage caused by microbial activity usually results in changes that are obvious such as colour, odour and flavour degradation. Based on the particular microorganism that is causing the spoilage and the prevailing condition, the food spoilage could be classified as being aerobic or anaerobic. Mould that depends on free oxygen to cause food spoilage is generally confined to food surfaces and such moulded food surface can be trimmed to make it acceptable for consumption. According to Doyle (2007) food which surfaces have been trimmed off as a result of mould infection might still have some of the microbial growth underneath the trimmed surface.

The microbial growth may go beyond the trimmed surface if the growth on the surface is intensive leading to penetration inside the food substance and making it toxic.

Food contamination as a result of anaerobic spoilage mostly occurs inside food products with or without the present of oxygen. For example souring of milk is caused by anaerobic bacteria that might have gained entry during processing and storage.

2.7.2. Chemical change

Increase microbial activity and load on food substance results in degradation of the food product (Jay, 2002). This occurs when the microorganism hydrolyse complex molecules into simpler compounds which serves as a source of nutrients for their survival. Most microorganisms prefer carbohydrates to other compounds such as protein as an energy source since they are more readily utilized for energy. Utilization of carbohydrate by microorganisms results in a variety of end products (Jay, 2002) such as alcohols and organic acids.

2.8. Foodborne Pathogens

Contaminated food and water are known to be sources of illnesses in human societies since antiquity (Campbell, 2011) and the CDC (2010) estimates that there are 31 pathogens known to cause foodborne illness in the United States of America. The problem of foodborne diseases is severe in developing countries due to difficulties in securing optimal hygienic food handling practices. In such developing countries, 70% cases of diarrhoeal diseases are associated with the consumption of contaminated food (Zeru and Kumie, 2007).

Food borne diseases (FBD) are defined by the World Health Organization as “diseases of infectious or toxic nature caused by or thought to be caused by consumption of food or water”

(Noosorn, 2009; Prescott *et al*., 2002). Symptoms vary widely depending on the etiological agents. Diarrhoea and vomiting are the most common effect (Negga *et al*., 2005; Le Loir *et al* 2003).

Among food borne diseases (FBDs), food borne infections are caused by many different disease causing pathogens that can contaminate food while food borne intoxication is caused by poisonous chemicals or other harmful substances that are present in food. Bacteria are the leading cause of FBD and appear to be the causative agents of more than two-thirds of the recorded foodborne outbreaks (Negga *et al*., 2005; Noosorn, 2009).

2.8.1. Classification and etiology of food borne diseases

Food borne diseases are classified into two major categories, that is, foodborne intoxication and foodborne infections (Le Loir et al, 2003) depending on the causative agents.

Table 1: Etiologies of some food borne infections and foods commonly involved Etiologic Diseases Causative organisms Foods Commonly involved Category

Bacterial	Typhoid Fever	*Salmonella typhi*	Raw vegetables and fruits, salads, unpasteurized milk and milk products, meat and water
	Paratyphoid Fever	*Salmonella paratyhi*	
	Shigellosis	*Shigella* species	All foods handled by unhygienic workers, potato or egg salad, lettuce, raw vegetables
	Cholera	*Vibrio Cholerae*	Fruits and vegetables washed with contaminated water
	Non-typhoid salmonellosis	*Salmonella* species e.g. Salmonella typhimurium	Eggs, poultry. undercooked meats, unpasteurized dairy products, seafoods, sausages
	Brucellosis	*Brucella* Species mostly *Brucella meliterisis*	Milk and dairy products from infected animals
	Anthrax	*Bacillus anthracis*	Contaminated raw and undercooked meat
	Bovine T B	*M. Bovis*	Unpasteurized milk or dairy products from T B cows, meat
	E. coli infections	*E. coli*	Beef, dairy products or raw produce (potato, lettuce, sprouts, fallen apples) salads
	Listeriosis	*Listeria monocytogens*	Milk, cheese, ice cream, poultry, red meat
2 Viral	Viral G E	Rota virus, Norwalk virus, Calici virus, astro virus	Any food of daily use with poor hygiene
	Viral hepatitis	Hepatitis A and E	Raw shellfish from polluted water, sandwich, salad and desserts
	Poliomyelitis	Polio virus	Any food of daily use with poor hygiene
	Rift valley fever	Rift valley fever virus	Any food contaminated with blood or aerosols from infected domestic animals or their abortuses
3 Parasitic	Taeniasis	*Taenia* Species	Raw beef, raw pork
	Amoebiasis	*Entomeba hystolitica*	Any food soiled with faeces
	Trichinosis	*Trichinella spirals*	Insufficiently cooked pork and pork products

Helmuth	Ascariasis	*Ascaris lumbricoids*	Foods contaminated with soil especially foods that are eaten raw such as salads and vegetables

	Glardiasis	*Glardia lanblia*	Any contaminated food item

Table 1 continued: Etiologies of some food borne infections and foods commonly involved

	Toxoplasmosis	*Toxoplasma gondii*	Raw or undercooked meat and any food contaminated with cat faeces
	Cryptosporidiosis	*Cryptospondium parvum*	Apple juice, any contaminated food item
	Hydated disease	*Echinococcus granulosus*	Any food contaminated with dog faeces
	Diphylobothriasis	*Diphylobothrium latum*	Raw or undercooked fish
	Trichuriasis	*Trichuris trichuria*	Any food contaminated with soil
4 Fungal	Fungal Infections	*Aspergillus* *Penicillium* Yeasts	Cereal, grains, flour, bread, cornmeal, popcorn, peanut butter, apples and apple products, moldy supermarket foods, cheese, dried meats, refrigerated and freezer pastries.

2.9. Prevention and Control of Food Borne Diseases

Regardless of the specific cause, the prevention and control of foodborne diseases are based on the same principles. Intervention of the source of infection may include;

Thorough cooking of raw food

Cooking or heating processed foods to at least the recommended temperature

Storing or holding foods at temperatures of below 4.4 ^{0}C or above 60 ^{0}C during preparation and holding for service

Reheating left-over food quickly to an internal temperature of at least 73.6 ^{0}C within two hours

Thoroughly washing raw vegetables with clean water

Keeping uncooked animal products far separate from cooked and ready to eat foods

Avoiding raw milk or foods made from raw milk

Appropriate treatment of food items before consumption

Using extreme care in storing and handling food prepared and ready to eat foods through hands, equipment and utensils, cleaning and sanitizing every food contact surfaces and equipment after every use to avoid cross contamination

Inspection of food

Washing hands, knives, cutting boards and utensils after coming into contact with uncooked food

Training and supervision of food handlers

Treatment of infections such as skin and throat in food handlers

2.9.1. Environment intervention

Control of flies, rats and cockroaches

Education on environmental and personal cleanliness

Maintenance of sanitary food area,

Proper handling and storage of left-over foods

Kitchen cleanliness

Careful storage and use of chemicals

2.9.2. Sanitation in the food industry

Sanitation within the food industry means, adequate treatment of food contact surfaces by a process that is effective in destroying vegetative cells of microorganisms of public health significance and in substantially reducing numbers of other undesirable microorganisms, but without adversely affecting the food or its safety for the consumer (U.S. FDA). Consumers prefer to select hotels considering the sanitation condition of the environment since this influence their choice. Cleanliness is therefore important to the protection of human health and therefore considered as an applied science. When sanitation is practiced properly, it can improve the aesthetic qualities and hygienic conditions of food service operations. Foods can be contaminated with spoilage microorganisms or those that cause food borne illness if proper sanitary practices are not followed. Most outbreaks of foodborne disease and illness are as a result of poor sanitary practices and food hygiene. There has been a lot of food safety issues and outbreak of foodborne illness most of it focusing on poor sanitary practices in the food sectors as shown in Table 2.

Table 2: Food safety incidents of major concern

AGENT	FOOD	EFFECT
S. enteritis	Ice-cream	224,000 ill
E. coli O157: H7	Hamburgers	732,000 ill, 4 deaths
Benzene	Mineral water	Worldwide recall of 160 million bottles
L. monocytogenes	Hot dogs	101 ill, 21 deaths

Source: Mariott and Gravani, (2006)

2.9.3. Use of sanitary equipment in the food preparation areas

In the food industry, the term sanitary equipment means equipment that is fully cleanable using clean-in-place (CIP) and sterilization-in-place (SIP) procedures that is fully drainable from cleaning solutions and other liquids. The design should have a minimum amount of deadleg or areas where the turbulence during cleaning is insufficient to remove food product deposits. To improve cleanability, this equipment should be made from stainless steel to reduce the possibility of bacterial adhesion (CODEX ALIMENTARIOUS, 2009).

2.10. The Benefits of Effective Food Sanitation

Food borne illness can be minimized if not controlled in food operations when proper sanitation measures are implemented. Effective sanitation programme helps to reduce lethal microbial population and this improve the quality of food products and increase the shelflife. Clean establishment as a result of improved working conditions result in fewer customer complaint as well as satisfied and delightful customers. Reduced public health risk increased trust of regulatory agencies and improves the morale of employees (Mariott and Gravani, 2006).

2.11. Hazards Related to Food

Any substance found in foods that have the potential to cause harm, injury or illness is a food safety hazard (Alli, 2004). Contaminant found in food products could be physical, chemical and biological and these are hazard that are capable of causing food poisoning and/or food borne disease (Purnomo, 2006). Poor food preparation, improper handling and storage are among the major cause of outbreaks found in the restaurants.

2.11.1. Biological or Microbiological hazard

Physical and chemical contaminants do not change the food itself but are potential hazards when consumed with the food. When undesired changes occur in the food itself, the food is considered to be spoilt and this is caused by biological hazard. Biological hazards include disease-causing microorganisms, certain plants and fish that carry toxins (poisonous). It can be referred to as microbial contaminants, microorganisms or pathogens. A biological hazard when found in food, may be very hard to kill or control. Bacteria and the toxins they produce may not have an odour or taste to help detect them. Bacteria can be a silent killer in foods. Some bacteria produce spores, thick-walled, protective structures that allow bacteria to survive cooking, freezing temperatures and some sanitizing mixtures.

Microorganisms grow when they have the right nutrients and conditions for growth. Although microorganisms release toxins onto food, processing involving heat may wipe out the pathogen but not the toxin which has been released already into the food (Purnomo, 2006; ISO, 2010). *Salmonella* spp, *Eschericha coli* O157:H7, *Lysteria monocytogenes*, *Clostridium perfringens*, *Clostridium botulinum*, *Staphylococcus aureus* and *Campylobacter jejeuni* are known to be the causative pathogens of food poisoning. The foods that are commonly involved in these food poisoning incidents include meat and poultry products, seafood and seafood products, egg and egg products, milk and dairy products, fruits and vegetable products, low-acid canned foods and water (Alli, 2004).

2.11.1.1. Viruses

Foods can be the medium for transmission of certain viruses. Examples of viruses that are known to be food safety hazards are the hepatitis A and E viruses, the Norwalk group of viruses, and rotavirus (Alli, 2004).

2.11.1.2. Parasites

A number of human parasites can be carried by food substances. The most frequent human parasites include parasitic protozoan species (e.g. *Entamoeba histolytica, Giardia lambia, Cryptosporidium parvum*) and parasitic worms (*Ascaris lumbricoides, Taenia solium, Trichinella spiralis*) (Alali, 2004). Love *et al*. (2002) in their study accounted an outbreak of acute gastroenteritis caused by parasite among groups of guest and hotel employees in Virginia. A similar observation was made by Florez *et al*. (2005) where *Shigella sonnei* was found to be the major causative organism that caused gastroenteritis outbreaks in both developing and industrialized countries. Hardie *et al*. (1999) in their study confirmed cases with diarrhoea being the infectious outbreak as occurred in Greece. Ingestion contaminated food has been a major reason of morbidity and a very important cause of death all over the world.

2.11.2. Chemical hazard

The FDA and the USDA have recognized the wide variety of chemicals used in food processing and have decided what chemicals are acceptable additives in food products and which chemical substances are strictly forbidden. Any substance used on crops must undergo thorough testing to see how effective it is. Foods are examined for residues by conducting tests to determine whether residues pose a health hazard. Naturally occurring chemicals which cause a health risk in food are mostly toxicants. Some of these poisonous substances do exist in the food itself and can directly cause illness after ingestion, for example, histamine found in seafood.

Some natural foods also contain slow acting toxicants, where if consumed over a period of time could potentially raise chronic diseases (Potter and Hotchkiss, 1995; O'Keefe and

Kennedy, 1998). Chemicals are divided into two primary categories; the prohibited substances and the unavoidable poisonous or deleterious substances. Unavoidable poisonous or deleterious substances have FDA tolerance levels or action levels. Products that fall into these categories include pesticides, herbicides, rodenticides, growth hormones and antibiotics, additives and processing aids, lubricants, paints, cleaners and sanitizers (Purnomo, 2006; Roberts, 2001). Substances that cannot be used or added directly to human food include; calamus (the dried rhizome of acorus), Cinnamyl anthranilate (C_{16} H_{16} NO_2) and Coumarine 1, 2 benzopyrone and they are prohibited because of toxicity to the liver.

2.11.3 Physical hazard

Physical hazard are substances that become part of a food mixture. They may not change or damage the food itself. However, their presence can create health hazards for the consumer. For instance, metal filings or broken pieces of glass have occasionally gotten into foods. These materials would not spoil food, but they could cause injury if swallowed. In addition, some physical hazards, depending on their size, shape, and texture, have the potential to cause choking if swallowed (Alli, 2004; Department of Health (DOH), 2010).

Frequent cause of physical hazard may be accidental and/or due to unacceptable food handling practices by food workers (DOH, 2010). Some of the common physical substances or hazards found in foods include tiny pebbles sometimes found in rice, beans sand peas. Potter and Hotchkiss (1995) found glass fragments, cuts of wood, stones, small piece of metal such as paper clip and other filth to be physical hazards that are mostly seen in food.

2.12. Hazard Analysis and Critical Control Point (HACCP) in Food Safety

HACCP is an internationally recognized food safety assurance system that concentrates on prevention strategies on known hazards with focuses on process control, and the steps within

that (Kirby, 1994; Worsfold and Griffth, 2003). According to Mariott and Gravani, (2006) HACCP programme is a preventive approach to ensure coherent safe food production. It establishes procedures where these hazards are reduced or eliminated and requires documentation of these control procedures (Codex, 1997).

The HACCP system is based on a universally recognized set of seven principles that are used for the development of an HACCP plan for a food. The principles reflect a framework that was developed on the basis of a combination of recognized, science-based, food safety considerations and quality system characteristics. This integration of basic food safety principles with the quality systems approach has been an important factor in the widespread recognition of the HACCP principles by food quality professionals. The universally recognized seven principles of HACCP are:

Principle 1:

Conduct hazard analysis

Identify hazards associated with a specific menu item

Prepare a flow diagram that outlines all handling/preparation steps from receiving to service

List likely hazards associated with each step

Identify how to prevent the hazards at each step

Hazards can be biological, chemical, or physical

List the hazards that are likely to occur and that will cause severe consequences if not controlled

Hazards that are low risk and not likely do not need to be considered

Principle 2:

Determine critical control points, that is, identify the critical points where control measures need be put in place to prevent, reduce or eliminate hazard.

Principle 3:

Establish critical limits, that is, to determine the anticipated hazard and the measures or the actions that are needed to prevent, reduce or eliminate the hazards.

Principle 4:

Establish monitoring procedures, that is, to monitor what is being done at each critical control point.

Principle 5:

Establish corrective action procedures

Corrective actions focus on what to do when a food does not meet the critical limit

Example of a corrective action:

The temperature of a hamburger is 140^{o}F (50^{o}C) after cooking (a CCP)

The critical limit is cooking the hamburger to 155^{o}F (68^{o}C) or hotter

Continue cooking the hamburger until it is 155^{o}F (68^{o}C) or hotter

Throwing out food might be a corrective action

Maintain records of all corrective actions taken

Principle 6:

Establish verification procedures, that is, to review the system to ensure that it is working, that hazards are identified, corrective actions are taken and that a safe food product is produced

Principle 7:

Establish record-keeping and documentation procedures

This is to ensure review of the HACCP where necessary (Alli, 2004)

2.13. Hygienic Practices for Food Handlers in the Hotel Industry

Mariott and Gravani (2006) emphasized that there must be an established protocol to ensure that employees adhere to and understand hygienic practices. There should be provision of amenities for upkeep of hygiene by providing healthful dressing rooms and wellbeing facilities. Management need to ensure that employees have gone through physical

examination to ensure good physical, mental, and emotional health. Food handlers having skin disease or infections need to be identified, quarantine and treated before they handle food to avoid any contamination.

Foakett and Ceserani (2007) also added that food handlers suffering from any form of illness need to report to the employer for the necessary action to be taken to protect food from the employee's illness or disease. During work-shift, proper hand washing and sanitizing need to be adhere to especially after using the toilet, handling garbage or other soiled materials, egg products, or dairy products. Also, hands must be washed thoroughly before handling food after handling money, smoking, coughing or sneezing. Food handlers who violate the established food safety practices need to be query thereby ensuring high standard sanitary practices (D.O.H. 2010).

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. MATERIALS

3.1.1. Location of study area

The study was conducted on five hotels in the Kumasi Metropolis in the Ashanti region of Ghana. Kumasi is the capital of the Ashanti Region of Ghana and the second largest city in Ghana with 1.5 million inhabitants (Ghana Statistical Service, 2010).

3.1.2. Ethical consideration

The data was collected after a written informed consent was obtained from all the study participants (Managers of the Hotels) and the study approved by the Committee for Human Research and Ethics (CHRE) at the School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana (Appendix A).

3.1.3. Sampling and data collection

A pre tested semi-structured questionnaire (Appendix B) was prepared for the first part of the study which was carried out from September 2012 to December 2012. A total of ten hotels with regular patronage viz., 3 ‘three star’, 3 ‘two-star’, 2 ‘one-star’ and 2 ‘budget hotels’ were selected from the study area using random sampling technique (Table 3). Five of these hotels were later selected for the second part of the study. Consent of the managers of the selected hotels was sought after explaining the nature of the study and confidentiality also assured.

A total of forty structured questionnaires with options for further responses were distributed to the cooks, chefs and their assistants since they were in direct contact with cooking of the food. The questions covered the demographic information of respondents, knowledge of food

hygiene and safety practices and kitchen sanitation. Staff members were given two weeks after which answered questionnaire were collected.

3.1.4. Sample collection

Samples of cooked food were aseptically collected in two batches from five hotels, only between 12:00 midday and 1:00 pm, each day with four foods in each batch. The foods were collected in the restaurant at the point of serving. The cooked food samples were kept in sterile stomacher bags (Sharpe and Jackson, 2000) and immediately stored inside an ice chest with ice packs while transporting them to the laboratory for analysis within two hours of collection. The foods collected were those that were available for the day and samples were examined the same day.

Table 3: Selected Hotels in Kumasi and the Star rating used in Phase-1 of study

Name of hotel	Star rating
Hotel-01	Three Star
Hotel-02	Three Star
Hotel-03	Three Star
Hotel-04	Two Star
Hotel-05	Two Star
Hotel-06	Two Star
Hotel-07	One Star
Hotel-08	One Star
Hotel-09	Budget
Hotel-010	Budget

Table 4. Selected Hotels and Food samples collected for Phase-2 of study

HOTEL	FOOD SAMPLE
01(***)	Chicken and vegetable sauce
(Three star)	Fresh pepper sauce
	Boiled plain rice
	Tossed mixed vegetables
	Beef sauce
	Fufu
	Goat light soup
	Boiled plain rice
02 (***)	Coleslaw
(Three star)	Fried rice
	Beef in vegetable sauce
	Potato chips
	Jollof rice
	Braised rice
	Chicken with noodles and vegetable
	Grilled steak
03(**)	Beef sauce
(Two star)	Tomato sauce
	Boiled plain rice
	Tossed mixed vegetables
	Tossed salad
	Fish light soup
	Fried fish
	Fried rice
04 (*)	Boiled plain rice
(One star)	Mixed salad
	Fried rice
	Fried chicken
	Jollof rice
	Vegetable sauce
	Beef sauce
	Fried rice
05 (B)	Goat light soup

(Budget)	Fried chicken
	Boiled plain rice
	Vegetable sauce
	Vegetable sauce
	Fried rice
	Boiled fish
	Jollof rice

3.2. METHOD

3.2.1. Determination of microbial counts on food samples

Microbial count on the collected food samples from the hotel kitchens were analyzed based on the method described by Herrera (2002) at the bacteriology laboratory of the Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR) at the Kwame Nkrumah University of Science and Technology, Kumasi. The samples were assessed for total coliform counts, total aerobic mesophilic counts (TAMC), total yeast and mould counts.

Pathogenic microbes such as *S. aureus*, *Salmonella* spp. and *E. coli* were also determined.

3.2.1.1. Homogenization of food samples

Ten grams of food was sampled and weighed into a stomacher bag as representative of the whole sample. Ninety milliliters of sterilized bacteriological peptone water (Oxoid LP0037 USA) was added to the sample, making a dilution of 1:10. This was then agitated vigorously for 2 minutes. Fifty milliliter of the liquid was transferred, after foam has dispersed, with a sterile pipette into a sterile 50 ml centrifuge tube.

3.2.1.2. Serial dilutions

One millilitre (1000 µl) of the sample from the 50 ml (10^{-1}) was pipetted into a separate tube containing 9 ml of peptone water to prepare a 1:100 dilution (10^{-2}). The liquids were carefully mixed by aspirating 10 times with a sterile pipette and again transferred with the same pipette 1.0 ml to another dilution tube containing 9 ml of dilution fluid and mixed with a fresh pipette

to obtain a 10^{-3} dilution. The above procedure was repeated to obtain a final dilution of 10^{-6}. Each successive dilution decreased the concentration 10-fold.

3.2.1.3. Spread plating of samples

For each of the dilutions, 0.1ml (100 µl), was transferred to the surfaces of the following agar; MacConkey Agar, Plate Count Agar, Violet Red Bile Glucose Agar, and Malt Extract Agar in triplicate using separate pipette for each dilution. The dilution (0.1 ml) was promptly spread on the surface of the agar plates using sterile glass spreader (Drigalsky spatulas) (Herrera, 2002). A separate spreader was used for each plate and the surface of each plate was allowed to dry for 15 min. The plates were then incubated in an inverted position for 72 h at 30 ^{0}C for bacteria and the plates containing the yeasts and moulds were incubated in a covered box at room temperature for between 5-7 days but not inverted.

3.2.1.4. Enumeration of microorganisms

Inoculated plates for containing the bacteria were removed from incubator after seventy two hours (72 h) for microbial colony counting. All colonies in the triplicate plates corresponding to one dilution and showing between 20-200 colonies were counted using a colony counter. Averages of the replicates were calculated and multiplied by their dilution factor as shown in appendix 1. This was then reported as the colony forming unit per gram.

Overcrowded colonies were not counted and were reported as “Too Numerous To Count” (TNTC) while colonies less than 20 were reported as “Too Few To Count” (TFTC). Inoculated plates for moulds and yeasts were counted after 3-5 days of incubation. The colonies were stored in the refrigerator for further analysis.

3.2.2. Inoculation of slopes

A flamed sterile inoculating pin was used to transfer the sub cultured colony in Eppendorf tubes by stabbing into the butt first and then withdrawn and streaking the slope in a zig-zag pattern. Tubes were then incubated for 24 h and then refrigerated until needed.

3.2.3. Gram staining of cultures

A sterile toothpick was used to transfer a colony to a slide. A drop of physiological saline was added to emulsify the colony using a sterile wire loop and to make a thin smear. The slides were left to air-dry and then further drying was done using a gentle heat of the flame of a Benson burner to properly fix the smear on the slides, as well as preserve microorganisms and prevent smears from being washed from the slides. Smears were allowed to cool before staining.

The fixed smear was covered with crystal violet stain for 60 sec and rapidly washed off with clean tap water. The smear was covered again with Lugol's iodine for 60 sec and washed off with clean tap water. Acid alcohol (Acetone) was used for only 10 sec to decolourize the stain and immediately washed off with clean tap water. The smear was covered again with neutral red stain (safranin) for 2 min and washed off with clean tap water. The slides were placed in a draining rack for the smear to air dry. The smears were examined microscopically with oil immersion objective.

3.2.4. Determination of microbiological safety of the cooking environment

Petri dishes containing nutrient agar were placed at the corners of the kitchen and restaurant of the various hotels to determine the microbial levels in the environments. Petri dishes were half opened for a period of one hour, covered and sent to the laboratory. They were incubated for 72 h at 30 ^{0}C.

3.2.5. Biochemical identification of microbes

3.2.5.1. Identification of bacterial colonies

Sub-culturing was done to provide pure colonies for identification on Nutrient agar. Petri dishes containing nutrient agar was dried on the surface in an incubator for 30-40 min at 35-37 ^{0}C (Cheesebrough, 2002). A flamed sterile loop was used to transfer a colony to a small area of the plate and then streaked in a zig-zag pattern. Plates were incubated immediately at 35-37 ^{0}C for 24 hours.

Isolates on nutrient agar were streaked on MacConkey agar (Fluka 71043) and incubated for 24 hours at 37 ^{0}C for the identification of lactose fermentors (LF) and non lactose fermentors (NLF). For the LF reddish or pinkish was expected and orange for the NLF. Colonies were inoculated onto Triple Sugar Iron Agar by stabbing the butt of the medium then streaking the surface of the agar slant. The tubes were then incubated for 24 hours at 37 ^{0}C. The fermented lactose produced acid which turned phenol red both in the Butt and in the Slant yellow. The organisms that generated gases also produced bubbles and cracks on the TSI. The Butt may also turn yellow when the lactose did not ferment. If on the slant, acid was oxidised to carbon dioxide and water by the organism then the slant turned red. Simmons citrate test was also conducted to identify Gram negative pathogens. Single isolated colonies were picked and lightly streaked on the surfaces of the slant of Simmons Citrate Agar (CMO 155 Oxoid). This was incubated at 35 ^{0}C for 24 hours. Only bacteria that could utilize citrate as the sole carbon and energy source were expected to grow on the Simmons citrate medium. The citrate positive growth on the slant surface, change from the original green colour to an intense Prussian blue colour. Oxidase test was conducted by smearing a colony of the microbe on a commercially prepared oxidase strip impregnated with NNNN'tetramethyl –phenyline-diamine dyhydrochloride for the detection of bacteria cytochrone oxidase enzyme. The result

was read within 5-10 seconds. A deep blue/violet colour indicated a positive reaction while a negative test showed delayed reacton or no colour change within 5-10 seconds.

In the Indole test, 1-2 drops of a commercially prepared indole reagent was dispensed into a cotton bud. With the hole of an applicator stick, an 18-24 hour old isolate was smeared and the cotton bud observed for colour development within 10-30 seconds. A positive test shows the development of a violet to purple colour within 10-30 seconds while a negative test showed a developed reaction of no colour development within 10-30 seconds. API 20NE TEST (bioMerieux Inc. Durham, NC.) was also used in the identification of the microbes. Using a loop, an isolated colony was picked and added to 2 ml saline. This was emulsified by stirring vigorously to a homogenous suspension. API tray was labelled and 5 ml of sterile distilled water was added to the tray to produce a moist atmosphere to prevent drying of the strips. The API strip was then placed in the incubation tray. The prepared suspension was inoculated into the first 8 capsules using a Pasteur pipette. Again 4 drops of the saline suspension was added to an API AUX and mixed well taking care not to add bubbles. This was then used to inoculate the remaining capsules. Mineral oil was overlaid on the wells and incubated at 35 ^{0}C for 24 hours. When the capsule is opaque then there is bacteria growth and the reaction was recorded on API result form.

3.2.5.2. Identification of the fungal colonies

Sub-culturing was done to provide pure colonies for identification on MEA and PDA. Slides of fungal cultures were prepared by gently lifting the mycelial mat with a sterile inoculation pin into a drop of lactophenol blue on a slide, teased, covered with a slip. This was observed under microscope. Different characteristic features of the isolated fungi were observed and used in their identification (Moss, 1998; Samson *et al*., B 2004).

3.2.6. Methods used for the personal observation studies of food safety practices

Personal observations (Appendix C) were conducted based on the results of the questionnaire administered to food handlers in some selected hotels in Kumasi. Information on the new harmonized standards for catering and hotel establishment in Ghana by the Ghana Standards Authority (2003) was also incorporated as a guideline. The hotels selected for the study were two "three-star" hotels, one "two-star" hotel, one "one-star" hotel and one budget hotel. A briefing visit was completed prior to the observation being conducted. Each hotel was visited at a mutually agreed time. The aims and objectives of the observational study were discussed with the manager. The food handlers were assured that any information gathered would be kept in the strictest confidence and also the name of the hotel would remain anonymous. Subsequent observation visit was made to each hotel during their busiest times that is, from 10 a.m. to 2 p.m. from Sunday to Friday which was the usual lunchtime preparation and cooking.

To ensure that the observer's presence may not affect the behaviour of those being observed, known as the reactivity or the Hawthorn effect (Roethlisburger and Dickson 1939), nothing was observed during the first half hour in order to allow food handlers time to acclimatize to the presence of the researcher. Secondly, protective clothing similar to those of the food handlers in each hotel was worn in an attempt to blend in with the surroundings (Breakwell *et al*., 2000).

3.3. Statistical Analysis

Statistical Package for the Social Sciences (SPSS version 17) was used to analyze survey data using descriptive analysis such as mean, frequencies, percentages and presented using tables, bar and pie charts. One way Analysis of Variance (ANOVA) was performed and

significant difference was determined at 95 % confidence level. Also qualitative analysis of data such as Chi Square Test was conducted for the questionnaire.

CHAPTER FOUR

4.0. RESULTS

4.1. Questionnaire

The first part of the chapter is the results of the questionnaire which include the demographic information of respondents, knowledge of food hygiene and safety practices and kitchen sanitation.

4.1.1. Demographic information of respondents

In the study, the dominance of women in the preparation of food in the hotels is shown in Figure 1. The gender of respondents was 38.5 % for males and 56.4 % with 5.1% not answering.

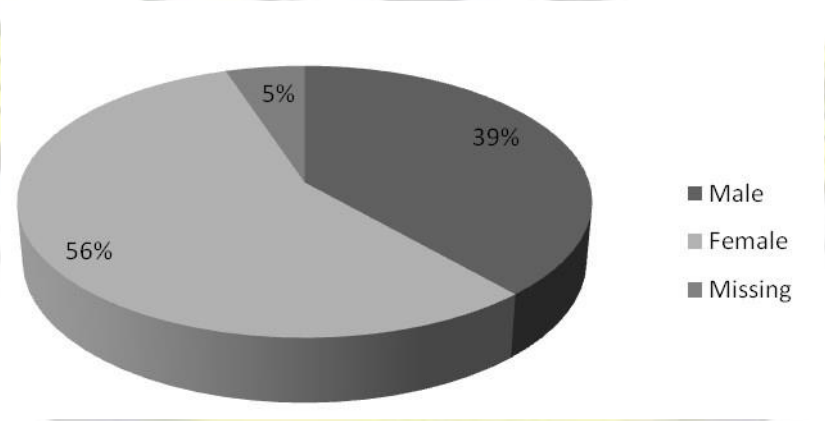

Figure1: Gender distribution of respondents (n=39)

The age range was between 15-44 years (Table 5) with majority of the respondents (35.9 %), being in the age range of 25 to 29 years while 20.5 % were between 20 and 24 years. Those in the age group ranging from 30 to 34 formed 15.4 % whilst those between 35 and 39 years formed 12.8 % of respondents. A reasonable level of education was recorded for the respondents. Table 5 indicates that 35.9 % of respondents attained vocational education, 17.9 % had senior high school education, 10.3 % had middle school education, and 5.1 % attained junior secondary school and/or primary school.

With regards to professional qualification, 23.1% of the respondents had ordinary certificate in catering while 17.9% had National Vocational Training Institute certificate. Higher National Diploma Certificate holders formed 15.4% of the respondents whilst 10.3% had Degree in Catering and Food and Beverage service. Only 2.6% respondent had 812/2 certificate. Those without certificate were 17.9% whilst only one 2.6 % had a different certificate.

Table 5. Demographic information of respondents

Variable	Frequency	%
Age (years)		
15-19	1	2.6
20-24	8	20.5
25-29	14	35.9
30-34	6	15.4
35-39	5	12.8
40-44	3	7.7
Missing (Did not answer)	2	5.1
Total	**39**	**100**
Education level		
Tertiary	8	20.5
Vocational	14	35.9
Senior secondary	7	17.9
Junior secondary	2	5.1
Primary	2	5.1
Middle school	4	10.3
Illiterate	1	2.6
Missing (Did not answer)	1	2.6
Total	**39**	**100**
Professional qualification		
Degree in catering and food and beverage service	4	10.3
HND certificate	6	15.4
Ordinary certificate in catering	9	23.1
812/2 certificate	1	2.6
NVTI certificate	7	17.9
No certificate	7	17.9
Others	1	2.6
Missing (Did not answer)	4	10.3
Total	**39**	**100.0**

4.1.2. Food safety knowledge and practices

The result in Table 6 indicates that 92.3% of respondents had knowledge about food poisoning and only 2.6% (1) had not heard about food poisoning. There were a few respondents, representing 7.7 %, who did not know that microorganisms could be found in refrigerated foods. Respondents who knew that microorganisms can grow on refrigerated foods represented 89.7 % (Table 6). In terms of hand washing, 87.3 % confirmed that when you touch something during food preparation the hands should be washed while 10.3 % responded that the hands should be washed only twice during food preparation. In terms of what needs to be used in hand wiping, most of the respondents (71.8 %) in the kitchen responded that they wiped their hands with kitchen napkins. Respondents who used paper towel were 15.4 % while 10.3 % used terry towel. It is noted in Table 6 that almost all the cooks in the kitchens had medical certificate (87.2 %) with only 7.7% who did not have medical certificate.

Table 6: Food Safety Knowledge and Practices

Knowledge about food poisoning	Frequency	%
Yes	36	92.3
No	1	2.6
Missing (Did not answer)	2	5.1
Total	**39**	**100.0**
Knowledge of microorganisms found in Refrigerated foods		
Yes	35	89.7
No	3	7.7
Missing (Did not answer)	1	2.6
Total	**39**	**100.0**
Number of times hands should be washed during food preparation		
Twice	4	10.3
Three times	1	2.6
When you touch something different from what is being cooked	34	87.2
Total	**39**	**100.0**
Items used to wipe hands after hands washing in the kitchen		
Terry towel	4	10.3
Paper towel	6	15.4
Kitchen napkin	28	71.8
Missing (Did not answer)	1	2.6
Total	**39**	**100.0**
Possession of medical certificate		
Yes	34	87.2
No	3	7.7
Missing (Did not answer)	2	5.1
Total	**39**	**100**

4.1.3. Sanitation knowledge and practices of respondents

Fly proof doors were common in the hotel kitchens that were used in the study. Almost all the kitchens (94.9 %) had fly proof doors installed and this is to prevent flies from fallen into food (Table 7).

Table 7: Respondent's knowledge on kitchen sanitation

Fly-proof doors for kitchen	**Frequency**	**%**
Yes	37	94.9
No	1	2.6
Missing (Did not answer)	1	2.6
Total	**39**	**100.0**
Disinfecting work surfaces		
Yes	38	97.4
No	1	2.6
Total	**39**	**100.0**

Contaminated food preparation surfaces, are just a few of the places where microorganisms can enter food. Almost all the respondents (97.4 %) disinfected their work surfaces regularly (Table 7).

The findings as presented in Figure 2 indicate that over half of respondents (66.7 %) swept the kitchen morning, afternoon and evening. Others (15 %) swept at different times and the rest (12.8 %) morning and evening.

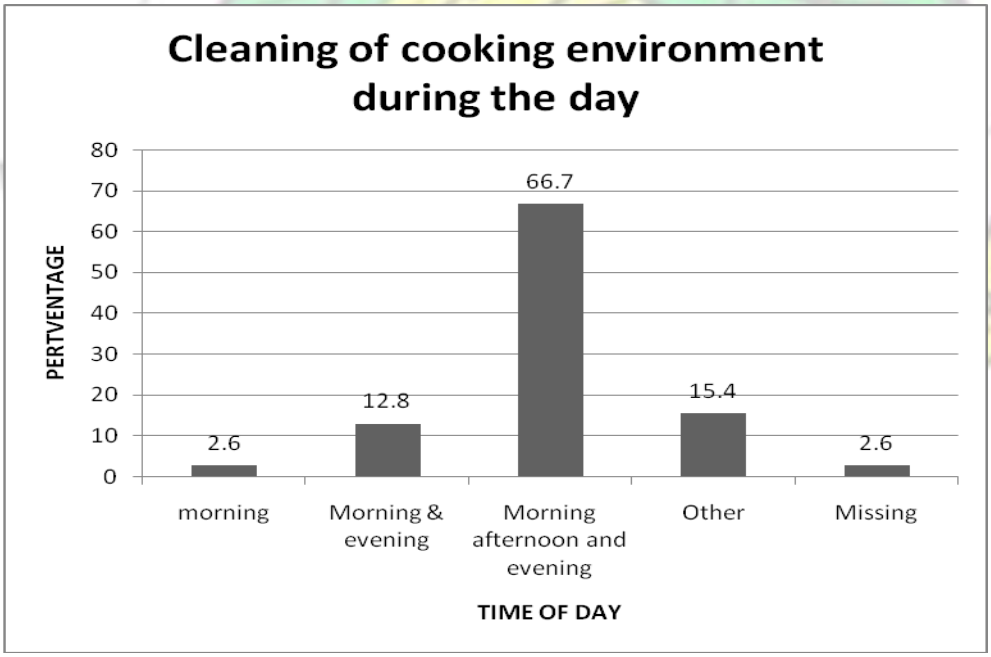

Figure 2. Time of the day the kitchen environment is kept clean

4.2. Fungi Enumerated

4.2.1. Colony count of fungi on foods from Hotel-01

From Table 8, the microbial count observed ranged from 5.0 x 10^1 to 1.0 x 10^4 cfu/g. The food with the highest colony count of 1.0 x 10^4 was chicken and vegetable sauce (shredded onion, green pepper and chicken fried in vegetable oil). The food with no observable growth was *fufu* (boiled plantain or cocoyam mixed with cassava and pounded). Chicken and vegetable sauce (1.0 x 10^4) was above the WHO acceptable limits for cooked foods (< 3.0 Log 10 cfu/g).

Table 8. Colony Counts on Foods from Hotel-01

Food	LOAD (cfu/g)	Log (cfu/g)
Fufu	NOG	- Boiled
plain rice	$5.0x10^1$	1.7
Beef sauce	$1.3x10^2$	2.0
Goat light soup	$2.3x10^2$	2.4
Tossed mixed vegetable	$2.7x10^2$	2.4
Fresh pepper sauce	$1.7x10^3$	3.2
Chicken and vegetable sauce	$1.0x10^4$	4.0

NOG = No Observable Growth

Plate 1: Sample of fungal growth observed on fresh pepper sauce from Hotel-01

4.2.2. Colony Count of Fungal Growth on Foods from Hotel-02

From Table 9, colony count ranged from 1.7 x 10^2 to 2.3 x 10^4 cfu/g. The food with the highest colony count was coleslaw (shredded cabbage and carrots mixed with mayonnaise sauce) with 2.3 x 10^4 cfu/g of food. Chicken with noodles and vegetables (cooked pasta mixed with chicken pieces and carrots and fried) and jollof rice (rice cooked in tomato sauce) had the same colony count of 1.0 x 10^3 cfu/g. The food with the least count was braised rice (fried onions mixed with rice and boiled) with growth of 1.7 x 10^2 cfu/g. All the foods in Hotel-02 had fungal loads that were above the WHO acceptable limits of < 3.0 Log 10 cfu/g except braised rice with count of 1.7 x 10^2 and grilled steak (fillet of beef brushed with salt, pepper and grilled) with count of 2.3 x 10^2 cfu/g.

Table 9: Colony counts on foods from Hotel-02

Food	LOAD (cfu/g)	Log (cfu/g)
Braised rice	$1.7x10^2$	2.2
Grilled steak	$2.3x10^2$	2.4
Chicken with noodles	$1.0x10^3$	3.0
Jollof rice	$1.0x10^3$	3.0
Fried rice	$5.3x10^3$	3.7
Potato chips	$5.7x10^3$	3.8
Beef in vegetable sauce	$6.7x10^3$	3.8
Coleslaw	$2.3x10^4$	4.4

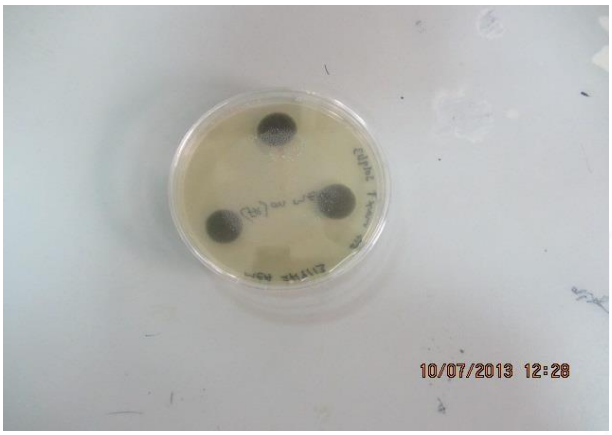

Plate 2: Sample of fungal growth observed on coleslaw from Hotel-02

4.2.3. Colony count of fungal growth on foods from Hotel-03

Colony counts on foods in Table 10 ranged from 1.0 x 10^2 cfu/g to 2.3 x 10^5 cfu/g. Tossed salad (lettuce, cucumber and onion tossed in oil and vinegar) recorded the highest fungal growth of 2.3 x 10^5 with fried fish recording growth of 1.7 x 10^2 whilst boiled plain rice had the least colony count of 1.0 x 10^3 cfu/g. The food with no observable growth of fungi was beef sauce. Fried rice (cooked plain rice mixed with fried onion, cabbage and soy sauce) and tomato sauce (blended tomatoes, onion and chilli pepper blended together and fried in oil) had the same colony counts of 1.0 x 10^2 cfu/g. The colony count recorded on fried fish was 1.7 x 10^2 cfu/g whilst that on boiled plain rice was 1.0 x 10^3 cfu/g. Fungal counts on boiled plain rice, fish light soup, tossed mixed vegetables and tossed salads were above the WHO acceptable limits of < 3.0 Log 10 cfu/g.

Table 10. Colony Counts on Foods from Hotel-03

Food	LOAD (cfu/g)	Log (cfu/g)
Beef sauce	NOG	- Tomato sauce
	$1.0x10^2$	2.0
Fried rice	$1.0x10^2$	2.0
Fried fish	$1.7x10^2$	2.2
Boiled plain rice	$1.0x10^3$	3.0
Fish light soup	$1.7x10^3$	3.2
Tossed mixed vegetable	$2.0x10^3$	3.3
Tossed salad	$2.3x10^5$	5.4

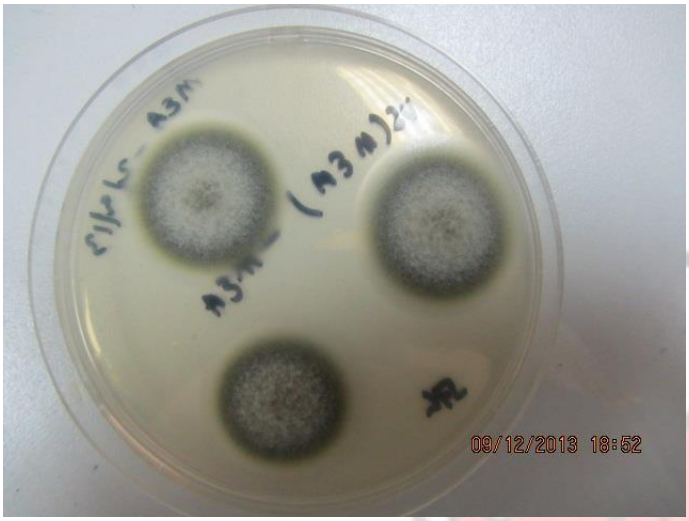

Plate 3: Sample of fungal growth observed on tossed salad from Hotel 03

4.2.4. Colony Count of Fungal Growth on Foods from Hotel-04

From Table 11, microbial count ranged from 1.7 x 10^2 cfu/g to 1.1 x 10^4 cfu/g. The food with the highest colony count was fried rice with a count of 1.1 x 10^4 cfu/g. Beef sauce recorded the least colony count of 1.7 x 10^2. There was no observable growth of fungi in jollof rice. The foods above the acceptable limits were vegetable sauce with a count of 1.1 x 10^3 cfu/g, mixed salad with 2.3 x 10^3 cfu/g, fried chicken with 1.3 x 10^3 cfu/g and fried rice with 1.1 x 10^4 cfu/g.

Table 11. Colony Counts on Foods from Hotel-04

Food	LOAD (cfu/g)	Log (cfu/g)
Jollof rice	NOG	- Beef
sauce	1.7x10^2	2.2
Boiled plain rice	3.3x10^2	2.5
Vegetable sauce	1.1x10^3	3.0
Fried chicken	1.3x10^3	3.1
Mixed salad	2.3x10^3	3.4
Fried rice	1.1x10^4	4.0

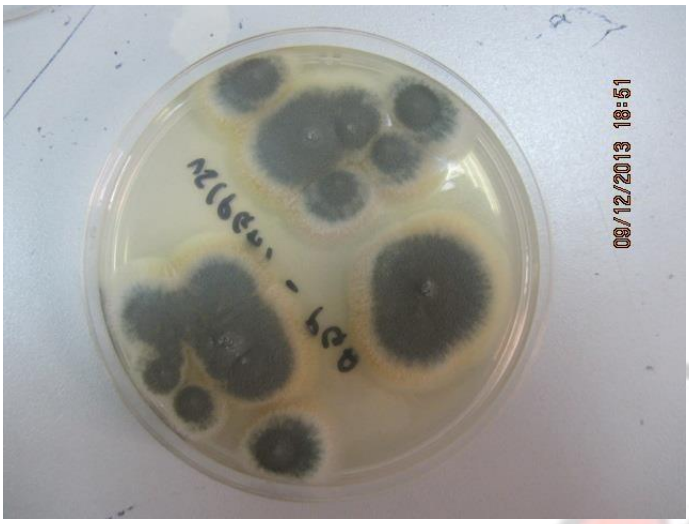

Plate 4: Sample of fungal growth observed on fried rice from Hotel 04

4.2.5. Colony count of fungal growth on foods from Hotel 05

Microbial count ranged from 1.3×10^2 to 1.3×10^6 cfu/g (Table 12). The food with the highest count was mixed vegetable salad with a count of 1.3×10^6 cfu/g. Boiled fish had the least colony count of 1.3×10^2 cfu/g with counts on foods such as goat light soup (1.0×10^3), fried chicken (1.0×10^4), fried rice (1.7×10^5) and mixed vegetable salad (1.3×10^6) exceeding the acceptable limit for food.

Table 12. Colony Count on Foods from Hotel-05

Food	LOAD (cfu/g)	Log (cfu/g)
Boiled fish	$1.3x10^2$	2.1
Jollof rice	$1.7x10^2$	2.2
Boiled plain rice	$2.0x10^2$	2.3
Vegetable sauce	$6.7x10^2$	2.8
Goat light soup	$1.0x10^3$	3.0
Fried chicken	$1.0x10^4$	4.0
Fried rice	$1.7x10^5$	5.2
Mixed vegetable salad	$1.3x10^6$	6.1

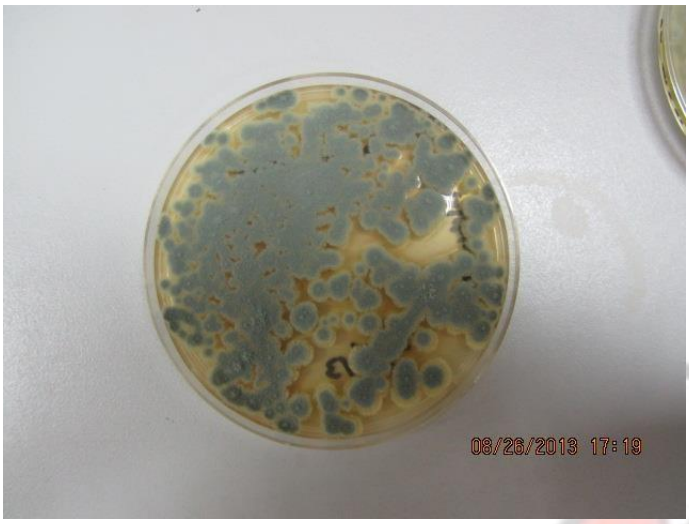

Plate 5: Sample of fungal growth observed on mixed vegetable salad from Hotel 05

4.2.6. Comparison of Fungal Counts on Common Foods from the sampled Hotels The common foods appeared in a maximum of four hotels as shown in Table13. Boiled plain rice was served in Hotels-01, 03, 04, and 05 with colony counts of 5.0×10^{1}, 1.0×10^{-3}, 3.3×10^{2} and 2.0×10^{2} cfu/g respectively. The highest count for boiled plain rice was 1.0×10^{-3} with the least count being 5.0×10^{1}. Fried rice was found in Hotels-02, 03, 04 and 05, with colony counts of 5.3×10^{3}, 1.0×10^{2}, 1.1×10^{4} and 1.7×10^{5} cfu/g, respectively. Salad served at Hotel-02 was 2.3×10^{4} cfu/g, that for Hotels-03 was 2.3×10^{5}, for Hotel-03 was 2.3×10^{3}, for Hotel-04, it was 1.3×10^{6} for 05. Jollof rice run through three Hotels and showing 1.0×10^{3} for 03, No observable growth on Hotel-04 while 1.7×10^{2} was recorded for Hotel-05.

Table 13. Colony Count for Fungal Growth on some Common Foods

FOOD	Hotel	LOAD (cfu/g)	Log (cfu/g)
BOILED PLAIN RICE	01	$5.0x10^{1}$	1.7
	03	$1.0x10^{3}$	3.0
	04	$3.3x10^{2}$	2.5
	05	$2.0x10^{2}$	2.3
FRIED RICE	02	$5.3x10^{3}$	3.7
	03	$1.0x10^{2}$	2.0
	04	$1.1x10^{4}$	4.0
	05	$1.7x10^{5}$	5.2
SALAD	02	$2.3x10^{4}$	4.4
	03	$2.3x10^{5}$	5.4
	04	$2.3x10^{3}$	3.4
	05	$1.3x10^{6}$	6.1
JOLLOF RICE	02	$1.0x10^{3}$	3.0
	04	NOG	NOG
	05	$1.7x10^{2}$	2.2

4.2.7. Identified fungi in the food samples

All the food examined showed presences of fungi contamination except those with no observable growth. Although some of the foods were within the acceptable limit, they depicted presence of fungi as presented in Table 14a and b. Most of the foods had more than one fungi being identified.

Table 14a: Isolated fungi on the various hotel foods

Fungal isolates	Food items							
	Beef sauce	Tossed salad	Tossed mixed veg.	Chicken & veg. sauce	Boiled plain rice	Fufu	Boiled fish	Fried chicken
Alternaria alternate	X							
Aureobasidium pullulans	X				X			
Aspergillus tamaric	X							
Candida albicans							X	
Cladosporium herbarum	X		X	X	X		X	X
Emericella nidulans	X							
Eurotium amsteloclami		X	X					
Eurotium herbariorum	X			X	X	X		
Fusarium oxysporum			X		X			
Gliocladium deliquescens	X						X	
Neurospora monilia/sitophila	X							
Penicillium citrinum	X							
Penicillium commune	X			X				
Penicillium verrucosum					X			
Penicillium viriclicatum	X							
Scopulariopsis candida	X							

X = fungi presence

Table 14b: Isolated fungi on the various hotel foods

Fungal isolate	Food items								
	Fresh pepper Sauce	Veg. sauce	Mixed veg. sauce	Fish light soup	Chicken with noodules & veg.	Goat light soup	Light soup	Kitche area	Restaurant area
Alternaria alternate								X	
Aspergillus niger		X	X						X
Aureobasidium pullulans				X		X			
Botrytis cinerea						X			
Cladosporium herbarum		X			X	X	X		
Emericella nidulans								X	
Eurotium chevalien	X								
Fusoma rubricosa							X		
Penicillium commune					X			X	
Penicillium polonicum				X					
Rhizoctonia solani							X		
Scopulariopsis candida				X					

X = fungi presence

4.3. Enumerated Bacteria on the Cooked Food Samples

4.3.1. Colony count of bacteria on foods from Hotel-01

From Table 15, the Total Mesophilic Count (TMC) ranged from 2.0 log_{10} cfu/g to 6.7 log_{10} cfu/g while Total Coliform Count (TCC) ranged from 2.0 log_{10} cfu/g to 7.0 log_{10} cfu/g. Total Enterobacteriaceae Count (TEC) ranged from 2.0 Log_{10} cfu/g to 6.4 Log_{10} cfu/g. The foods with the highest TMC were fresh pepper sauce (6.7 Log_{10} cfu/g) and fufu (6.7 Log_{10} cfu/g), that of TCC was fufu (7.0 Log_{10} cfu/g) while the highest TEC was *fufu* (6.4 Log_{10} cfu/g).

Table 15. Bacterial counts on foods from Hotel-01

FOOD	COLONY COUNT (cfu/g)	Log	TMC	TCC	Log	TEC	Log
Chicken and vegetable sauce	$1.0x10^2$	2.0	NOG	-	NOG	-	
Beef sauce	$2.7x10^2$	2.4	$1.0x10^2$	2.0	$1.0x10^2$	2.0	
Goat light soup	$2.7x10^4$	4.4	$7.3x10^3$	3.9	$5.4x10^3$	3.7	
Tossed mixed vegetable	$3.3x10^4$	4.5	$1.7x10^4$	4.2	$3.7x10^2$	2.6	
Boiled plain rice	$1.1x10^5$	5.0	$6.7x10^4$	4.8	$1.0x10^4$	4.0	
Fresh pepper sauce	$4.5x10^6$	6.7	$1.3x10^4$	4.1	$1.7x10^4$	4.2	
Fufu	$5.5x10^6$	6.7	$1.1x10^7$	7.0	$2.3x10^6$	6.4	

TMC = Total Mesophilic Count; TCC = Total Coliform Count; TEC = Total Enterobactriaceae Count; NOG = No Observable Growth

4.3.2. Colony Count of Bacteria on Foods from Hotel-02

Total Mesophilic Count (Table 16) ranged from 4.6 log_{10} cfu/g to 7.0 Log_{10} cfu/g while TCC was between 4.6 log_{10} cfu/g and 6.8 log_{10} cfu/g. The lowest count of TEC was 4.0 log_{10} cfu/g and the highest was 6.7 log_{10} cfu/g. The foods with the highest TMC were braised rice and chicken with noodles and vegetables while that of TCC were braised rice, grilled steak and coleslaw.

Braised rice also recorded the highest TEC.

Table 16. Bacterial counts on foods from Hotel-02

FOOD	COLONY COUNT (cfu/g) TMC	Log	TCC	Log	TEC	Log
Fried rice	$3.7x10^4$	4.6	$2.2x10^4$	4.3	$9.3x10^3$	3.0
Potato chips	$4.5x10^5$	5.7	$5.7x10^6$	6.8	$1.7x10^5$	5.2
Jollof rice	$4.4x10^6$	6.6	$2.6x10^6$	6.4	$4.2x10^6$	6.6
Coleslaw	$4.7x10^6$	6.7	$4.6x10^6$	6.7	$4.2x10^6$	6.6
Grilled steak	$5.6x10^6$	6.7	$5.5x10^6$	6.7	$1.2x10^6$	6.1
Beef in vegetable sauce	$6.2x10^6$	6.8	$4.7x10^6$	6.8	$6.9x10^5$	5.8
Chicken with noodles and vegetables	$9.3x10^6$	6.9	$2.0x10^6$	6.3	$3.7x10^4$	4.6
Braised rice	$9.6x10^6$	6.0	$5.4x10^6$	6.7	$5.1x10^6$	6.7

TMC = Total Mesophilic Count; TCC = Total Coliform Count; TEC = Total Enterobactriaceae Count; NOG = No Observable Growth

4.3.3. Colony Count of Bacteria on Foods from Hotel-03

The TMC (Table 17) ranged from 3.5 log_{10} cfu/g to 4.6 log_{10} cfu/g while that of TCC ranged from 2.4 log_{10} cfu/g to 4.5 Log_{10} cfu/g. The TEC, on the other hand, ranged from 3.0 Log_{10} cfu/g to 4.2 Log_{10} cfu/g. Beef sauce recorded the highest TMC and TCC with no TEC observed. TEC were also not observed in tossed mixed vegetables and tomato sauce.

Table 17. Bacterial counts on foods from Hotel-03

Food	COLONY COUNT (cfu/g)					
	TMC	Log	TCC	Log	TEC	Log
Tossed salad	TNTC	-	TNTC	-	TNTC	-
Fried rice	TNTC	-	TNTC	-	TNTC	-
Boiled plain rice	$3.0x10^3$	3.5	$2.7x10^2$	2.4	NOG	-
Fish light soup	$3.7x10^3$	3.6	$8.0x10^2$	2.9	$1.0x10^3$	3.0
Fried fish	$4.3x10^3$	3.6	$1.7x10^3$	3.2	$1.7x10^4$	4.2
Tossed mixed vegetable	$1.0x10^4$	4.0	$2.0x10^3$	3.3	NOG	-
Tomato sauce	$2.3x10^4$	4.4	$1.3x10^4$	4.1	NOG	-
Beef sauce	$4.3x10^4$	4.6	$3.0x10^4$	4.5	NOG	-

TMC = Total Mesophilic Count; TCC = Total Coliform Count; TEC = Total Enterobactriaceae Count; NOG = No Observable Growth; TNTC = Too Numerous To Count

4.3.4. Colony count of bacteria on foods from Hotel-04

Total Mesophilic count (TMC), TCC and TEC ranged from 3.0 log_{10} cfu/g to 6.5 log_{10} cfu/g, 3.1 log_{10} cfu/g to 6.4 log_{10} cfu/g and 3.1 log_{10} cfu/g to 6.3 log_{10} cfu/g respectively. Boiled plain rice recorded the highest TMC and TCC while mixed salad recorded the highest TEC. Jollof rice had no observable TCC and TEC. Vegetable sauce and beef sauce also had no observable growth of TEC (Table 18). In this 1 star hotel, TMC in their vegetable sauce was 3.0 log_{10} while TCC was 3.1 log_{10} with no observable growth of TEC.

Table 18. Bacterial counts on foods from Hotel-04

Food	COLONY COUNT (cfu/g) TMC	Log	TCC	Log	TEC	Log
Vegetable sauce	$1.0x10^3$	3.0	$1.4x10^3$	3.1	NOG	-
Jollof rice	$4.7x10^3$	3.7	NOG	-	NOG	-
Fried rice	$2.4x10^4$	4.4	$1.8x10^4$	4.3	$1.4x10^3$	3.1
Beef sauce	$2.7x10^5$	5.4	$2.6x10^3$	3.4	NOG	-
Fried chicken	$1.3x10^6$	6.1	$1.0x10^5$	5.0	$7.0x10^4$	4.8
Mixed salad	$2.8x10^6$	6.4	$8.7x10^5$	5.9	$1.8x10^6$	6.3
Boiled plain rice	$3.3x10^6$	6.5	$2.6x10^6$	6.4	$7.3x10^5$	5.9

TMC = Total Mesophilic Count; TCC = Total Coliform Count; TEC = Total Enterobactriaceae Count; NOG = No Observable Growth

4.3.5. Colony count of bacteria on foods from Hotel-05

From Table 19, it is observed that the TMC ranged from 3.2 Log_{10} cfu/g to 6.9 Log_{10} cfu/g, while the TCC was between 3.1 Log_{10} cfu/g and 7.2 Log_{10} cfu/g. In the case of TEC, it ranged from 2.1 Log_{10} cfu/g to 6.8 Log_{10} cfu/g. Vegetable sauce had the highest counts of TMC, TCC and TEC. There were no observable growths of Mesophiles and Enterobacteriaceae on jollof rice.

Table 19. Bacterial counts on foods from Hotel-05

Food	COLONY COUNT (cfu/g)	Log	TMC	TCC	Log	TEC	Log
Jollof rice	$1.7x10^3$	3.2	NOG	-		NOG	-
Boiled fish	$5.3x10^4$	4.7	$1.2x10^3$	3.1		$1.3x10^2$	2.1
Fried chicken	$1.6x10^5$	5.2	$2.6x10^3$	3.4		$1.3x10^3$	3.2
Mixed vegetable salad	$2.3x10^5$	5.4	$2.7x10^6$	6.4		$2.3x10^5$	5.4
Boiled plain rice	$6.2x10^5$	5.8	$2.7x10^4$	4.4		$5.9x10^5$	5.8
Fried rice	$7.0x10^5$	5.8	$4.0x10^4$	4.6		$4.7x10^3$	3.7
Goat light soup	$2.9x10^6$	6.5	$1.7x10^4$	4.2		$4.1x10^5$	5.6
Vegetable sauce	$8.2x10^6$	6.9	$1.5x10^7$	7.2		$5.9x10^6$	6.8

TMC = Total Mesophilic Count; TCC = Total Coliform Count; TEC = Total Enterobactriaceae Count; NOG = No Observable Growth

4.3.6. Colony counts on common foods from the five hotels

Table 20 shows some common foods prepared by some of the hotels. Boiled plain rice was recorded in four of the Hotels. Hotel-01 had 5.0 Log_{10} cfu/g of TMC, 2.8 Log_{10} cfu/g of TCC and 4.0 Log_{10} cfu/g of TEC. In Hotel 03, boiled plain rice recorded TMC of 3.5 Log_{10} cfu/g, 2.4 Log_{10} cfu/g of TCC with no observable growth of Enterobacteriaceae. Hotel 04 had 6.5 Log_{10} cfu/g as the TMC, 6.4 4 Log_{10} cfu/g as the TCC and 5.9 Log_{10} cfu/g as the TEC while Hotel-05 had 5.8 Log_{10} cfu/g as the TMC, 4.4 Log_{10} cfu/g as the TCC, and 5.9 Log_{10} cfu/g as the TEC. Fried rice was also obtained from four of the five hotels and as shown on the Table 19, Hotel-02 had 4.6 Log_{10} cfu/g as the TMC, 4.3 Log_{10} cfu/g as the TCC and 4.0 Log_{10} cfu/g as the TEC. For Hotel-03, the Mesophiles, Coliforms and the Enterobacteriaceae were too numerous to count (TNTC), whilst Hotel-04 recorded 4.4 Log_{10} cfu/g as the TMC, 4.3 Log_{10} cfu/g as the TCC and 3.1 Log_{10} cfu/g as the TEC. In the case of Hotel-5, count on fried rice was 5.8 Log_{10} cfu/g for TMC, 4.6 Log_{10} cfu/g for TCC and 3.71 Log_{10} cfu/g for TEC. The colony counts on salad ranged between 6.6 Log_{10} cfu/g and 6.7 Log_{10} cfu/g for TMC, TCC and TEC for Hotel-02 while colony counts were all too numerous to count (TNTC) for Hotel-03. Hotel-04 had 6.4 Log_{10} cfu/g as the

TMC, 5.9 Log_{10} cfu/g as the TCC and 6.3 Log_{10} cfu/g as the TEC. In the case of Hotel-05, salad had 5.4 Log_{10} cfu/g as the TMC, 6.4 Log_{10} cfu/g as the TCC and 5.4 Log_{10} cfu/g as the TEC. Jollof rice was obtained from three of the hotels. Hotel-02 had 6.6 Log_{10} cfu/g, 6.4 Log_{10} cfu/g and 6.6 Log_{10} cfu/g as the TMC, TCC and TEC respectively. For Hotel-04, 3.7 Log_{10} cfu/g was recorded as the TMC, while there was no observable growth (NOG) of Mesophiles and Enterobacteriaceae. The colony count for Hotel-05 was 3.2 Log_{10} cfu/g for TMC while there were no observable TCC and TEC recorded.

Table 20. Colony counts on some common foods from the five Hotels

HOTEL	FOOD	TMC(Log_{10})	TCC(Log_{10})	TEC(Log_{10})
01	Boiled plain rice	5.0	2.8	4.0
03		3.5	2.4	NOG
04		6.5	6.4	5.9
05		5.8	4.4	5.8
02	Fried rice	4.6	4.3	4.0
03		TNTC	TNTC	TNTC
04		4.4	4.3	3.1
05		5.8	4.6	3.7
02	Salad	6.7	6.7	6.6
03		TNTC	TNTC	TNTC
04		6.4	5.9	6.3
05		5.4	6.4	5.4
02	Jollof rice	6.6	6.4	6.6
04		3.7	NOG	NOG
05		3.2	NOG	NOG

4.4. Identified Bacteria in Foods From the Five Hotels

4.4.1. Bacteria identified in foods from Hotel-01

Figure 3 depicts the percentages of the bacteria isolated whilst Table 21 shows their occurrence in foods from Hotel-01. Coagulase negative *Staphylococcus* (27.3 %) was identified in three of the

foods sampled namely; goat light soup, boiled plain rice and beef sauce whilst *Bacillus* spp (18.2 %) was also identified in two of the food samples; namely tossed mixed vegetables and fresh hot pepper sauce. The rest which occurred once were, *Enterobacter asburiae* (9.1 %) in goat light soup, *Staphylococcus aureus* (9.1 %), *Burkholderia cepaceae* (5.1 %), *Pseudomonas* spp (9.1 %) *Acinetobacter* spp (9.1%) and *Enterobacter cloaceae* (9.1 %) in *fufu.*

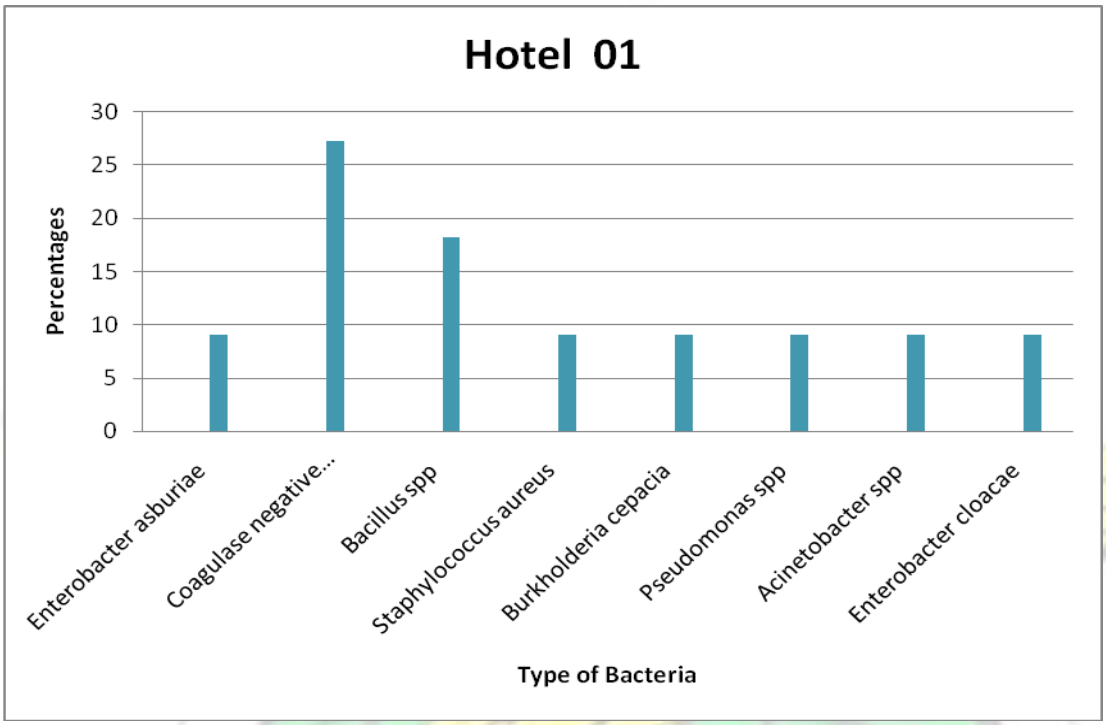

Figure 3. Percentage distribution of bacteria on foods from Hotel-01

Table 21. Bacteria identified on foods from Hotel-01

BACTERIA	FOOD
Enterobacter asburiae	Goat light soup
Coagulase negative Staphylococcus	Boiled plain rice Beef sauce Goat light soup
Bacillus spp.	Tossed mixed vegetables Fresh hot pepper sauce
Staphylococcus aureus	*Fufu*
Burkholderia cepacia	*Fufu*

Pseudomonas spp.	*Fufu*
Acinetobacter spp.	*Fufu*
Enterobacter cloacae	*Fufu*

4.4.2. Bacteria Identified in Foods from Hotel-02

Eleven pathogenic bacteria were isolated in food samples examined from Hotel-02. Grampositive rods (35.3 %) appeared six times in the following foods such as jollof rice, fried rice, chicken with noodles and vegetables, grilled steak, braised rice and coleslaw. *Klebsiella pneumoniae* (11.8 %) occurred twice: one in chicken with noodles and vegetables and the other in braised rice. *Escherichia coli* (5.9 %) *Raoutella planticola* (5.9 %), *Acinetobacter* spp. (5.9 %), *enterobacter gergoviae* (5.9 %), *Salmonella typhi* (5.9 %), *Enterobacter* spp. (5.9 %), *Burkholderia gladioli* (5.9 %), *Kluyvera* spp. (5.9 %), and *Bacillus* spp. (5.9 %) occurred once in the jolloff rice, fried rice, chicken with noodles and vegetables, grilled steak, braised rice, coleslaw and potato chips respectively (Figure 4 and Table 22).

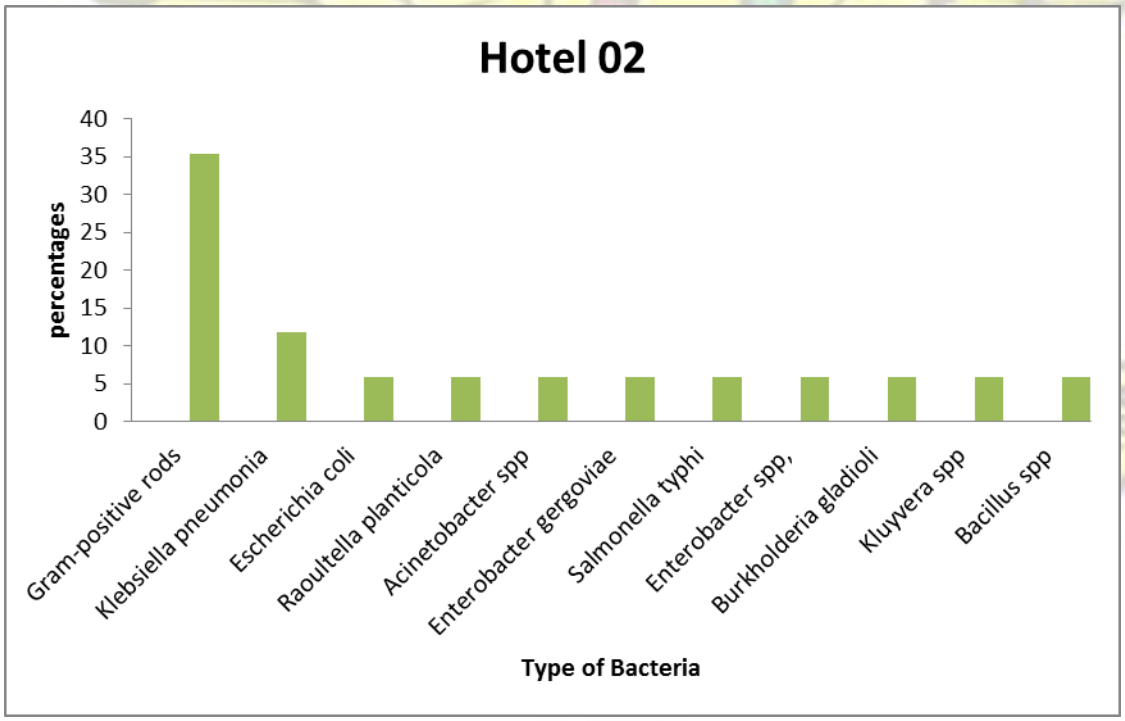

Figure 4. Percentage distribution of bacteria on foods from Hotel-02

Table 22. Bacteria identified on foods from Hotel-02

ISOLATE	FOODS
Gram-positive rods	Jollof rice Fried rice Chicken with noodles and vegetables Grilled steak Braised rice Coleslaw
Klebsiella pneumoniae	Chicken with noodles and vegetables Braised rice
Escherichia coli	Jollof rice
Raoultella planticola	Fried rice
Acinetobacter spp.	Chicken with noodles and vegetables
Enterobacter gergoviae	Chicken with noodles and vegetables
Salmonella typhi	Grilled steak
Enterobacter spp.	Chicken with noodles and vegetables
Burkholderia gladioli	Braised rice
Kluyvera spp.	Coleslaw Beef in vegetable sauce
Bacillus spp.	Potato chips

4.4.3. Bacteria identified in foods from Hotel-03

Figure 5 shows Gram-positive rods (100 %) as the only pathogenic bacteria isolated in foods from Hotel-03. Gram-positive rods appeared once in four different foods samples namely; fried rice, beef sauce, fried fish and tossed salad (Table 23)

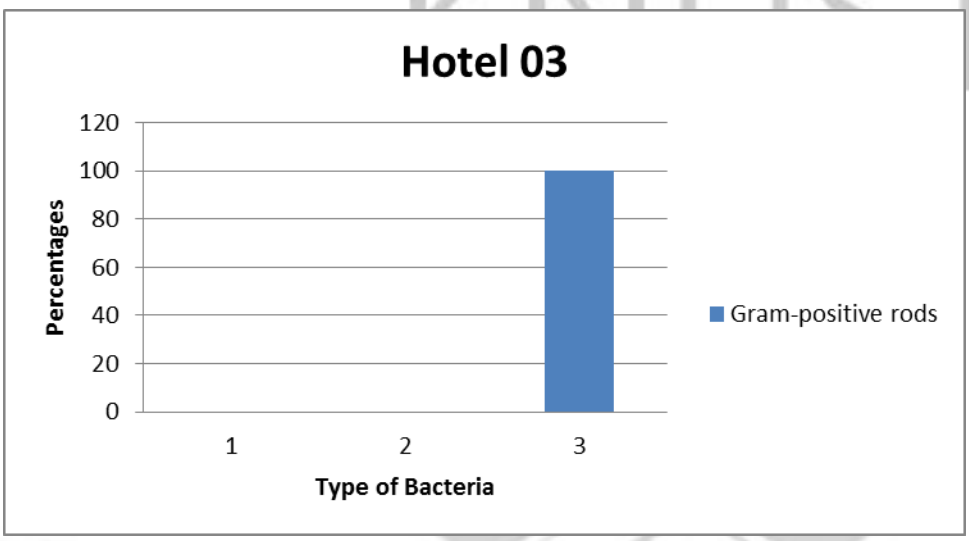

Figure 5. Percentage distribution of bacteria on foods from Hotel-03

Table 23. Bacteria identified on foods from Hotel-03

BACTERIA	FOODS
Gram-positive rods	Fried rice Beef sauce Fried fish Tossed salad

4.4.4. Bacteria identified in foods from Hotel-04

Eight pathogenic organisms were isolated from foods from Hotel-04 (Figure 6 and Table 24). *Acinetobacter* spp. (22.2 %) was identified in two different foods (mixed salad and beef sauce) whilst *Pseudomonas putida* (11.1%) and *Klebsiella pneumoniae* (11.1 %) appeared once in the salad, *Pseudomonas leuteola* (11.1 %), Coagulse negative *Staphylococcus* (11.1 %), as well as *Bacillus* spp. (11.1 %) also occurred once in jolloff rice. *Proteus spp* (11.1 %) and Gram-positive rods (11.1 %) were also isolated in fried rice and vegetable sauce respectively.

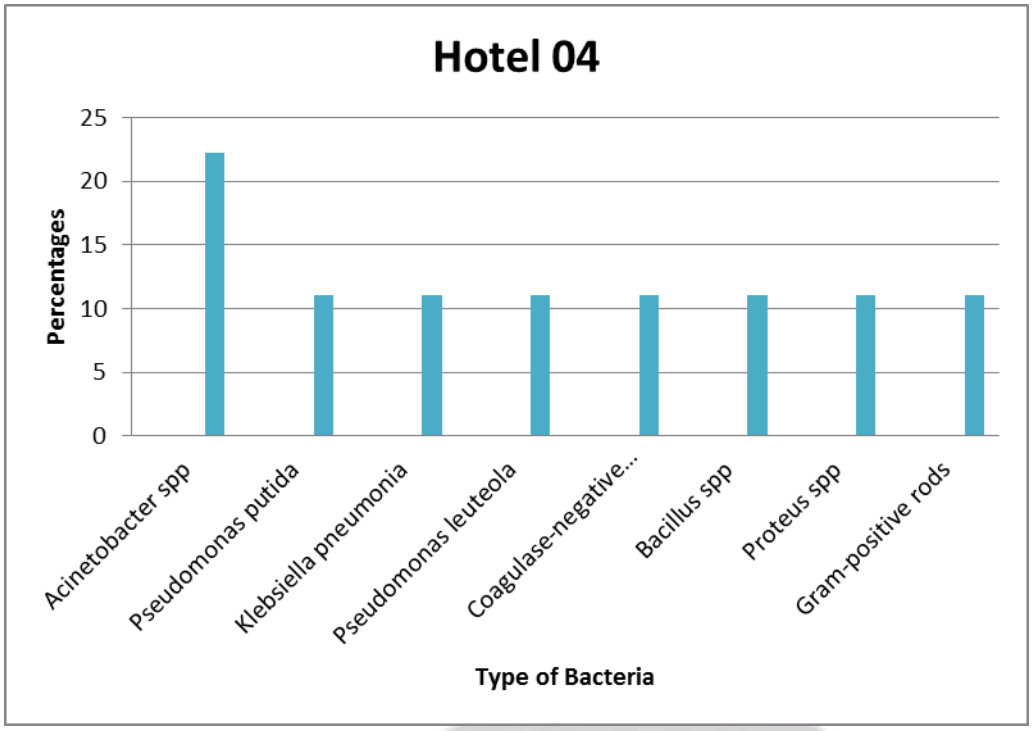

Figure 6. Percentage distribution of bacteria on foods from Hotel-04

Table 24. Bacteria identified on foods from Hotel-04

BACTERIA	FOODS
Acinetobacter spp	Mixed salad Beef sauce
Pseudomonas putida	Mixed salad
Klebsiella pneumoniae	Mixed salad
Pseudomonas leuteola	Jollof rice
Coagulase-negative staphylococcus	Jollof rice
Bacillus spp	Jollof rice
Proteus spp	Fried rice
Gram-positive rods	Vegetable sauce

4.4.5. Bacteria Identified in Foods from Hotel-05

From Figure 7 and Table 25, the bacteria which occurred most in the foods sampled from Hotel05 was *Klebsiella pneumoniae* (27.3 %) identified in jolloff rice, boiled plain rice and boiled fish. *Pseudomonas aeruginosa* (18.2 %) occurred twice; one in jolloff rice and the other in boiled fish. Coagulase-negative *Staphylococcus* (18.2 %) also occurred twice in jolloff rice and vegetable sauce. *Acinetobacter junii/johnsonii* (18.2 %), *Salmonella typhi* (18.2 %) *Pseudomonas putida* (18.2 %) and *Staphylococcus aureus* (18.2 %) occurred once in fried rice.

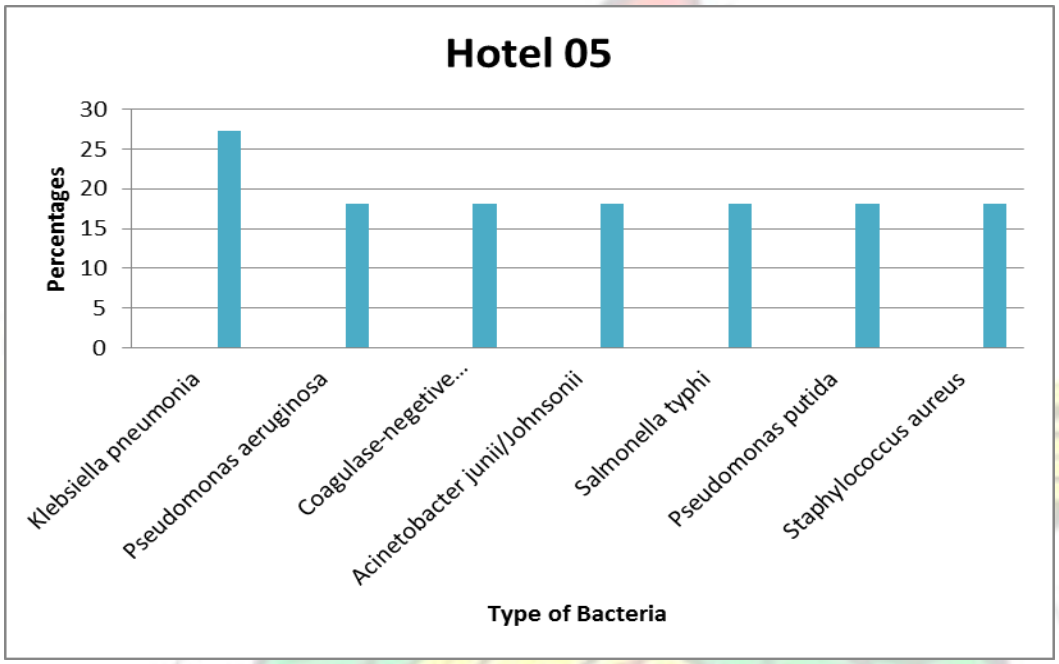

Figure 7. Percentage distribution of bacteria on foods from Hotel-05

Table 25. Bacteria identified on foods from Hotel-05

ISOLATE	FOODS
Klebsiella pneumoniae	Jollof rice Boiled plain rice Boiled fish
Pseudomonas aeruginosa	Jollof rice Boiled fish
Coagulase-negetive *Staphylococcus*	Vegetable sauce Jollof rice
Acinetobacter junii/Johnsonii	Fried rice
Salmonella typhi	Fried rice
Pseudomonas putida	Fried rice
Staphylococcus aureus	Fried rice

4.5. Microbiological Safety of the Kitchen and Restaurant Environment

4.5.1. Microbiological safety of the kitchen environment

Table 26 demonstrates the isolated microbes from the kitchen environment of the hotels under study. From Hotel-01, the microbes isolated were *Staphylococcus* and Gram-positive rods whilst *Staphylococcus* and Coagulase-negative *Staphylococcus* were isolated from Hotel-02. One organism each namely; Gram-positive rods and Coagulase-negative *Staphylococcus* were isolated from Hotel-03 and Hotel-04 respectively. The isolates obtained from Hotel-05 were Coagulase-negative *Staphylococcus* and Filamentous Gram-positive rods.

Table 26. Microbes identified from the selected hotel kitchens

HOTEL	ISOLATED ORGANISMS
01	*Staphylococcus* Gram-positive rods
02	*Staphylococcus* Coagulase-negative *Staphylococcus*
03	Gram-positive rods
04	Coagulase-negative *Staphylococcus*
05	Coagulase-negative *Staphylococcus* Filamentous Gram-positive rods

4.5.2. Microbiological Safety of the Restaurant Environment

Isolated microbes from Hotel-01 and Hotel-02 were Gram-positive rods and Coagulase-negative *Staphylococcus* whilst Gram-positive rods and *Staphylococcus* isolates were also prevalent in Hotel-03. Microbes isolated in Hotel-04 were Coagulase-negative *Staphylococcus* and *Acinetobacter* spp. from Hotel-05 as seen in Table 27.

Table 27. Isolated organisms from the selected hotel restaurants

HOTEL	ISOLATED MICROBE
01	Gram-positive rods
02	Coagulase-negative *Staphylococcus*
03	Gram-positive rods *Staphylococcus*
04	Coagulase-negative *Staphylococcus*
05	*Acinetobacter* spp.

4.6. Observed Microbiological Safety Measures at the Hotels

4.6.1. Microbiological safety measures observed at the Hotel-01

Safety measures observed at Hotel 01(Appendix C 1) indicate that the food workers wore protective uniforms and suitable footwear. Changing room facilities were provided as well as clean toilet and washing facilities. In terms of hand washing, cold water, ordinary soap and napkins were used. The hotel workers washed their hands before start of work, had adequate food preparation area, provided with separate chopping boards for different foods, had safe thawing of foods with

adequate stoves, grills, and ovens. The Hotel-01 also provided suitable storage facilities for cooked foods, safe cooking temperatures and left over foods were recovered for chilling or frozen. Chilled and ambient storage facilities, adequate frozen facilities, and pest control system were all in place with enough ventilation in the kitchen (Table 28b). It was also observed that the Kitchen building was suitable for catering activities. Hotel-01, however, did not provide basin for hand washing, warm water for hand washing, disposable paper towel for drying hands, hot air dryer for drying wet hands, roller towel for drying wet hands, nor basin and water. There were also no safe holding temperatures and all foods were not consumed within two
(2) hours.

4.6.2. Microbiological safety measures observed at the Hotel-02

In this hotel, food workers wore protective uniforms and suitable footwear (Appendix C 3 and 4). Changing room facilities were provided as well as clean toilet and washing facilities. In terms of hand washing, cold water, ordinary soap and napkins were provided for use by the kitchen staff. Hotel-02 also scored Yes for the following actions; washing of hands before start of work; adequate food preparation area; safe thawing of food; separate chopping boards for different foods; with adequate stoves, grills and ovens also provided. The hotel also scored Yes for suitable storage facilities for cooked foods; safe cooking temperatures; left over foods recovered for chilling or frozen and available chilled and ambient storage facilities. Safety measures such as adequate frozen facilities, food defrosted safely in hot kitchen, pest control system in place, enough ventilation in the kitchen and kitchen building suitable for catering activities were observed. On the other hand, safety measures such as the provision of sink for hand washing, hot water for hand washing, disposable paper towel for drying hands, hot air dryer for drying wet hands, roller towel for drying wet hands, washing hands in between work, and adequate basin and water were not

observed. There were also no safe holding temperatures and all foods were not consumed within 2 hours.

4.6.3. Microbiological safety measures observed at the Hotel-03

The results from Appendix C 5 and 6 indicate that food workers wore protective uniforms and suitable footwear. Changing room facilities were provided as well as clean toilet and washing facilities. In terms of hand washing, cold water, ordinary soap and napkins were used. Hotel-03 practiced washing of hands before start of work, had adequate food preparation area, safe thawing of food, separate chopping boards for different foods with adequate stoves, grills and ovens provided. The hotel also provided suitable storage facilities for cooked foods with safe cooking temperatures whilst left over foods were recovered for chilling or frozen. Chilled and ambient storage facilities were used. The hotel had adequate frozen facilities and foods were defrosted safely. Pest control system was also in place with enough ventilation in the kitchen and kitchen building suitable for catering activities. The hotel, however, had no provision of basin for hand washing, warm water for hand washing, disposable paper towel for drying hands, hot air dryer for drying wet hands and a roller towel for drying wet hand. Hands of staff were not washed in-between work, with adequate basin and water also not provided. Again, there were no safe holding temperatures and all foods were not consumed within 2 hours (Appendix C 6).

4.6.4. Microbiological safety measures observed at the Hotel-04

Results in Appendix C7 and 8 indicate that food workers at Hotel-04 wore protective uniforms and suitable footwear. Changing room facilities were provided as well as clean toilet and washing facilities. In terms of hand washing, cold water, ordinary soap and napkins were used. This hotel made sure that there was washing of hands before start of work, adequate food preparation area apart from the main kitchen, safe thawing of frozen foods in the hot kitchen, separate chopping boards for different foods and adequate stoves, grills and ovens. There were also suitable storage

facilities for cooked foods, and safe cooking temperatures. Left-over foods were recovered for chilling or freezing with available chilled and ambient storage facilities provided. The hotel also provided adequate frozen facilities and foods were defrosted safely. Pest control system was in place at the hotel and there was enough ventilation in the kitchen and the kitchen building was suitable for catering activities. The hotel did not provide basin for hand washing, hot water for hand washing, disposable paper towel for drying hands, hot air dryer for drying wet hands, roller towel for drying wet hands. Hands were not washed in-between work and there was no basin and water. Finally, the hotel did not provide safe holding temperatures and all foods were not consumed within 2 hours (Appendix C 8).

4.6.5. Microbiological safety measures observed at the Hotel-05

Food workers in Hotel-05 wore protective uniforms and suitable footwear (Appendix C 9). Changing room facilities were provided as well as clean toilet and washing facilities. In terms of hand washing, cold water, ordinary soap and napkins were provided and the workers washed their hands before start of work. There was adequate space for the preparation of raw foods, safe thawing of food, separate chopping boards for different foods and adequate stoves, grills and ovens. The hotel also provided suitable storage facilities for cooked foods with safe cooking temperatures. Left-over foods were recovered for chilling or frozen and available chilled and ambient storage facilities were also provided. Additionally, there were adequate frozen facilities and foods were defrosted safely. There were also pest control system in place, enough ventilation in the kitchen and the kitchen building was suitable for catering activities. In this hotel, however, was no provision of basin for hand washing, no warm water for hand washing, no disposable paper towel or hot air dryer for drying wet hands. Hands were not washed in-between work, and there was also no basin and water. Additionally, there were no safe holding temperatures to keep food hot for a period of time and all foods were not consumed within 2 hours (Appendix C 10)

CHAPTER FIVE

5.0. DISCUSSION

Customer satisfaction in hotels is actually based on the quality of hygiene of food, taste, kitchen and the dining experience as well as the affordability. Food handlers with infections can bring pathogens to the food preparation area and therefore it is important to know whether hotel staff members have any knowledge of food safety. Clayton and Griffith (2004) found the use of questionnaire as an appropriate tool to examine the knowledge, attitude and self-reported practices of food handlers as this provide general indication of the food safety practices undertaken by commercial food handlers.

In this study, the dominance of women in the preparation of food in the hotels is shown in Figure 1. This confirms works by Annor and Baiden (2011) as well as works by Tomlin *et al.* (2002), who concluded that food is mostly prepared by women in Ghana and that they do form the highest number in restaurant operations. Other studies conducted in Kumasi on street foods also showed that most of the food handlers were females (Ababio and Adi, 2012). In the Ghanaian culture, women form the majority of food preparers and this is evident in the hotels industries in Ghana, however, the head cooks (chefs) were mostly men with the women being their assistants. The ages were between 20 and 24 years representing 20.5% (8) (Table 1), described by Ababio and Adi (2012) as the active work group. Other studies done in some hotels in Accra, Ghana by Annor and Baiden (2011), also showed that majority of respondents were under thirty years. At this age, respondents are very young and are able to manage any work given to them. The result of the educational background (Table 4), however, is in contrast with the findings of Addo *et al*. (2007), who indicated that most food preparers in hotels in Accra had barely any formal education. The study is, however, in line with Tonder *et al*. (2007), who reported that 74 % of food handlers sampled in their study had some basic education.

The high number of respondents with professional qualifications (Table 4) could be due to the fact that, polytechnics and several vocational schools as well as some of the universities offer courses in cookery for the hotel industry at various levels. It is, therefore, not surprising to find a lot of professionally qualified food handlers with various categories of certificates working in hotels. It must be emphasized, however, that the fact that one is professionally qualified does not mean what is learnt is being put into practice because it has been found that 70 % of all bacterial food poisoning is caused by caterers with some form of education (Annor and Baiden, 2011).

Respondents' level of education may contribute to their knowledge of food poisoning which is important to the prevention of food borne illness in the hotel industry. The Centre for Disease Control in the USA estimates that food handler errors, for instance, contribute to about 97% of all cases of food-borne disease in food-service establishments (Clayton and Griffiths, 2004). In this study, majority of the respondents had professional certificates and food hygiene is likely to be found in their syllabus. It is presumed that they might have learnt about food poisoning in their various schools.

Responses to the number of times the hands should be washed were very encouraging. Chen *et al*. (2001) reported that proper hand washing has been recognized as one of the most effective measures to prevent cross-contamination and minimize transfer of microorganisms to ready-toeat foods in large-scale kitchens. Almost all people do not recognize that as part of the normal flora, one conveys a great deal of diverse disease causing microorganisms on the hands. It is estimated that approximately 40-50 % of all healthy humans carry the *Staphylococcus* bacterium. This normally happens when one uses the hands to blow the nose. About 60-70% of healthy humans carry *Clostridium perfringens,* which can also be easily transmitted onto foods with hands. Thus, workers should thoroughly wash their hands prior to contact with food and periodically during the time that contact is necessary (Mariott and Gravani, 2006; Vaclavik and

Christian, 2008). Wiping hands with kitchen napkin is not a good hygiene practice because bacteria can be transmitted from the napkin onto food after being used repeatedly. Probably, respondents in this study were not exposed to the correct hand wiping materials when they were in school and/or the managers in the study hotels might not have provided the appropriate hand wiping materials for the cooks because they may be costly.

Cesereni and Foskett (2007), maintain that after washing, hands should be cleaned and dried on a clean towel, suitable paper towel or by hot air dryer. Transmission of microorganisms is much more efficient as a result of using the same kitchen napkin repeatedly. The use of hands free towel dispenser for drying hands after washing has been more effective in reducing crosscontamination (Merry *et al*., 2001; Harrison *et al*., 2003).

Almost all the cooks (87.2 %) in this study had medical checkup certificates (Table 6) in contrast to studies carried out by Abera *et al.* (2010), on food handlers in Ethiopia, where none of them had medical checkup. Medical assessments are carried out to ensure that food handlers are fit to work in the environment. In a preliminary report on a study conducted by Zain and Naing (2002) on Socio-demographic characteristics of food handlers and their knowledge, attitude and practice towards food sanitation, 61.9 % had undergone medical examination which is a good food safety practice. Food handlers in the kitchens can easily transfer diseases they have onto food which can infect consumers. Even those with medical checkup certificates are advised to periodically go for review.

Flies are insects that harbour contaminant in their feeding activities as they feed on decayed animal and human wastes. They collect disease-causing microorganisms on their feet, mouth, gut and wings and transmit them to humans through food (Foskett and Ceserani, 2007). Mensah *et al*. (2002) also noted that the contamination from the adult housefly is a risk factor in the transmission of diarrhoea pathogens. Also *Salmonella typhimurium* and shigellae have been shown to multiply in the gut of the house fly (Vaclavik and Christian, 2008).

Contaminated food preparation surfaces, are just a few of the places where microorganisms can enter food. Mariott and Gravani (2006), have emphasised that work surfaces can collect microorganisms and other debris from the air, as well as from employees and materials in the kitchen. Furthermore, Chen *et al*. (2001), confirmed that during food handling and preparation, microorganisms on raw foods can be transferred to various surfaces, such as cutting boards and tap water handles. Work conducted by Addo *et al*. (2007), showed 70 % coliform contamination of work surfaces after cleaning. Thus, surfaces can be improved with hygienic designs by using materials such as stainless steel and marble especially in a hotel.

The respondents in this study understood the importance of sweeping to reduce the risk of contamination which is a sign of good hygiene practice. This is important because throughout food operation bacteria are capable of being transfer in the food preparation environment as the environment becomes more conducive for their growth. Relative humidity has been shown to be one of the significant determinants for bacteria endurance in the food preparation and processing environment. Bacteria are generally found to be more pronounced in low relative humidity than where the relative humidity is very high (Todd *et al*., 2009).

The fungal isolates identified in foods in the various hotels are shown in Table 14a and b. The hotels used in this study were rated "three-star to budget" and well patronized. A total of 40 food samples were analyzed from the five hotels with some of the foods contaminated with unacceptable levels of fungi. For instance, fresh pepper sauce (Table 8), coleslaw (Table 9), tossed salad (Table 10), mixed salad (Table 11) and mixed vegetable salad (Table 12) were all above the WHO acceptable limits of < 3.0 log 10 cfu/g (Ameko *et al*., 2012). Raw vegetables used in the preparation of fresh pepper sauce and salads are always eaten without further cooking. Perhaps the holding temperatures were not adhered to. These foods should be kept cool at holding temperatures of 1^{o}C-5^{o} C before and during service (Ceserani and Foskett 2007). The fungal contamination on these foods may also be due to the fact that the vegetables must have come from the farm where polluted

water is used for irrigation which can cause contamination (Gosh *et al*., 2007; Donkor *et al*., 2008). Studies in Kumasi-Ghana by Feglo and Sakyi (2012) also revealed high levels of contamination in salads. These salads contain raw onion and probably may contain toxigenic species of *Penicillium* spp. and *Aspergillus* spp. (El-Nayerabi and Abdallah, 2004).

Again, these fungi adhere to plant surfaces as black moulds, therefore, improper washing by food preparers can cause contamination of the food. Samson *et al*., (2001) maintained that thousands of fungal species for example *Aspergillus niger* are commonly found in indoor environment and can easily contaminate the environment in food processing areas which can spread onto food and cause contamination. Recent evidences suggest some true *A. niger* strains do produce ochratoxin (Samson *et al*., 2004) and this calls for regular and proper sanitation in the kitchens.

Boiled plain rice (Table 10) was above the acceptable limits of < 3 Log10 cfu/g and the organism identified was *Penicillium verrucosum* (Table 14a and b). This compares with a study by AnnanPrah *et al*. (2011) who also found *Fusarium* spp. in cooked rice. From personal observations at the hotels, it was found that, imported rice especially parboiled and perfumed was used in all the rice dishes. According to Anderson and Thraine (2006), stored rice from Argentina and Paraguay is dominated by *Penicillium citrinum*, *Aspergillus niger*, *Aspergillus flavus* and *Alternaris* spp. and these microbes have also been isolated from different rice samples. They added that the mycobiota of rice may establish itself on parboiled rice even though this mycobiota has been eliminated by boiling. This, thus, calls for proper washing of the rice before cooking. The cooks can also introduce the fungi onto food through talking in the kitchen and this may occur when supervision is poor.

The fungal counts on fried rice was 3.7 Log_{10} cfu/g (Table 9), 3.2 Log_{10} cfu/g (Table 13), 4.0 Log_{10} cfu/g (Table 11), 5.2 Log_{10} cfu/g, and 5.1 Log_{10} cfu/g (Table 12) which were all above the acceptable limits of < 3.0 Log_{10} cfu/g. Fried rice may be susceptible to microorganism because of its composition. The rice is cooked first, and then mixed with chopped fried vegetables and soy

sauce which may create a favourable condition for the growth of fungi. The high level of fungi in fried rice observed in this study was consistent with work done by Wogu *et al*. (2011) in Benin City, Nigeria, when studying microbial load as found in ready-to-eat rice.

Jollof rice (Table 9) did not meet the acceptable levels and the contamination may be due to spores and mycelium fragments from the environment. The ingredients used in the preparation of the jollof rice could have been mouldy but were not visible. In this case, if the washing was not done properly, the food may be contaminated when ready for service. Contamination could have also occurred during blending of the vegetables from the blender since blending of ingredients is part of the jollof preparation. This result is in contrast with Addo *et al*. (2007) who did not find any contamination in jollof rice in their study of hotels in Accra.

Braised rice, which was above the acceptable limits (Table 9), is prepared with fried chopped onions then rice and water is added and allowed to boil till cooked. *Aspergillus tamaric* was identified in braised rice (Table 14a and b). *Aspergillus* spp. has been identified in stored rice (Samson *et al*., 2001) and therefore, inadequate washing can contribute to the contamination. Again the organism identified in the braised rice could be thermophillic and therefore was able to survive the high temperatures of the cooking. Vegetable sauce (Table 11) had a fungal count of 3 Log_{10} cfu/g.

All the sauces (chicken and vegetable sauce, beef sauce and beef in vegetable sauce) as shown in Tables 8 and 9 did not meet the WHO acceptable levels (< 3.0 Log_{10} cfu/g). This is in agreement with Mensah *et al.* (2002) who also found sauces to be above the acceptable levels. The organisms identified in the sauces are shown in Table 14a and b. *Cladosporium herbarum* dominated the organisms identified in the sauces. *Cladosporium* spp. includes some of the most common indoor and outdoor moulds and grows indoors where moisture is present.

In the process of sauce preparation a lot of steam is generated which will allow the organism to enter the sauce and hence the contamination. Steam generation is common in food preparation

kitchens and it is important to have extractor fans installed in the kitchen walls to remove the steam to avoid contamination by moulds. In this regard, regular cleaning and sanitation of the kitchen is very important. Equipment for cooking should be sanitized regularly to remove organisms that adhere to the surfaces. There are no major mycotoxins produced from the identified *Cladosporium* spp. which is rarely pathogenic but can cause infection of the skin and toenails.

Fish light soup (Table 10) and goat light soup (Table 12) were above the acceptable limit. The possible causes of the contamination can be poor personal hygiene by the food handlers and can be corrected by proper kitchen supervision. Potato chips (3.8 $\log_{10}$ cfu/g) and chicken with noodles (3 $\log_{10}$ cfu/g) as shown in Table 9 did not meet the acceptable limits. The possible causes may be due to the use of defective equipment such as chipped saucepans that can habour germs, pollution of kitchen environment, staff conversing during food preparation, poor storage of ingredients and insufficient cleaning of meat and vegetables.

There were no observable growths for TCC and TEC for chicken and vegetable sauce (Table 15), whilst TMC was within the WHO acceptable limit of < 3.0 $\log_{10}$ cfu/g. Beef sauce was also within the acceptable limit. Perhaps the methods of cooking these sauces may have contributed to their acceptable levels. The preparation of beef sauce, for instance, involves long cooking of the cut beef until tender and then added to tomato sauce and allowed to continue cooking again. Poultry and meat are some of the potentially hazardous foods because of their high protein content. This thorough cooking kills most harmful bacteria and makes them safe to eat as emphasized by DOH (2010). This compares favourably with a study by Mensah *et al*. (2002), on the microbiological quality of different types of foods collected from hotels in Accra, Ghana.

Meat stew recorded no growth of *Staphylococcus aureus*, *Salmonella* spp. and *E. coli*. Despite the long boiling time used for Goat light soup, TMC, TCC and TEC were above the acceptable limits (Table 15). This is not surprising because other ingredients like ginger, aniseed and garlic are normally added to spice the soup and are potential vehicles of microorganisms and toxins despite

the long boiling time (Mariott and Gravani, 2006). Mensah *et al*. (2002) in their studies found soup to be more contaminated with enteroaggregative *E. coli*. The goat meat may also have been contaminated during slaughtering and also poor preparation procedures might have contributed to the contaminated soup.

Tossed mixed vegetables had high TMC and TCC (Table 15). The cut vegetables are tossed very quickly in hot oil for a few seconds and this may not be long enough for the destruction of all microorganisms. Secondly, the vegetables are cut on a chopping board and this board could have been a contributing factor to the contamination. Sneed *et al*. (2004) found that food contact surfaces are notorious when it comes to bacterial contamination. Not properly washing organisms that adhere to the surface of the vegetables due to poor supervision may cause contamination. Mensah *et al*., (2002) likewise found microbes on vegetables to be above the acceptable limits and attributed it to inadequate cooking and defective cooking utensils.

Boiled plain rice had high TMC and TEC (Table 18) and this study confirms the results of Mensah *et al*. (2002) where a large proportion of dishes including rice were heavily contaminated. Wogu *et al*. (2011) also isolated four different bacteria from boiled rice namely; *Bacillus cereus, Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumoniae.* Rice is prepared everyday in most of these hotels and therefore the needed precaution, such as checking for foreign bodies and thorough washing before cooking, may have been taken for granted and hence the contamination. The contamination could have also come from the serving spoon which may not have been wiped properly with a clean napkin not leaving out poor personal hygiene of the food handlers and incorrect holding temperature. Perhaps the microorganism may have originated from the natural microflora of the rice which in most cases may have no discernible effect and the rice may be consumed without objection (Adams and Moss, 2000).

The colony count for fresh pepper sauce was above the acceptable levels of WHO < 3 $\log_{10}$.

This result is in line with previous studies conducted by Mensah *et al*. (2002) and Feglo and Sakyi, (2012) who found contamination in fresh pepper sauce. In a normal traditional Ghanaian way, fresh pepper sauce is made from fresh pepper, fresh tomatoes and fresh onions blended in an earthenware bowl and a wooden masher without the application of heat. Thus, bacteria that find their way into the food during its preparation live on and reproduce if confined for too long at ambient temperatures (Ghana Standards Authority, 2003). Thus, if the same blender is used in blending other foods, it could accumulate bacterium and introduce contamination into the pepper sauce. Other factors could be insufficient washing, poor storage and talking during preparation. The TMC and TCC for *fufu* (7.0 $\log_{10}$/g and 6.4 $\log_{10}$/g respectively) exceeded the acceptable level. *Fufu* is a Ghanaian traditional food which is prepared by excessive handling after cooking.

Pathogens can easily be transmitted into the *fufu* by the food handler's hands (Todd *et al*., 2007). The organisms may multiply if the *fufu* is not eaten immediately. In such a situation, there should be a thorough hand washing which is the first line of defence against bacteria, before preparing the *fufu*. The use of bare hands in preparing the *fufu* could be a problem because one of the easiest ways to spread bacteria is through dirt under the fingernails (Mariott and Gravani, 2006). Normally untrained persons are employed to pound the *fufu* without hair cover and this can lead to hair falling into the *fufu* unnoticed. Mariott and Gravani (2006) added that microorganisms especially staphylococci are found on hair and may easily contaminate the *fufu*. A study by

Mensah *et al*. (2002) and Feglo and Sakyi (2012), found unacceptable levels of bacteria in *fufu*. The contamination of the *fufu* was observed in a three-star hotel where foods prepared are expected to be of highest microbiological standards

The chances of fried rice (Table 16) getting contaminated with bacteria are very high because of its preparation and the ingredients involved. Plain boiled rice is allowed to cool, then vegetables like carrots and cabbage are fried quickly and added to the rice together with soy sauce and some spices making this food very rich and could account for the level of contamination. The

contamination may come from the vegetables which are irrigated with polluted water (Gosh *et al*., 2007; Donkor *et al*., 2008), therefore, if not washed and cooked properly, a high level of contamination is not unexpected. This food is also prepared in ambient temperatures which are suitable for bacteria growth and the serving dish and spoon if not sterilized but just wiped with a kitchen napkin can introduce contaminants.

Surprisingly potato chips (Table 16), which are fried longer enough at very high temperatures, had microbes that were above the acceptable limits. This could be attributed to failure to change frying oil which has been used repeatedly, dirty knives used in peeling the potatoes coupled with excessive handling during peeling. During frying the food which is not covered is likely to be contaminated by organisms suspended in the kitchen environment.

The TMC, TCC and TEC for jollof rice (Table 16) were above the acceptable limits of < 3.0 $\log_{10}$. Jollof rice is a rich rice dish which is cooked in rich tomato sauce with a lot of meat stock and water. The contamination may have come from improper storage of the rice in the store room where cleaning is perhaps inadequate with poor ventilation as observed during the study. Water used in cooking may also be a source of contamination since water is a major ingredient in the dish or perhaps the washing of the ingredients prior to cooking was inadequate. This result is in contrast with results of Addo *et al*. (2007) who found microbes in jollof rice to be within the acceptable limits.

Colony count for coleslaw was above the WHO acceptable limits of < 3.0 $\log_{10}$ (Table 16). Mishandling of ingredients during preparation may perhaps be the major cause for the contamination. Eghan *et al*. (2007) investigating food hygiene training in the commercial sector found that, mishandling of food plays a significant role in the occurrence of food borne illnesses. They are argued that improper washing of the vegetables used for the coleslaw may result in contamination since sewage, sludge, manure, and compost of animal and human origin are commonly used as organic fertilizer (FDA, 2006). Gloves were worn during the preparation of the

salad but from personal observations these gloves were never changed during the entire process. Previous studies carried out by Yeboah-Manu *et al*., (2010) in Accra also found salad to be above the acceptable limits. Another possible reason could also be due to the unsterilization of the serving equipment to kill and reduce the number of harmful bacteria that could be introduced (FDA, 2006). The ingredients throughout the preparation stage should be kept as cold as possible. There could also be cross-contamination if chopping boards are not washed properly. The salad in this study had salad cream containing egg yolk which could possibly be a medium for supporting microbial growth (Ameko *et al*., 2012). The Ghana Health Services (2007), reported of school children who developed gastroenteritis after being fed with salad among other things and this was attributed to cross contamination.

The grilled steak had TMC of 6.7 $\log_{10}$, TCC of 6.7 $\log_{10}$ and TEC of 6.1 $\log_{10}$ (Table 16). Grilled steak is prepared from lean sliced beef, seasoned with spices and cooked under the grill. The meat may have been contaminated from the slaughter house (Jay, 2002). If the grilling is not done properly, the middle part of the meat will become undercooked and will not kill the most heat-resistant spores resulting in contamination. The grilling which is done outside the kitchen could be contaminated with air-borne microorganisms from the cooking environment. The metal bars on the grill if not cleaned properly may also introduce organisms onto the grilled meat and cause contamination.

Beef sauce also had high bacteria count which was above the acceptable limits. The contamination of this food could possibly be due to unsanitized serving dishes and spoons which were wiped with kitchen napkin. This result compares with works by Mensah *et al*., (2002) who revealed that contamination of utensils is possible during serving. Talking and sneezing during the serving of food may also introduce organisms onto the food. Some chefs do not cover their hair fully and this was seen during the observational studies and this may result in hair falling onto food and contaminating it. Equipment observed in the kitchen do not promote regular hand washing and this

could contribute to contamination of the food when cooking. The vegetables in this food could also be a source of contamination if not cooked properly.

The TMC on chicken with noodles and vegetables was 7.0 $\log_{10}$, TCC was 6.3 $\log_{10}$ and 4.6 $\log_{10}$ for TEC. This could be attributed to the rich nature of this particular food which can create conditions for growth of microorganisms. The chicken which is high in protein and very susceptible to bacteria is cooked before cutting and mixing it with cooked noodles and the vegetables. Probably the chicken was undercooked. *Salmonella* and *Campylobacter* can be present in undercooked chicken. Maybe the use of the same cutting board for meat and vegetables might have contributed to the high colony count since this is a major source of contamination. Also inadequate hand washing coupled with wrong serving temperature could contribute to the contamination. Throughout the preparation time, bacteria from the kitchen environment can possibly contaminate the food (Todd *et al*., 2009a). If during the preparation some of the cooks were infected, the food can possibly be contaminated.

Braised rice in Hotel-02 was above the acceptable limits. This food is prepared by frying chopped onions in very little oil for a few seconds then rice and enough water added and allowed to cook. This method makes the food very rich in nutrients and open to microbial attack. The preparation of this food calls for proper hand washing which was lacking in this hotel since there was no special hand washing facilities provided. Sometimes there was shared equipment whereby equipment used in preparing high risk foods were also used in preparing other dishes and if cleaning is not done effectively, foods easily get contaminated. Flies are associated with eating and food preparation areas and could be so small that they cannot be seen in the kitchen and are able to contaminate food. Contamination can also result from improper storage of the rice where the layout and poor ventilation encourage insects to enter the storeroom and contaminate the rice. Previous studies conducted on microbiological quality of cooked rice from restaurants indicated that rice from

Indian premises was of poorer microbiological quality than those from Chinese and other premises (Nichols *et al*., 1999).

In Table 17, tossed salad and fried rice had organisms that were too numerous to count. This agrees with the results obtained by Ameko *et al.* (2012) where bacteria counts exceeded WHO acceptable levels. Tossed salad contains raw vegetable and the fried rice is also full of undercooked vegetables. The contamination of the vegetables could have resulted from the farm where poultry dropping and other faecal materials are used as manure (Samarajeewa, 2005). The method of preparation and contaminated hands of food handlers can also be a source of transmission of microorganism. Contaminated hands of food handlers could carry *Micrococcus* spp, *Salmonella* spp, and *Shigella* spp. Unfortunately, there was inappropriate hand washing facilities provided in the kitchens of Hotel-03. At this hotel, boiled plain rice recorded TMC of 3.5 $\log_{10}$ which was slightly above the acceptable limits and 2.4 $\log_{10}$ as the TCC which was within the acceptable limits.

The bacteria contamination in Hotel-03 compares with works by Annan-Prah *et al*. (2011) who also found soup in their study to be above the acceptable limits. The contamination of fish light soup is surprising since it involves a long boiling process. Contamination may have come from the water used in the soup preparation since water is one of the major ingredients in soup preparation. If raw sewage which contains pathogens from human and other materials from the environment flow into potable water through faulty plumbing, the water may contain microorganisms and can cause typhoid and paratyphoid fever. The contamination might have come from airborne microorganisms from the unclean air present in the kitchen which may gain access due to uncovered pot.

Mensah *et al*. (2002) found that fish can be an additional source of pathogens. The contamination may probably be due to excessive handling of the fish before frying which is a normal practice in Ghana. Kwaasi (2003) also confirmed in his research on microorganisms that, fish can easily be

contaminated by human pathogens during handling. The handling in the kitchen involves the removal of scales, gills, fins and the gut with a knife and the bare hands. The hands may not be washed before this operation and can possibly contaminate the fish. Kwaasi (2003) again added that the skin, gills and alimentary track carry large numbers of bacteria and could be as high as 10^7 on the skin and up to 10^9 in gills and gut, and these are mainly Gram-negative of the genera *Pseudomonas, Shewanella,, Psychrobacter, Vibrio, Flavobacterium* and *Cytophaga* as well as a few Gram positive micrococci and Coryneforms. To save money, oil for frying may not be changed regularly and this can possibly contaminate the fish. Contamination of fresh fish could also come from wholesalers during packaging.

TMC on tossed mixed vegetables was 4.0 $\log_{10}$ while TCC was 3.3 $\log_{10}$. There was no TEC observed. The organisms found in tossed vegetables may have originated from their natural microflora coupled with excessive handling involving cutting and transferring onto another pan for frying (Adams and Moss, 2008). This dish is fried for a short period which may allow thermophiles to survive and cause contamination. Serving equipment coupled with unclean napkins might have contributed to the contamination.

The contamination on tomato sauce could be attributed to contaminated tomatoes from the farm where contaminated water might have been used for irrigation. In the hotels, tomatoes among other things are bought in bulk and refrigerated. However, some microorganisms such as psychrophiles (e.g. *Alcaligenes* and *Pseudomonas*) are able to thrive and in some cases multiply when refrigerated (Samarajeewa, 2005). This finding agrees with studies of Mensah *et al*., (2002), where enteroaggregative *E. coli* was isolated from tomato sauce. The tomatoes are also cut on a board before blending in a liquidizer and this process could be a contact point for bacterial contamination. Food contact surfaces and cutting boards have been identified by Sneed *et al*. (2004) as contact point for contamination in most kitchens

Beef sauce was also contaminated, probably; the beef used in the preparation of the beef sauce was contaminated at the time of purchase coupled with wrong storage temperature in the hotel. Within the beef slaughter and dressing process, carcass skinning and evisceration process have been identified as probable introduction point of major contamination (Mariott and Gravani, 2006; Adams and Moss, 2008). Contact between carcass and hide allows a mixture of microorganisms to be introduced onto the carcass. These microorganisms may be of faecal, soil, water or feed origin and may also emanate from process workers and the processing environment (Kwaasi, 2003). This initial quality of beef if not handled properly, may affect the final microbiological quality of the food. Another contributing factor may involve the prolong storage of beef which may lead to further contamination.

The results in Table 18 were very close to the acceptable limits of the WHO. The layout and workflow pattern of this kitchen was very poor during the observation with no hand washing facilities. Food handlers wore uniforms and headgears alright but some of the preparation bowls were found on the bare floor. The contaminations in Hotel-04 could result from the poor kitchen environment, ineffective supervision, inappropriate storage of the vegetables as well as cheap supplies (Mensah *et al*., 2002).

The TMC on jollof rice was observed to be 3.7 $\log_{10}$ but no observable growth of coliforms and enterobacteria. This is in contrast with works by Wogu *et al*. (2011) where jollof rice collected from two standard restaurants had the lowest microbial load for bacteria.

Fried rice recorded TMC of 4.4 $\log_{10}$ cfu/g, TCC of 4.3 $\log_{10}$ cfu/g and 3.1 $\log_{10}$ cfu/g (Table 18) that were all above the acceptable levels. Wogu *et al*., (2011) also found microorganisms in fried rice in comparison to other rice preparations. The result is also similar to that reported by Patricia and Azanza (2005). The long handling of fried rice and addition of spices could have contributed to the contamination.

Again in Table 18, the TMC for beef sauce was 5.4 $\log_{10}$ cfu/g whilst TCC was 3.4 $\log_{10}$ cfu/g, however, there was no observable growth for enterobacteria. These results are above the acceptable levels. The beef sauce in Hotel-03 also did not meet the acceptable levels. These confirm the good knowledge but poor practices by the hotel food handlers. Food handlers in these two hotels might not have adhered to good food preparation practices and hence the contamination. Contamination could also come from the slaughter house, poor storage temperature, the cutting and other food contact surface in the kitchen (Addo *et al*., 2007).

Fried chicken had a TMC of 6.1 $\log_{10}$ cfu/g, TCC of 5.0 $\log_{10}$ cfu/g and TEC of 4.8 $\log_{10}$ cfu/g. From personal observations at this hotel (Hotel-04), the chicken was boiled slightly before frying. Nevertheless if the boiling is not done thoroughly before being fried contamination can still occur. The result is in contrast with that obtained from studies conducted in some hotels in Accra by Addo *et al*. (2007) where chicken sampled was negative for total aerobic count, coliform and enterobacteriaceae. Chicken is vulnerable to contamination especially *Salmonella* and *Campylobacter* species during processing such as defeathering and evisceration (Mariott and Gravani, 2006). Unsanitary food handling such as irregular hand washing, not having separate knives and cutting boards for raw meat as well as inappropriate storage temperature can further contaminate the chicken. Again proper cleaning and sanitizing procedures were not observed in this kitchen, however, unsanitized serving dishes can easily contaminate fried chicken.

Mixed salad had counts above the acceptable limits. For instance 6.4 $\log_{10}$ cfu/g was recorded for TMC, 5.9 $\log_{10}$ cfu/g for TCC and 6.3 $\log_{10}$ cfu/g for TEC. This is consistent with works done on salads in Ghana by Mensah *et al.* (2002), where bacteria counts of 6.3 ± 0.78 log10 cfu/g total was reported. Ameko *et al.* (2012) had microbial loads on salads in excess of 4.7 $\log_{10}$ cfu/g whilst Feglo and Sakyi (2012) also had 5.13 $\log_{10}$ cfu/g with Olukoya *et al*., (1991) having a count of 5.0 $\log_{10}$ cfu in Johannesburg (Olukoya *et al*., 1991). In a similar study Adu-Gyamfi and Nketsia-Tabiri (2007), reported aerobic mesophilic count of 6.9 and 7.6 $\log_{10}$ cfu/g, coliform counts of 5.7

$\log_{10}$ cfu/g and moulds and yeasts counts of 4.9 and 5.4 $\log_{10}$ cfu/g respectively as observed in early mornings and late evening salad samples. Processing of raw vegetable for salads creates the environment and chances for the propagation of pathogenic microorganisms.

Salads from Hotel-02 (3 star) and Hotel-03 (2 star) were also all above the acceptable limits. This is a cause for concern for salad preparations in these hotels. From personal observation, these hotels had no cold kitchens where salads and other cold foods like fruits were prepared.

The best way of serving salads are on bed of ice instead of placing them on serving tables.

The results of boiled plain rice showed TMC of 6.5 $\log_{10}$, TCC of 6.4 $\log_{10}$ and TEC of 5.9 $\log_{10}$. The results are in contrast with the results of Mensah *et al*. (2007), where rice samples collected from hotels were within acceptable limits with *Staphylococcus aureus, Salmonella* and *E. coli* species not detected. Personal observations made in this particular kitchen suggested that the workers contributed to the contamination of the rice. For instance, not all the food handlers had their hair covered. In the questionnaire studies, 23% of the food handlers had ordinary catering certificates confirming their food safety knowledge. Responses in the questionnaire again indicated that all food handlers washed their hands during food preparation; however, personal observation revealed that there was no hand washing facility in the kitchen of Hotel-04 which could be a contributing factor to the contaminations observed. Another reason could be the warm temperature in the kitchen. Rice may become contaminated during growth, harvesting and other agricultural operations such as processing and handling (Haque and Russel 2005) and therefore can contain bacteria that may survive the cooking process. For instance, *Bacillus cereus* may be present in rice as spores until water is added when they start to grow. Since the cooking will not kill the most resistant spores it is better to keep the cooked rice at 60 ^{o}C to 70 ^{o}C and should also not be kept for more than 4 days in the refrigerator.

In Table 19, TMC for jollof rice was 3.2 $\log_{10}$ cfu/g and no TCC and TEC. This could be attributed to the long cooking process which is applied to jolloff rice. This is commendable for Hotel-05

because jollof rice can contain enteropathogens (Patricia and Azanza, 2005). The slight contamination could perhaps be attributed to contaminated serving dish and spoon which were used. The contamination result on the jollof rice is in accordance with Patricia and Azanza (2005) who reported bacteria colony count of > 10^5 cfu/g. Again in the findings of Wogu *et al*. (2011), jollof rice did not contain a significant number of microorganisms. The slight contamination in this study could be attributed to additional ingredients added during cooking. Boiled fish in this hotel (Hotel-05) had TMC of 4.7 $\log_{10}$ cfu/g, TCC of 3.1 $\log_{10}$ cfu/g and TEC of 2.1 $\log_{10}$ cfu/g which is a credit for Hotel-05. The long boiling period of the soup may be a contributing factor to the low count for enterobacteriaceae. Fish can be easily contaminated by human pathogens especially during its storage and handling. The contamination could perhaps be attributed to the poor ventilation of the hot kitchen environment and direct contact of the fish with bare hands during cooking. The contamination may also come from the source of supply. For instance, Jayasinghe and Rajakaru, (2005) found in their study in Sri Lanka that the conditions of 50% of fish stalls were not within the stipulated standards. They inferred that, Fish that live in water polluted with human and animal faecal matter may carry substantial numbers of bacteria which may contaminate food (Jayasinghe and Rajakaru, 2005).

The TMC on fried chicken was 5.2 $\log_{10}$ cfu/g, TCC was 3.4 $\log_{10}$ cfu/g whilst TEC was 3.4 $\log_{10}$ cfu/g. These results were better than those obtained for fried chicken from Hotel-04. Processing methods may have contributed to this difference. The higher colony counts on chicken in the hotels are a cause for concern. The chicken used in these hotels are bought already dressed, cut and frozen and it may be possible that the frozen conditions in these hotels are not up to the required standards as well as thawing procedures. *Salmonella* and *Campylobacter* are known to contaminate poultry carcasses and parts (Simmons *et al*., 2003). High levels of faecal bacteria and various enteric pathogens have also been found in chicken (Sackey *et al*., 2001).

The results for mixed vegetable salad were 5.4 $\log_{10}$ cfu/g, 6.4 $\log_{10}$ cfu/g and 5.4 $\log_{10}$ cfu/g for TMC, TCC and TEC, respectively and these findings were above the acceptable limits. These findings were similar to results obtained for Hotel-02 (Table 16), Hotel-03 (Table 17) and Hotel04 (Table 18) with all the salads above the acceptable limits. These results may probably be attributed to the methods of preparing the salads, poor hygiene, sources of the ingredients and the temperatures under which they were served. Lawley (2009), in his study also suggested that poor hygiene in the catering industry could result in the contamination of salads served. He continued that a disproportionate number of outbreaks often large and occasionally international, had been associated with leafy salad greens especially lettuce where contamination seem to have occurred before harvesting and that the number of such outbreaks have been growing in recent years.

In this hotel (Hotel-05), boiled plain rice recorded TMC of 6.8 $\log_{10}$ cfu/g, 4.4 $\log_{10}$ cfu/g of TCC and TEC of 5.8 $\log_{10}$ cfu/g and was all above the acceptable limits. Boiled plain rice in Hotel-01 and Hotel-04 were also above the acceptable limits, however, Hotel 01 had coliform counts that were within the acceptable limits. This shows that cooked rice may be a potentially hazardous food especially in tropical countries where it is common to keep food at ambient temperatures. Studies conducted by Sandra *et al*. (2012) to determine the presence of *Bacillus cereus* and *Bacillus thuringiensis* found *Bacillus cereus* in the boiled rice. Tang *et al*. (2013) in their study on survivability of *Vibrio cholera* in cooked rice among other rice preparations also found plain boiled rice to be contaminated with *Vibrio cholera*, probably, due to holding the food for an extended period of time at room temperature. Perhaps the warm temperature of the dining rooms of these hotels under study could be a major contributing factor to the unacceptable levels. The TMC for fried rice in this hotel (Hotel-05) was 5.8 $\log_{10}$ cfu/g, TCC was 4.6 $\log_{10}$ cfu/g whilst TEC was 3.7 $\log_{10}$ cfu/g. These results are above the WHO acceptable limits and are comparable to the counts obtained for fried rice in Hotel-02, Hotel-03 and Hotel-04 which were all above the acceptable limits. The preparation of bulk rice in anticipation of subsequent need especially for fried rice

should be discouraged in the hotels. Inadequate personal hygiene may perhaps be a major contributing factor to the contamination.

The goat light soup (Table 19) was contaminated with microbes and this is very consistent with a study by Mensah *et al*. (2002) where soup among other foods appeared to be more contaminated with *Salmonella arizonae*. The goat meat can be an additional source of pathogens. In contrast, Addo *et al*. (2007) found the microbial quality of soup among other foods to be satisfactory. In this study, the key factors could be unsanitized equipment used coupled with poor ventilation in the kitchen with no hand washing facilities observed.

Vegetable sauces (Table 19) were all above the acceptable limits. This could be attributed to the cutting boards on which the vegetables were cut prior to addition to the sauce. Most of the food cutting surfaces were either made of wood or plastic and this offers a viable surface for bacteria to adhere (Addo *et al*., 2007).

Some common foods run through the five hotels sampled. Considering Table 20, the foods with the highest colony count were salads, and fried rice and as discussed already this is a cause for concern for the preparation of these foods in the hotels. These results may probably be attributed to the methods of preparing the salads and the fried rice, poor hygiene, sources of the ingredients and the temperatures under which they are served. Hotel-02 is a three star hotel where foods including salads are expected to be of high standard than Hotel-03 (two-star), Hotel-04 (one-star and Hotel-05 (budget) but this was not found to be so. Tossed salad contains raw vegetable and the fried rice is also full of undercooked vegetables. Considering the method of preparation, contaminated hands of food handlers can easily contaminate the vegetables. For instance, contaminated hands of food handlers can carry *Micrococcus* spp, *Salmonella* spp, and *Shigella* spp. Table 20 also shows high colony counts of microbes in jollof rice, especially in Hotel-02 which is a three-star hotel. The contamination could perhaps be attributed to unsanitized serving dishes and spoons. Boiled plain rice was served in all five hotels except Hotel-02 with Hotel-04

having very high colony counts. Observations made in this particular kitchen (Hotel-04) suggested their contribution to the contamination of the rice. Not all the food handlers had their hair covered. Responses in the questionnaire again indicated that all food handlers washed their hands during food preparation; however, personal observations revealed that there was no hand washing facility in the kitchen which could be a contributing factor to the contamination. Another reason could be the warm temperature in the kitchen where the rice is served leading to sweating of the kitchen staff which may also contaminate the food.

In Table 13, the common foods found in all the hotels for fungal growth were boiled plain rice, fried rice and salad from four hotels and jollof rice from three hotels. The food with the most fungal growth in the four hotels was salad with the highest being 6 Log_{10} cfu/g from Hotel-05 which is a budget hotel. The kitchen staffs of this hotel were fully aware of safe food hygiene practices in theory but much was not done in practice hence the high contamination of the salad. Improper washing of the salad ingredient could be a major reason. Fungi adhere to plant surfaces as black moulds and, therefore, improper washing by food preparers can cause contamination. The next food with high fungal contamination was fried-rice with fungal contaminants of 5 log_{10} cfu/g also from Hotel-05. Weak supervision during food preparation with no hand washing facilities before start of work, as was seen during the observation as well as wearing of casual clothing, could be the reason for the high fungal growth.

Findings from this study revealed bacteria growth in almost all the food samples investigated from the five hotels. The most prevalent bacteria from Hotel-01 to Hotel-05 were Coagulasenegative *Staphylococcus*, Gram positive rods, *Acinetobacter* spp. and *Klebsiella pneumoniae*. In Hotel-01, which is a "three star" hotel, Coagulase-negative *Staphylococcus* (CNS) (27.3%) (Figure 3) was detected in goat light soup, boiled plain rice and beef sauce (Table 21). Again in Hotel-04, jollof rice and vegetable sauce tested positive for Coagulase-negative *Staphylococcus* (11.1 %) (Figure 6 and Table 24). The same organism (18.2%) was identified in Hotel-05 for (Figure 7) in vegetable sauce and jollof rice (Table 25). These findings compares with work done

by Feglo and Sakyi (2012) who identified Coagulace Negative *Staphylococcus* in red pepper sauce, salad and macaroni. It is possible this contamination may have come from the hands of the food handlers during food preparation since parts of the body that contribute to the contamination of food include the skin, hands, hair, eyes, mouth, nose, nasopharynx, respiratory tract, and excretory organs. These contamination sources act as carriers through direct or indirect transmission of detrimental microorganisms (Food Standard Agency, 2007). This result correlate with that of the personal observations made, where no proper hand washing facility was provided by the management of the hotel, an evidence of poor hand hygiene. Previous studies by Udo *et al*., (1999) has shown that enterotoxigenic CNS were detected from food handlers' hands in Kuwait and suggested that such strains may contribute to food poisoning if food is contaminated by them and held in conditions that allow their growth. CNS resides on skin and mucous membrane and is opportunistic bacteria which undoubtedly are able to cause severe infections in humans (Vincent *et al*., 1998).

The other most prevalent organisms were Gram-positive rods (35.3 %) (Figure 4) which were isolated from six different foods in Hotel-02 namely; jollof rice, beef in vegetable sauce, chicken with noodles and vegetables, grilled steak, braised rice, and coleslaw (Table 22). All organisms isolated in Hotel-03 (Figure 5) were Gram-positive rods (100 %) and the foods were: fried rice, beef sauce, fried fish and tossed salad (Table 23). Figure 6 also exhibits Gram- positive rods (11.1%) isolated from vegetable sauce (Table 24). The high prevalence of Gram-positive rods observed in this study, may be attributed to ignorance of kitchen hygiene rules or different methods of food preparation using different ingredients with poor storage conditions in the various hotels which may lead to the risk of contamination by the food handlers. In these hotels, cleaning of equipment was performed with cold water and soap with poor washing facilities as shown in the personal observations. However, *Salmonella* was found to persist on chicken after cleaning with just hot water and soap. Cogan *et al*. (1999) suggested hypochlorite as a much more effective agent

for destroying these organisms. *Clostridium perfringens* which is a Grampositive bacterium is the third most common cause of food poisoning in the United Kingdom and the United States, though it can sometimes be ingested with no harm caused (Le Loir *et al* 2003). The spores of *Clostridium* are found in soil, air and all environment of the body which means foods can easily get contaminated by them during processing and handling if the necessary precaution is not taken, for instance, if vegetables and other food ingredients are washed with untreated water. *Streptococcus*, *Staphylococcus* and *Bacilli* are also Gram-positive rods. *Staphylococcus aureus,* which is a leading cause of gastroenteritis resulting from the consumption of contaminated food was reported by El-Shatal *et al*. (1998) and Sameer *et al*. (2008). Abdalhamid *et al*. (2013) in their study found the presence of *Staphylococcus aureus* in ready-to-eat foods in Misurata City, indicating lack of hygiene in its production. Moreover such organisms may take the chance to multiply in the product during storage and produce their enterotoxins which constitute a public health hazard to the consumers. *Staphylococcal* food poisoning can be endemic in the processing environment due to the absorption of Staphylococcal enteritoxins preformed in the food.

In this study, *Staphylococcus aureus* (9.1 %) was isolated from *fufu* in Hotel-01 (Figure 3 and Table 21). Foods that are handled frequently during preparation just like *fufu* and also vegetables that are shredded on chopping boards and used in preparing the fried rice are prime targets for *Staphylococci* contamination (Gosh *et al*., 2004). *Staphylococcus aureus* is found on the skin and in the nose and throat of most healthy people. Indeed, small cuts or burns on the hands in food preparation settings can become infected and contain millions of *S aureus* cells which can cause heavy contamination of foods handled by the cooks. It is also possible that injuries and/or infections on the hands of cooks could reduce the inclination of workers to wash and dry their hands properly and frequently and may thence contamination foods they are in touch with. *S. aureus* are also widespread in untreated water, raw milk and sewage (Nyenje *et al*., 2012). When

Staphylococcus aureus is allowed to grow in foods, it can produce toxins that cause illnesses. Although cooking destroys the bacteria, the toxin produced by *Staphylococcus aureus* is heat stable and may not be destroyed even by heating, let alone by refrigeration (Nyenje *et a*l 2012). Previous studies conducted by other authors also recorded the presence of *Staphylococcus aureus* in their results (Kumar *et al*., 2006; Gosh *et al*., 2007; Yeboah-Manu *et al*., 2010; Nyenje *et al*., 2012). For instance Kumar *et al* (2006) in their study, reported a high prevalence of *Staphylococcus aureus* in street vended foods whilst Gosh *et al* (2007) observed a high prevalence (60%) in coriander sauce and ready-to-eat salads (86%) in India. Nyenje *et al* (2012) also obtained a prevalence of 3.2% from rice and chicken stew samples in roadside cafeterias in

Alice, South Africa. Other studies conducted in Ghana by Yeboah-Manu *et al,*. (2010) on Bacteriological Quality of Ready-to-eat foods sold on and around University of Ghana Campus showed the presence of *Staphylococcus aureus*. In Accra, Ghana out of 511 menu items studied from streets vendors by Mensah *et al*., (2002), 163 (31.9%) *Staphyloccocus aureus* were isolated.

Bacillus spp. in Hotel-01 was 18.2 % and was present in tossed mixed vegetables and fresh hot pepper sauce (Figure 3 and Table 21). *Bacillus* spp. (5.9 %) were again isolated from potato chips in Hotel-02 (Figure 4 and Table 22), and jollof rice in Hotel-04 (11.1 %) (Figure 6 and Table 24). Similarly, *Bacillus cereus* was isolated from different foods by various authors in Accra, Benin city and Kumasi. In Accra, Yeboah-Manu *et al*., (2010) isolated *Bacillus* spp. from ready-to-eat foods sold on and around the University of Ghana Campus. Wogu *et al*., (2011) also isolated *Bacillus cereus* in boiled plain rice from high class restaurants whilst Feglo and Sakyi

(2012) isolated *Bacillus* spp. from red pepper sauce, salad, macaroni and *fufu* in street vending foods in Kumasi. *Bacillus* spp. are non-pathogenic and most commonly found in soil and the environment. Probably the foods were served in restaurant environments contaminated with the *Bacillus* spp. or there might have been inadequate washing of the vegetables prior to use. The

spores commonly contaminate raw foods and food materials particularly foods in contact with soil and vegetable origin. The spores of some species can, however, survive cooking for example *Bacillus cereus* and the *Bacillus subtillis* group. *Bacillus* spp. was prevalent in tossed mixed vegetables and fresh hot pepper sauce as shown in Figure 3 and Table 21. Setlow (2006), found that spores of *Bacillus* commonly contaminate raw foods particularly foods in contact with soil and of vegetable origin. In this study, salad as well as fresh hot pepper sauce were prepared from raw vegetables whilst the tossed mixed vegetables were minimally cooked hence the possibility of existence of the organism. Vegetables have been associated with food borne outbreak in many countries and may be contaminated from the farm with human sewage and from the irrigation water. Unsafe water used for rinsing and sprinkling to keep them fresh by sellers is other possible sources of contamination (Bukar *et al*., 2010). As most of these produce are eaten raw or with minimal cooking, their microbial content may represent a risk factor for the consumer (Falomir *et al*., 2010). The *Bacillus* spp. can germinate and grow in warm kitchens. Consumption of foods with large numbers of *Bacillus* spp. can cause gastrointestinal illnesses either by the consumption of pre-formed toxin or toxins produced by these bacteria in the gut. A fatal case of liver failure after the consumption of pasta salad was described by Dierick *et al*., (2006). *B. cereus* can cause systemic and local infections including meningitis, brain abscesses, pneumonia and gas gangreane like cutaneus infections (Butone, 2010) and two types of food borne illness; the emetic and diarrhoeal syndrome (Stenfors Amesom *et al.*,2008). Other reasons for the contamination could come from a previously cooked food which was reheated for insufficient time or inadequate temperature.

Acinetobacter spp. was prevalent in four of the five hotels sampled for the study. *Fufu* from Hotel-01 (9.1 %) (Figure 3 and Table 21), chicken with noodles and vegetables (5.9 %) from Hotel-02 (Figure 4 and Table 22), beef sauce and coleslaw (22.2 %) from Hotel-04 (Figure 5 and Table 28) and fried rice (18.2 %) from Hotel-04 (Figure 6 and Table 29) were all contaminated

with *Acinobacter*. The contamination may possibly have stemmed from the use of defective equipment which because of cost some managers find it difficult to replace. *Acinetobacter* spp. is ubiquitous inhabitants of soil, water and sewage environment and can survive on moist and dry surfaces. They are also part of the natural microbial flora of the skin and occasionally the respiratory tract of healthy individuals. Bergogne-Berenzin (2001) found that in the USA, *Acinetobacter* spp. was isolated in 38% of ground water and are often detected in treated drinking water. In human health, they are opportunistic pathogens that may cause urinary tract infections, pneumonia, bacteraemia, secondary meningitis and wound infections (RokhbakhshZamin *et al*., 2011). Their presence may be indicative of poor hygiene, cross-contamination and dampness in the kitchen environment.

Klebsiella pneumoniae (11.8 %) (Figure 4), was prevalent in chicken with noodles and vegetables and braised rice in Hotel-02, (Table 22). *Klebsiella pneumoniae* (11.1 %) (Figure 6) was detected in mixed salad (Table 24) in Hotel-04 and was also present (27.3 %) (Figure 7) in jollof rice, boiled fish and boiled plain rice (Table 25) in Hotel-05. These results agree with results from Yeboah-Manu *et al* (2010) who isolated *Klebsiella pneumoniae* in ready-to-eat foods as well as Feglo and Sakyi (2012) who also identified the same bacteria in salad (4.4%), and macaroni (5.9 %). The study of Wogu *et al* (2011) and Ameko *et al* (2012) also isolated *Klebsiella pneumoniae* in ready-to-eat rice and salads respectively. From the study, *Klebsiella pneumoniae* isolates were high in Hotel-05, which is a budget, where staff toilet facilities were very poor coupled with unavailability of hand washing facility in the kitchen. Water was also kept in a receptacle and was not flowing through the tap. Observations revealed poor equipment washing facility. *Klebsiella pneumoniae* is found in the normal flora of the mouth skin and intestines and also in human stool (faeces). This calls for good catering practices by the food preparers since *Klebsiella* can cause healthcare associated infections including pneumonia, bloodstream infections and meningitis (CDC, 2009)

The study isolated *Salmonella typhi* (5.9 % and 18.2 %) in some foods from Hotel-02 (Figure 4) and Hotel-05 (Figure 7) respectively. These foods were: grilled steak (Table 22) and fried rice (Table 25). This report, however, differs from that from Addo *et al*. (2007) who did not find any *Salmonella typhi* in the food samples tested during their study of food and its preparation conditions in some hotels in Accra, Ghana. The bacteria in these foods may be due to the use of left over foods as well as the hands of infected food preparers in the kitchen who may not wash their hands well after visiting the toilet. Faecal contamination of fingers and fingernails occurs more often in individuals with diarrhoea and can be caused by *Salmonella* spp. It is possible that some of the cooks in this hotel were infected but failed to report to the managers for fear of losing their salary or jobs. The encounter of bodies of humans to *S. typhi* is through faecal-oral route from infected individuals to healthy ones. In the developing world, transmission is through contaminated food and water. Infectious carriers pose great risk to public health due to their lack of disease related symptoms (Kidgell *et al*., 2002). In this study, other causes may be due to cross-contamination, inappropriate storage and probably an infected food handler. *Salmonella* spp. has been recognized as human and animal pathogen for over a century. They are estimated to cause about 1.3 million non-typhoidal infections per year in the U.S with approximately 378 deaths and over 19,000 people requiring hospitalization (Scallan *et al*., 2011).

Enterobacter asburiae (9.1 %) and *Enterobacter cloacae* (9.1 %) (Fugure 3) were prevalent in goat light soup and fufu respectively (Table 21) from Hotel-01. *Enterobacter gergoviae* (5.9 %) and *Enterobacter* spp. (5.9 %) (Figure 4) were mainly isolated from chicken with noodles and vegetables (Table 22) in Hotel-02. Feglo and Sakyi (2012) and Mensah *et al.* (2002) reported a high prevalence of *Enterobacter cloacae* in fufu in Kumasi and Accra respectively. In his study, Shaker *et al* (2007) isolated *Enterobacter cloacae* from infant milk formula. *Enterobacter* species have been reported as frequently isolated from different environments including soil, rats, flies, milk powder factories, chocolate factories and households (Nyenje *et al*., 2012).

Enterobacter spp. are in the family *Enterobacteriaceae* and are commonly found in soil and water (Hart, 2006). *E. cloacae and E. aerogenes* can inhabit the intestines of humans and animals and can also be found in sewage (Hart, 2006). *E. aerogene*s has also been found in dairy products (Abbot, 2007). Bacteria can be transferred from contaminated hands or contaminated urinals (Hart, 2006). *Enterobacteriaceae* can also be spread through the faecal-oral route (Bayda *et al.*, 2007). *Enterobacter* spp., particularly *E. aerogenes* and *E. cloacae*, have been associated with nosocomial outbreaks, and are considered opportunistic pathogens (Pagotto *et al*., 2003; Hart, 2006). *Enterobacter* spp. can cause numerous infections, including cerebral abscesses, pneumonia, meningitis, septicemia, and wound, urinary tract (particularly catheter-related UTI), and abdominal cavity/intestinal infections (Farmer *et al*., 2007; Russo and Johnson 2008). *E. cloacae* and *E. aerogenes* were responsible for the majority of Enterobacter infections, 65-75 % and 15-25 %, respectively according to Russo and Johnson (2008). *Enterobacteriaceae* are primarily colonizers of the lower gastrointestinal tract of humans and animals and are primarily colonizers of the lower gastrointestinal tract of humans and animals (Ryan, 2004).

Escherichia coli (5.9 %) was present in jollof rice (Table 22) from Hotel-02 (Figure 4). Sources of *E. coli* include contaminated food especially ground beef, unpasteurised milk and raw fruits and vegetables, contaminated water and faeces of infected people. Jollof rice is cooked for a very long period and therefore undercooking may not be the cause of the bacteria growth in this study, probably, improper handling during preparation and poor hand washing by the food preparers and some bad habits by food handlers might have resulted in contamination. An infected worker could also be a potential source of contamination. Results from this study is in agreement with that reported in Benin City by Wogu *et al*., (2012) who found *E. coli* in ready-to-eat jollof rice from high class restaurants. Yeboah-Manu *et al*., (2010) also isolated *E. coli* from ready-to-eat foods sold on and around the University of Ghana Campus in Accra, Ghana. *E. coli* lives in the intestines of humans and animals and most types are harmless but some types can cause sickness, for

example, *E. coli* 0157: H7 which causes bloody diarrhoea and can sometimes cause kidney failure and even death. *E. coli* makes a toxin called Shiga toxin (STEC). Other severe complications associated with *E. coli* infection is haemolytic uremic syndrome (HUS), with toxic substances that destroys red blood cells, causing kidney injury.

In Hotel-01, *Pseudomonas* spp. (9.1 %) (Figure 3) was isolated from fufu (Table 21). In Hotel04, *Pseudomonas leuteola* (11.1 %) (Figure 6) was again prevalent in jollof rice (Table 29) whiles *Pseudomonas putida* (11.1 %) (Figure 6) was isolated from mixed salad (Table 24).

Pseudomonas aeruginosa (18.2 %) (Figure 7) was isolated in jollof rice (Table 25) from Hotel05. *Pseudomonas* can be found almost everywhere; in soil, water, plants and animals and it is the most famous opportunistic human pathogen most commonly affecting immunocompromised patients (Franzetti and Scarpellini, 2007). Most members are psychrotrophic, can be fluorescent or non-fluorescent, and have long been known to be responsible for chilled food spoilage. Psychrotrophic *Pseudomonas* species, pose significant food spoilage problems in refrigerated meat, fish, shell fish and dairy products. Probably, the jollof rice in study was refrigerated and reheated inadequately hence the contamination. *Pseudomonas* as well can lead to problems in water systems and this could also be the source of the contamination in the food industry. The principal microbial population of many vegetables in the field consists of species of the genus *Pseudomonas*, especially the fluorescent forms. During storage and processing their numbers increase and in Minimally Processed Vegetables (MPV) they play an important role in the phenomenon of browning because of their pectinolytic activity (Ngyen-The and Carlin, 1994;

Riva *et al.*, 2001). Feglo and Sakyi (2012) in their study of street vending foods in Kumasi also identified 0.7 % and 1.5 % *Pseudomonas aeruginosa* from *fufu* and salad respectively. The study agrees with Mensah *et al*., (2002) who isolated *Pseudomonas aeruginosa* from salad during their study on street foods in Accra, Ghana. *Pseudomonas* spp. also plays an important role in milk spoilage. During the storage of raw milk they produce many thermo-tolerant lipolytic and

proteolytic enzymes that reduce both the quality and shelf life of processed milk (Wiedmann *et al.*, 2000; Dogan and Boor, 2003).

The isolates with the highest percentages were: Coagulase-negative *Staphylococcus* (27.3 %), *Bacillus* spp. (18.2 %) all from Hotel-01. Gram-positive rods occurred in Hotel-02 (35.3 %) and Hotel-03 (100 %). *Acinetobacter* spp. (22.2 %) from Hotel-04 and *Klebsiella pneumoniae* (27.3%) from hotel 05 was observed. The most common bacteria *Salmonella typhi* and *Escherichia coli* were also prevalent in this study. In Hotel-02, *Salmonella typhi* was 5.9 % and 18.2 % in Hotel-05 whilst *E. coli* was 5.9 % in Hotel-02. These findings demonstrate that foods prepared in some hotels in Kumasi constitute likely potential hazards to human health from three star hotels to budget and this is as a result of poor habits of some food handlers. Foskett and Ceserani (2007) found that poor temperature control or prolonged holding in the danger zone can result in food poisoning bacteria multiplying to large numbers and contaminating the food. Thus, there must be an established protocol to ensure that employees adhere to and understand hygienic practices. There should also be provision of welfare facilities such as dressing room to help improve the well-being of the employees at the catering services.

Environmental investigations in both kitchen and restaurant were conducted in parallel with the practical microbial food safety analysis since the environment also plays an important role in transmitting microbial agents' to food. A total of four different kinds of isolates were detected from the kitchens under study (Table 27). The isolates were; *Staphylococcus*, Gram-positive rods, Coagulase-negative *Staphylococcus* and Filamentous Gram-positive rods. These organisms were also isolated from some of the foods used for the study. *Staphylococcus*, for instance, was prevalent in four out of five kitchens. Presence of *Staphylococcus* poses a potential hazard for consumers. Most staphylococcal food poisoning outbreak investigations successfully traced food handlers as a source of contamination (Kadariya and Smith, 2014). Pathogens from the kitchen environment can originate from workers with infections that are contagious and raw foods that are brought into

the kitchen. In the kitchen, any surface touched by an infected worker while preparing food can easily become contaminated and then be present in the environment (Zhao *et al* 1998). Diarrhoea and vomiting are the main symptoms and it is likely that the food preparers in this study did not report the symptoms for them to be excluded from work. Flushing of toilets that are close to the kitchen can probably disperse enteric pathogens into the immediate environment including the kitchen area if there are gaps between the doors and the kitchen area. Additionally, the cooks may talk, sneeze or cough during food preparation and could generate small and large droplets which can transmit bacteria into the kitchen environment. Another possible cause may come from a food preparer who may vomit in the toilet close to the kitchen and contaminate the food preparation area, sinks, dishwashing stations as well as the food worker's clothing and then return to the kitchen afterwards with hands that are improperly washed. Todd *et al.* (2009b) found vomit to be splendid medium for bacteria transfer because of its thyrotrophic (gluey) nature. During cooking, especially boiling of food, the kitchen may become moist and could encourage microorganisms to survive in the environment. Other activities that can cause the kitchen environment to become moist include wringing out a dishcloth or a sponge, scraping and cutting up carrots and opening the tap several times. Microorganisms that are capable of causing gastroenteritis are able to thrive on moist surfaces for hours and even days (Kramer *et al*., 2006; Scatter *et al*., 2002; Rzezutka and Cook, 2004). Again soil that are left on surfaces such as spills on stoves as well as work tops and are left to be cleaned later can possibly assist in the spreading of pathogens within the kitchen.

Other means of spreading pathogens in the kitchen environment may be the foods in packages that are brought into the kitchen such as chicken, fish, meat and crates of eggs which can contaminate the kitchen environment during unpacking and preparation. Harrison *et al*. (2001) in their study in South Wales detected the presence of Campylobacter on the external surface of food package product. Perhaps there should be greater control of cleaning with written cleaning procedures

highlighting correct cleaning compounds to use on equipment, surfaces and on hands to reduce environmental microorganisms to a minimum level.

The study found that apart from Hotel-05 where *Acinetobacter* spp. was isolated from the restaurant environment, the rest of the hotels had similar pathogens compared to those of the kitchens (Table 27). Surprisingly, Hotel-01 and Hotel-02 which are 'three star' hotels and of high standard had bacteria in the restaurant environment. In the restaurant, transmission of pathogens could occur from contaminated fabrics like the curtains, cushioned chairs, carpets and rugs. Also chandeliers, if not cleaned regularly, could harbour microorganisms that may be able to survive in the environment. Boone and Gerba (2007) found that vacuuming an unclean rug in the restaurant may agitate airborne pathogens that might have ensconced and could be transferred to other areas in the restaurant. Additionally, there are various surfaces in the restaurants where microorganisms can probably remain viable, for example, glass doors and windows, framed pictures on the walls, ceramics, wood and stainless steel. Todd *et al.* (2009b) found that guests who patronized the restaurant could introduce microorganisms into the environment through some items brought along with them. These may include keys, handbags, shoes, handkerchiefs and paper currencies. Michaels (2002), in his study found money handling as a means of microbial contamination when handling or serving ready-to-eat. This lead to the withdrawal of paper currency when notes were detected to be heavily contaminated with microorganism as found in Nigeria and Philippines. Questionnaire which was administered to food handlers at the beginning of this study showed that there are regular inspections in the food preparation areas in the hotels under study. The results of this study have, however, revealed that those inspections concentrated on the overall appearance and physical conditions of the food preparation and service buildings but not safety standards practiced by the food preparers. The results of this study have highlighted the need for microbiological analysis on food and the environment of eating premises in Ghana.

The cooks would be educated to know that if, for instance, *E. coli* is found in the food, and then the causes could be faecal contamination from food handlers, poor personal hygiene and unsafe food and water supplies. If on the other hand, *Staphylococcus aureus* is found in the food, the reasons could be improper and insufficient hand washing, allowing sick food handlers to prepare food and improper heating and refrigeration of food.

CHAPTER SIX

6.0. CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

Food safety problems may arise at any stage from food production to consumption. Generally, it was observed that respondents were knowledgeable about food safety, hygiene and sanitation which are cardinal points of the international codes of practice by the Codex Alimentations Commission. Their food safety knowledge and the professional qualification indicated that foods from these hotels would be safe and suitable for consumption as well as improve the reputation and consumer confidence of the hotels. Some organisms were identified in the kitchen and restaurant environment which could easily serve as a source of the food contaminations identified.

As determined in the study, there were microbial contaminations in the foods examined despite the awareness of food hygiene and qualification of the kitchen staff. The contamination levels were above the WHO acceptable levels and thus consumption of such foods can endanger the health of the people who patronize these foods. Foods like salads, fried rice, fufu, boiled plain rice and even jollof rice were above the acceptable limits. Some of the bacteria isolated such as *Staphylococcus aureus* and *Escherichia coli* are potential enteric pathogens and are known to cause gastro enteritis. The study has also demonstrated that most of the foods served in some hotels have fungal contaminants such as *Penicillium viliclicatum* from plain boiled rice and *Aspergillus niger* from vegetable sauce which is used in the preparation of salads. Observation and personal conversation during the study showed that some of the hotel managers knew very little about food preparation. Poor kitchen sanitation in the low level and in some of the high level hotels accounted for the contamination of the foods.

6.2. Recommendations

The following are further recommended based on the findings of this study:

- Similar study should be conducted in other hotels in the Kumasi Metropolis.
- Bacteriological studies should be done on more common foods to determine their contamination levels.
- Prevalence of resistant strain should also be investigated and the genes encoding the resistance also determined.
- It is also recommended that there should be a policy by the Kumasi Metropolitan Assembly (KMA) whereby hotel inspections should include microbial test on foods.

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APPENDIX

APPENDIX A: Ethical clearance on human Research and Publication

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY
COLLEGE OF HEALTH SCIENCES
SCHOOL OF MEDICAL SCIENCES / KOMFO ANOKYE TEACHING HOSPITAL
COMMITTEE ON HUMAN RESEARCH, PUBLICATION AND ETHICS

Our Ref: CHRPE/AP/038/13

27th February, 2013.

Mrs. Sophia Darko
Department of Food
Science and Technology
KNUST.

Dear Madam,

LETTER OF APPROVAL

Protocol Title: ***Investigations of Microbiological Food Safety Standards Practiced by some Hotel Industries in Kumasi.***

Proposed Site: ***Eleven (11) Selected Hotels in Kumasi.***

Sponsor: ***Principal Investigator.***

Your submission to the Committee on Human Research, Publications and Ethics on the above named protocol refers.

The Committee reviewed the following documents:

- A number of notification letters from the study sites was reviewed.
- A completed CHRPE Application Form.
- Participant Information Leaflet.
- Research Proposal.
- Questionnaire.

The Committee has considered the ethical merit of your submission and approved the protocol. The approval is for a fixed period of one year, renewable annually thereafter. The Committee may however, suspend or withdraw ethical approval at anytime if your study is found to contravene the approved protocol.

Data gathered for the study should be used for the approved purposes only. Permission should be sought from the Committee if any amendment to the protocol or use, other than submitted, is made of your research data.

The Committee should be notified of the actual start date of the project and would expect a report on your study, annually or at close of the project, whichever one comes first. It should also be informed of any publication arising from the study.

Thank you Madam, for your application.

Yours faithfully,

Osomfuor Prof. Sir J. W. Acheampong MD, FWACP
Chairman

Room 7 Block J, School of Medical Sciences, KNUST, University Post Office, Kumasi, Ghana
Phone: +233 3220 63248 Mobile: +233 20 5453785 Email: chrpe.knust.kath@gmail.com / chrpe@knust.edu.gh

Appendix B: Questionnaire for Chefs, Assistant Chefs, Cooks and Assistant Cooks

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY KUMASI

DEPT OF FOOD SCIENCE AND TECHNOLOGY.

QUESTIONNAIRE FOR CHEFS, ASSISTANT CHEFS, COOKS AND ASSISTANT COOKS

Please complete this questionnaire by ticking the relevant answers or by writing the answer in the spaces provided

Section A. Demographic information

1. Gender: [a] Male [b] Female

2. Age: [a] 15 -19 [b] 20-24 [c] 25-29 [d] 30-34 [e] 35-39 [f] 40-44 [g] 45 and above.

3. Religion: [a] Christian [b] Muslim [c] other?
   (Please specify).....

4. Marital status: [a] Married [b] Single

5. Nationality: [a] Ghanaian [b] Non-Ghanaian

6. Educational level: [a]Tertiary [b] Vocational [c] Senior secondary [d] 'O'level [e] Junior secondary [f] Primary [g]Middle school [h] Illiterate.

7. Professional qualification: [a]Degree in catering and Food and beverage service [b] Higher National Diploma Certificate [c]Ordinary Certificate in Catering

[d] 812/2 Certificate [e] 812/1 Certificate [f] National Vocational Training Institute (NVTI) Certificate [g] No Certificate. [h] Other (specify)

.....

8. How many years have you been working here? [a] Less than one year [b]1 yr. [c] 2 yrs. [d]3 yrs. [e] 4 yrs [f] 5yrs and above

Section B: Food safety knowledge/ practices

1. Which section of this establishment do you work? [a] preparation [b] production. [c] Washing of dishes [d] other (specify)…….
2. Do you know or have you heard about food poisoning? [a] Yes [b] No
3. If yes, Which of these do you think can cause food poisoning?

   [a]Eating cold food

   [b] Eating uncooked food

   [c] Eating contaminated food/food with germs

   [d]Drinking cold water

   [e] Other (specify)…….
4. Are you also aware that microorganisms can be found in refrigerated foods?

   [a] yes [b] no
5. What effect does food poisoning have on the individual? .....
6. Is it necessary to wash your hands with soap and hot water before food preparation? [a] Yes [b] No
7. Give reasons to your answer in question 6

   .....
   ……

   ……
8. Is it necessary to wash your hands during food preparation? [a] Yes [b] No.
9. If yes, how many times should the hands be washed during food preparation.

[a] Once

[b] Twice

[c] Three times

[d] When you touch something different from what is being cooked

10. What do you use to wipe your hands in this kitchen after washing your hands in this kitchen?

[a] Terry towel [b] Paper towel [c] Kitchen napkin [d] Hot air dryer [e] Other (specify)…….

11. Do you have a special hand washing basin in the kitchen? [a] Yes [b] No

12. Do you wear special uniform during food preparation [a] yes [b] no.

13. If yes, state the colour of the uniform [a] white [b] yellow [c] blue [d] green [e] red

[f] Other (specify)……..

14. Where do you change your house dress? [a] in the kitchen [b] changing room [c] Other (specify)……..

15. Do you cover your hair during food preparation? [a] Yes [b] No

16. If yes, Is the covering done [a] fully [b] partially [c] Other (specify)…….....

17. Do you think it is important to cover the hair during food preparation? [a] Yes [b] No

If yes, give your reason(s) 1)……

2)……...
3)……..

18. How many chopping boards do you have in your production kitchen?

[a] one [b] two [c] three [d] separate board for different ingredients.

19. Which type of surface do you cut your vegetables?

[a] wooden [b] Tiles [c] Cement [d] Plastic [e] Metal [f] Other

(specify)…….

20. Do you keep separate knives for particular ingredients?

[a] yes [b] no [c] no idea

21. If yes, why? ……..

…….

22. How long does it take to sell all the prepared food [a] within 1-2 hrs [b] within 3-4 hrs [c] within 5-6 hrs [d] 6hrs and above

Are there instances, where you have left-over foods? [a] Yes [b] No

23. If yes, how do you store your left-over foods?

[a] keep covered till morning in the kitchen at room temperature

[b] Kept in the refrigerator

[c] Leave it on the stove

[d] Discard

24 Do you sometimes have to reheat food for sale? [a] Yes [b] No

25. How is the cooked food kept on hold for sale? [a] on the stove [b] on a table

[c] Hot cupboard [d] Other (specify)……

26.. Do you have separate refrigerators for fresh meat, fruits and vegetables and dairy? products? [a] Yes [b] No

27 How is the cooked foods dished-out to customers?

[a] Separate spoon for each food

[b] Few spoons for all dishes

[c] One spoon for all the dishes

[d] Other (specify)……..

28. What kind of water do you use to wash your dishes [a] Running water [b] Water in receptacles [c] Hot water [d] Running hot water [e] Other (specif)

,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, .

29. Do you have a medical certificate? [a] Yes [b] No

Section C: Sanitation

1 Are there fly -proof doors for the kitchen? [a] Yes [b] No

2 Where do you keep dustbins during food preparation?

[a] in the kitchen covered

[b] in the kitchen opened

[c] outside the kitchen opened

[d] outside the kitchen covered

3. How often is the cooking environment cleaned during the day?

[a] Mornings [b] Morning and afternoon [c] Morning and evening [d] Morning, afternoon and evening [e] Other specify……………..

4. How many times are the walls cleaned and disinfected in the kitchen?

[a] Weekly [b] Twice a week [c] Monthly [d]every six months [e] once in a year [f] not cleaned

5. How many times are the ceilings cleaned and disinfected in a year?

[a] Weekly [b] Twice a week [c] Monthly [d]every six months [e] once in a year [f] not cleaned

6. Do you disinfect the work surfaces? [a] Yes [b] No

7. If yes, how often is this done in a day ……………………………………………………………

8. What cleaning agent do you use to clean the kitchen work surfaces?

[a] Ordinary soap [c] Antibacterial cleaner [d] Water [e] Other

(specify) ....

9 Are there special employees solely responsible for cleaning the kitchen?

[a] Yes [b] No

10 Is there regular inspection by regulatory body in this kitchen? [a] Yes [b] No

11. Which regulatory body does the inspection? ....

12. Is there regular fumigation in this kitchen? [a] Yes [b] no

13. If yes, how often is this done ? [a] Yearly [b] Every 6 months [c] Quarterly [d] Other specify……………………

14. Have you heard of microorganisms? [a] Yes [b] No

15. Are you aware that apart from food, microorganisms can also be found in the kitchen environment? [a] yes [b] no

16. Do you participate in food safety workshops? [a] Yes [b] No

17. What measures do you take to prevent food pests in the kitchen?

[a] spray occasionally [b] spray every weekend

[c] nothing is done [d] other? (Please specify) …………………………….

Questionnaire No... Date.................

Sample Area..

Appendix C: Observational Criteria used at the Kitchen of the Various Hotels 1.

Observational criteria used at the Kitchen of Hotel-01

CRITERIA	**SCORE**	
	YES **Period of 5 Days**	**NO** **Period of 5 Days**
Personal Hygiene		
Provision of protective clothing	√ √ √ √ √ √ √	
_ √		√ √ √ √ √

Wearing of casual clothing	√ √ √ √ √ √
Suitable footwear	
Provision of changing room facilities	√ √ √ √ √ √ √
Provision of clean toilet and washing facilities	√ √ √ √ √ √
Special basin provided for hand washing	√ √ √ √ √ √ √
Hot/warm water for hand washing	√ √ √ √ √ √ √
Cold water for hand washing	√ √ √ √ √ √ √
Soap for hand washing	√ √ √ √ √ √ √
Napkins used for drying hands	√ √ √ √ √ √ √
Disposable paper towel for drying hands	√ √ √ √ √ √ √
Hot air dryer for drying hands	√ √ √ √ √ √ √
Drying hands with roller towel	√ √ √ √ √ √ √
Hands washed before start of work	√ √ √ √ √ √ √
Hands washed in between work	√ √ √ √ √ √ √
Availability of adequate food preparation areas	√ √ √ √ √ √ √
Can thawing be carried out safely?	√ √ √ √ √ √ √
Adequate sink and water	√ √ √ √ √ √ √
Separate chopping boards for different foods	√ √ √ √ √ √ √
Are adequate stoves, grills and ovens available?	√ √ √ √ √ √ √
Can safe cooking temperature be achieved?	√ √ √ √ √ √ √
Are there suitable storage facilities for cooked foods?	√ √ √ √ √ √ √
Suitable storage facilities for cooked foods	√ √ √ √ √ √ √
Can safe holding temperatures be achieved?	
Is all foods consumed within 2 hours?	√ √ √ √ √ √ √

2. Observational criteria used at the Kitchen of Hotel-01

CRITERIA	SCORE	
	YES Period of 5 Days	NO Period of 5 Days
Are left over foods recovered for chilling or frozen?	√ √ √ √ √ √	√
How are serving spoons kept during service? In a bowl of water		
Are chilled and ambient storage facilities available?		
Is there enough supervision?	√ √ √ √ √ √ √ √ √ √ √ √ √ √	
Are adequate frozen facilities available?	√ √ √ √ √ √ √	
Can food be defrosted safely?	√ √ √ √ √ √ √	
Is there pest control system in place?	√ √ √ √ √ √ √	
Is the swill area kept clean?	√ √ √ √ √ √ √	
Is there enough ventilation in the kitchen?	√ √ √ √ √ √ √	
Is the kitchen building suitable for catering activities?	√ √ √ √ √ √ √	

3. Observational criteria used at the Kitchens of Hotel-02

CRITERIA	SCORE	
	YES Period of 5 Days	NO Period of 5 Days
Personal Hygiene		
Suitable protective clothing	√ √ √ √ √ √ √	
Casual clothing		√ √ √ √ √ √ √
Suitable foot wear	√ √ √ √ √ √ √	
Provision of changing room facilities	√ √ √ √ √ √ √	
Provision of clean toilet and washing facilities	√ √ √ √ √ √ √	
Special sink provided for hand washing		√ √ √ √ √ √ √
Hot water for hand washing		√ √ √ √ √ √ √
Cold water for hand washing	√ √ √ √ √ √ √	
Soap for hand washing	√ √ √ √ √ √ √	

Napkins used for drying hands	√ √ √ √ √ √ √
Disposable paper towel for drying hands	
Drying hands with hot air dryer	√ √ √ √ √ √ √
Drying hands with roller towel	√ √ √ √ √ √ √
Hands washed before start of work	√ √ √ √ √ √ √
Hands washed in between work	√ √ √ √ √ √ √
Are adequate food preparation areas available?	√ √ √ √ √ √ √
Can thawing be carried out safely?	√ √ √ √ √ √ √
Adequate sink and water	√ √ √ √ √ √ √
Separate chopping boards for different	√ √ √ √ √ √ √
Are adequate stoves, grills and ovens foods	√ √ √ √ √ √
	√ available?
Can safe cooking temperature be achieved?	√ √ √ √ √ √ √

4. Observational criteria used at the Kitchens of Hotel-02

Are there suitable storage facilities for cooked foods?	√ √ √ √ √ √ √
Suitable storage facilities for cooked foods	
Can safe holding temperatures be achieved?	√ √ √ √ √ √ √
Is all foods consumed within 2 hours?	√ √ √ √ √ √ √
Are left over foods recover for chilling or frozen?	√ √ √ √ √ √ √
How are serving spoons kept during service? Kept in a bowl of water	
Is there enough supervision?	√ √ √ √ √ √ √
Are chilled and ambient storage facilities available?	√ √ √ √ √ √ √
Are adequate frozen facilities	√ √ √ √ √ √ √
Can food be defrosted safely?	√ √ √ √ √ √ √
Is there pest control system in available?	√ √ √ √ √ √ √
Is the swill area kept clean? place?	√ √ √ √ √ √ √

Is there enough ventilation in the kitchen?	√	√	√	√	√	√√
Is the kitchen building suitable for catering activities?	√	√	√	√	√	√√

5. Observational criteria used at the Kitchens of Hotel-03

CRITERIA	SCORE: YES Period of 5 Days	SCORE: NO Period of 5 Days
Personal Hygiene		
Suitable protective clothing	√ √ √ √ √ √ √	
Casual clothing		√ √ √ √ √ √ √
Suitable foot wear		
Provision of changing room facilities	√ √ √ √ √ √ √	
Provision of clean toilet and washing facilities	√ √ √ √ √ √ √	
Special sink provided for hand washing		√ √ √ √ √ √ √
Hot water for hand washing		√ √
Cold water for hand washing	√ √ √ √ √ √ √	
Soap for hand washing	√ √ √ √ √ √ √	
Napkins used for drying hands	√ √ √ √ √ √	
Disposable paper towel for drying hands		√ √ √ √ √ √ √
Drying hands with hot air dryer		√ √ √ √ √ √ √
Drying hands with roller towel		√ √ √ √ √ √ √
Hands washed before start of work	√ √ √ √ √ √ √	
Hands washed in between work		√ √ √ √
Are adequate food preparation areas available?		√ √ √ √
Can thawing be carried out safely?	√ √ √ √	√ √ √
Adequate sink and water	√ √ √ √	√ √ √
Separate chopping boards for different foods	√ √ √ √	√ √ √
Are adequate stoves, grills and ovens available?	√ √ √ √ √ √ √	

. Observational criteria used at the Kitchens of Hotel

	SCORE NO Period of 5 Days
Can safe cooking temperature be achieved?	√ √ √ √ √ √ √
Are there suitable storage facilities for cooked foods?	√ √ √ √ √ √ √

6 **-03**

CRITERIA	SCORE YES Period of 5 Days	 YES Period of 5 Days
Suitable storage facilities for cooked foods		
Can safe holding temperatures be achieved?		
Is all foods consumed within 2 hours?		
	Are left over foods recovered for chilling or frozen?	√
√ √ √ √ √ √		
How are serving spoons kept during service? In a bowl of water		
Is there enough supervision?		
Are chilled and ambient storage facilities available?	√ √ √ √ √ √	_____
Are adequate frozen facilities available?	√ √ √ √ √ √ √	
Can food be defrosted safely?	√ √ √	√ √ √ √
Is there pest control system in place?	√ √ √	√ √ √ √
Is the swill area kept clean?	√	
	√ √ √ √ √ √	√ √ √ √ √ √ √
Is there enough ventilation in the kitchen?	√ √ √ √ √ √ √	

√ √ √ √ √ √ √

. Observational criteria used at the Kitchens of Hotel

Is the kitchen building suitable for catering activities?	√ √ √ √ √ √ √

7 -04

CRITERIA	**YES Period of 5 Days**
Personal Hygiene	
Suitable protective clothing	√ √ √ √ √ √ √
Casual clothing	√ √ √ √ √ √ √
Suitable foot wear	√ √ √ √ √ √ √
Provision of changing room facilities	√ √ √ √ √ √ √
Provision of clean toilet and washing facilities	√ √ √ √ √ √ √
Special sink provided for hand washing	√ √ √ √ √ √ √
Hot water for hand washing	√ √ √ √ √ √ √ √ √ √
Cold water for hand washing	√ √ √ √ √ √ √
Soap for hand washing	√ √ √ √ √ √ √
Napkins used for drying hands	√ √ √ √ √ √
Disposable paper towel for drying hands	√ √ √ √ √ √ √

. Observational criteria used at the Kitchens of Hotel

	SCORE	
		NO Period of 5 Days
Drying hands with hot air dryer		√ √ √ √ √ √ √
Drying hands with roller towel		√ √ √ √ √ √ √
Hands washed before start of work	√ √ √ √ √ √ √	
Hands washed in between work		√ √ √ √ √ √ √
Are adequate food preparation areas available?	√ √ √ √ √ √ √	

8 -04

CRITERIA	**SCORE**	
	YES Period of 5 Days	**YES Period of 5 Days**
Can thawing be carried out safely?		√ √ √ √ √ √ √
Adequate basin and water		√ √ √ √ √ √ √
Separate chopping boards for different foods	√ √ √ √ √ √ √	

. Observational criteria used at the Kitchens of Hotel

Are adequate stoves, grills and ovens available?	√	√	√	√	√	√	√
Can safe cooking temperature be achieved?	√	√	√	√	√	√	√
Are there suitable storage facilities for cooked foods?					√	√	√ √
Suitable storage facilities for cooked							
Can safe holding temperatures be foods achieved?	√	√	√	√	√	√	√
Is all foods consumed within 2 hours?					√	√	√ √ √ √ √ √
Are left over foods recovered for chilling or frozen?					√	√	√ √ √ √ √ √
How are serving spoons kept during service? On the lids of the cooking pot.							
Is there enough supervision?					√	√	√ √ √ √ √ √
Are chilled and ambient storage facilities available?	√	√	√	√	√	√	√
Are adequate frozen facilities available?	√	√	√	√	√	√	√ √ √ √ √ √ √ √ √ √ √ √ √
Can food be defrosted safely?							
Is there pest control system in place?							
Is the swill area kept clean?	√	√	√	√	√	√	√
Is there enough ventilation in the kitchen?	√	√	√	√	√	√	√
Is the kitchen building suitable for catering activities?	√	√	√	√	√	√	√
					√	√	√ √ √ √ √ √

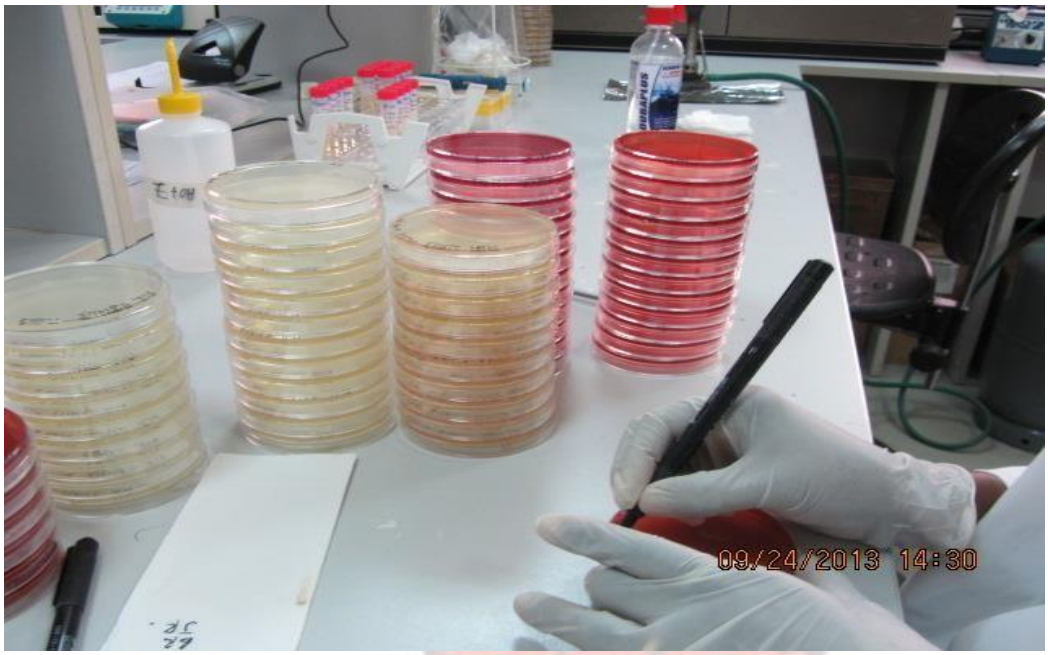
9. Observational criteria used at the Kitchens of Hotel-05

CRITERIA	SCORE YES Period of 5 Days	SCORE NO Period of 5 Days
Personal Hygiene		
Suitable protective clothing		√ √ √ √ √ √ √
Casual clothing	√ √ √ √ √ √ √	
Suitable foot wear	√ √ √	√ √ √ √
Provision of changing room facilities	√ √ √	√ √ √ √
Provision of clean toilet and washing facilities	√ √ √	√ √ √ √
Special sink provided for hand washing	√ √ √	√ √ √ √
Hot water for hand washing		√ √
Cold water for hand washing	√ √ √ √ √ √ √	
Soap for hand washing	√ √ √ √ √ √ √	
Napkins used for drying hands	√ √ √ √ √ √	
Disposable paper towel for drying hands	√	√ √ √ √ √ √ √
Drying hands with hot air dryer		√ √ √ √ √ √ √
Drying hands with roller towel		√ √ √ √ √ √ √
Hands washed before start of work	√ √ √ √ √ √ √	
Hands washed in between work		√ √ √ √ √ √ √

10. Observational criteria used at the Kitchens of Hotel-05

CRITERIA	SCORE	
	YES	**NO**
	Period of 5 Days	**Period of 5 Days**
Are adequate food preparation areas available?	√ √ √ √ √ √ √	
Can thawing be carried out safely?	√ √ √ √ √ √	
Adequate sink and water		√ √ √ √ √ √ √
Separate chopping boards for different foods		√ √ √ √ √ √ √
Are adequate stoves, grills and ovens available?	√ √ √ √ √ √ √	
Can safe cooking temperature be achieved?	√ √ √ √ √ √ √	
Are there suitable storage facilities for cooked foods?	√ √ √ √ √ √ √	
Suitable storage facilities for cooked foods		√ √ √ √ √ √ √
Can safe holding temperatures be achieved?	√ √ √ √ √ √ √	
Is all foods consumed within 2 hours?	√ √ √ √ √ √ √	

Appendix D

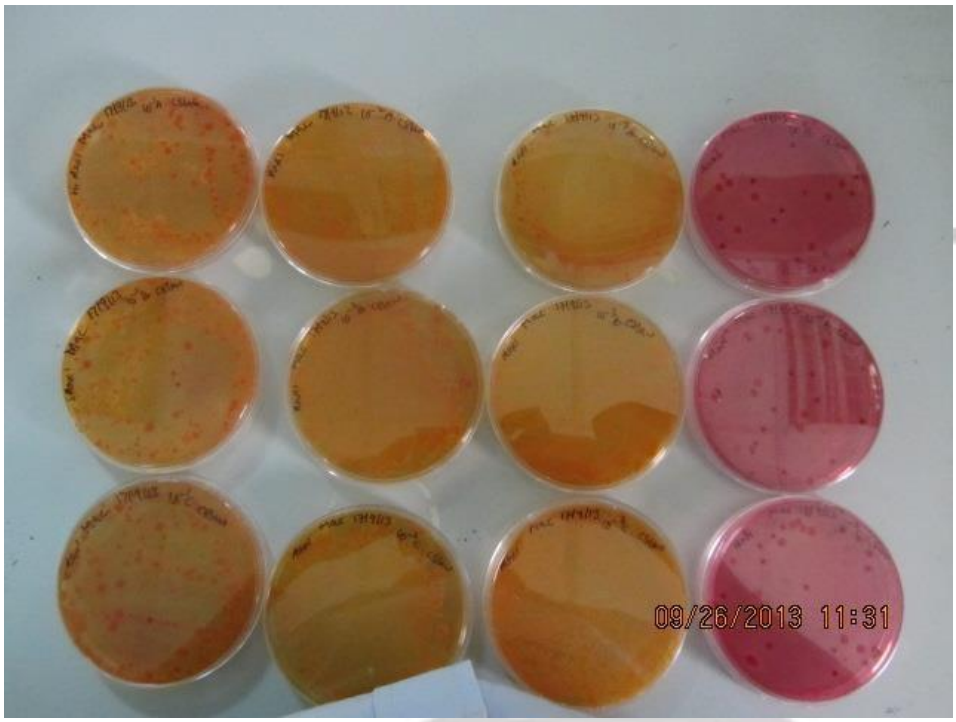


Labelling of agar plates in readiness for incubation

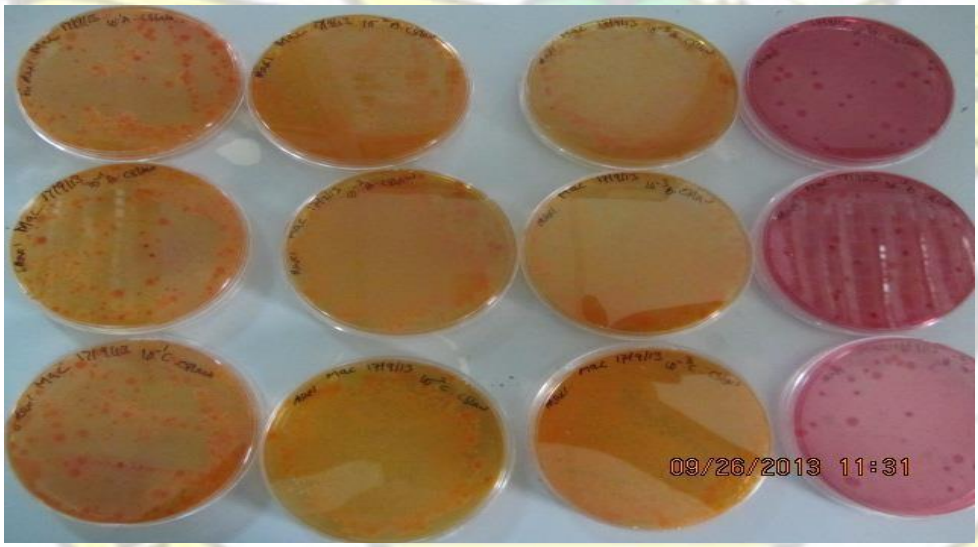
Plated Nutrient and Mac Conkey agar

Water bath used to keep prepared agar at constant temperature

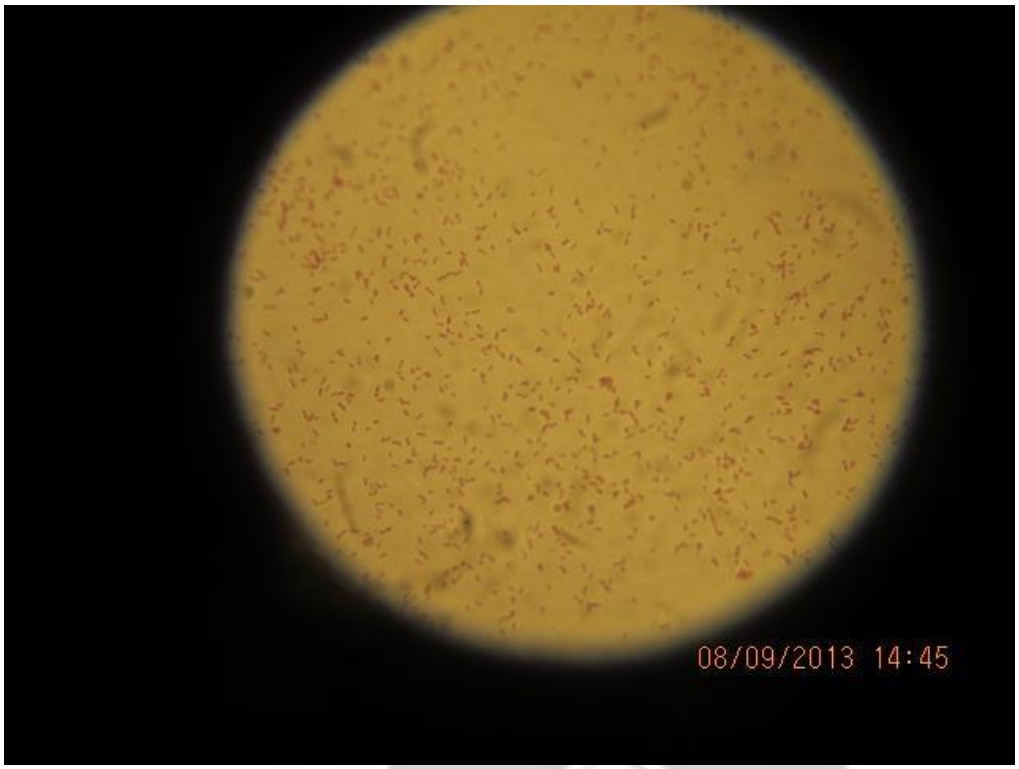
Incubator used in the study to dry agar plates



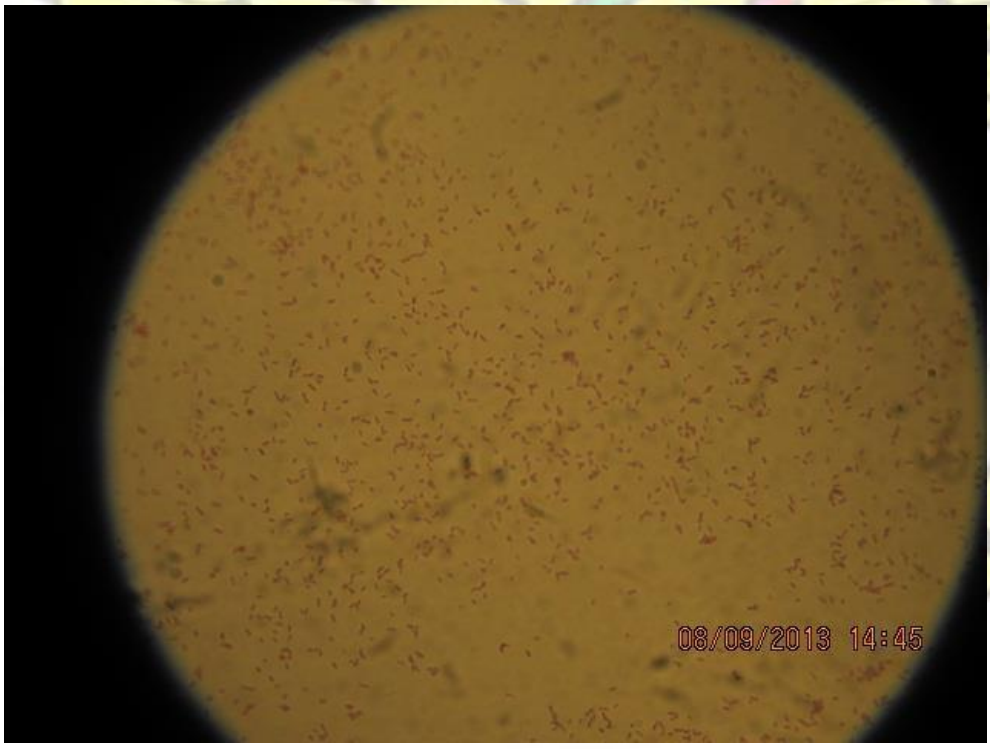
Bacteria growth on Violet Red Bile Glucose Agar Mac Conkey Agar.plates



Bacteria growth on Violet Red Bile Glucose Agar an d Mac Conkey Agar.plates

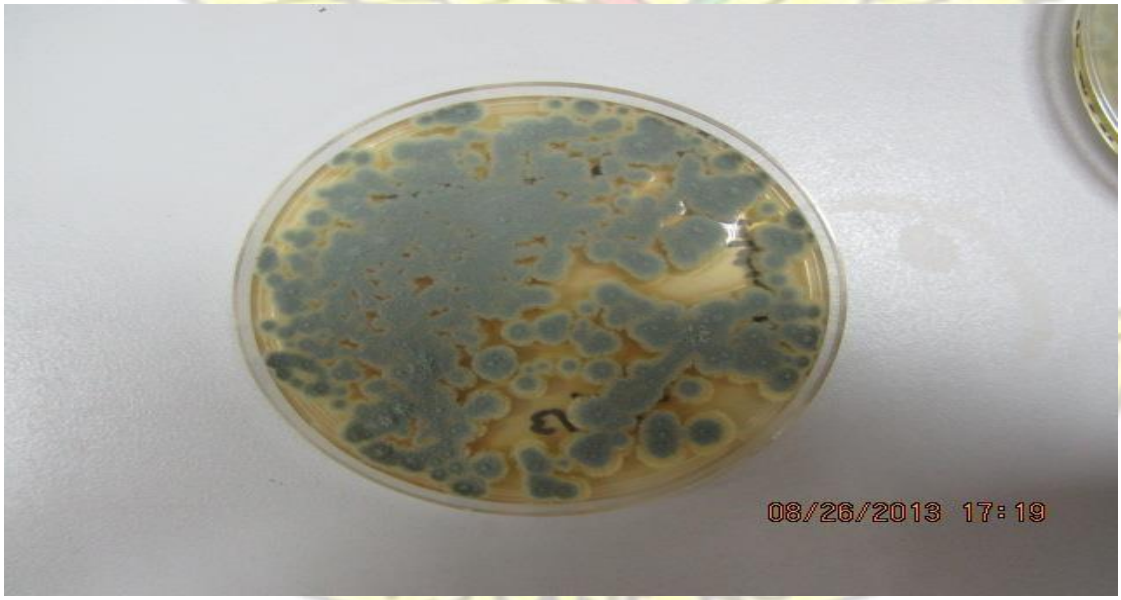


Bacteria growth on food in agar plates

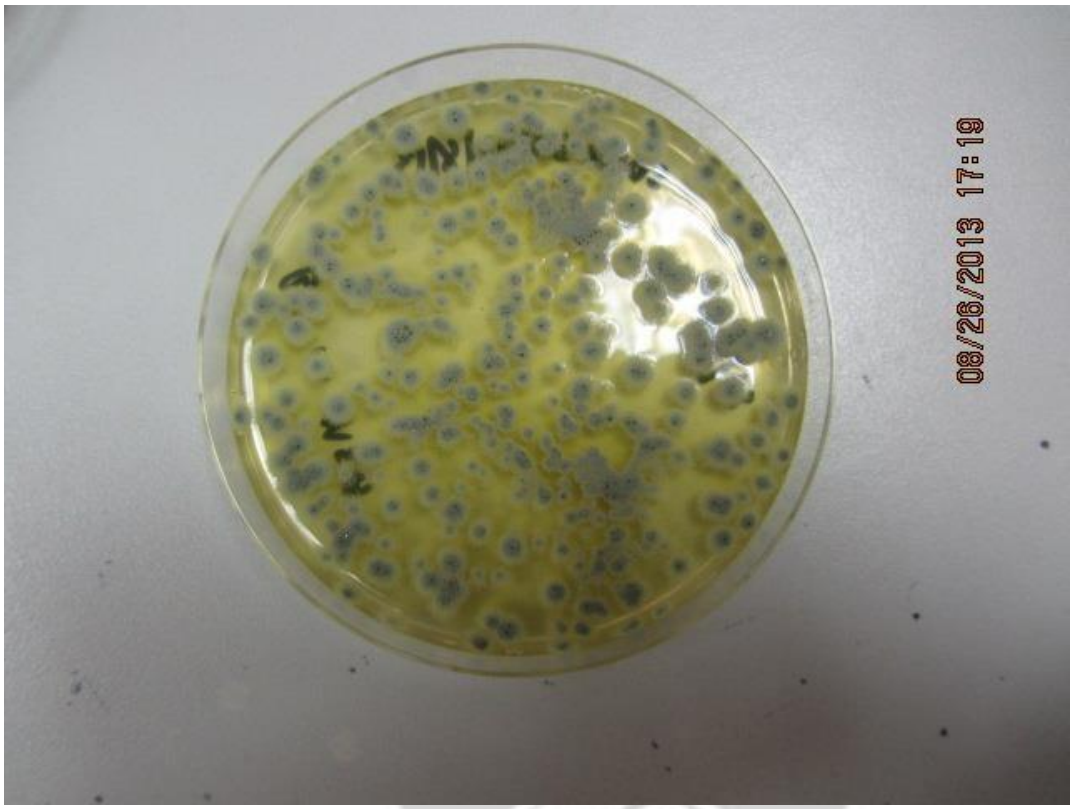


Bacteria growth on food in agar plates

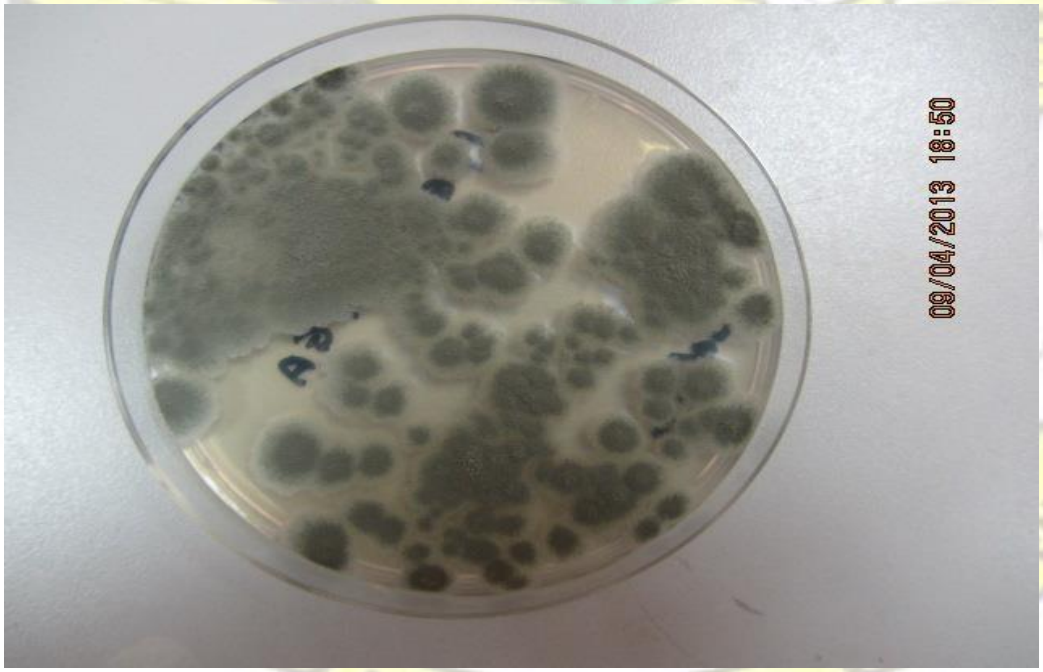
Streaked isolates on Mac Conkey Agar



Fungal growth on Malt Extract Agar

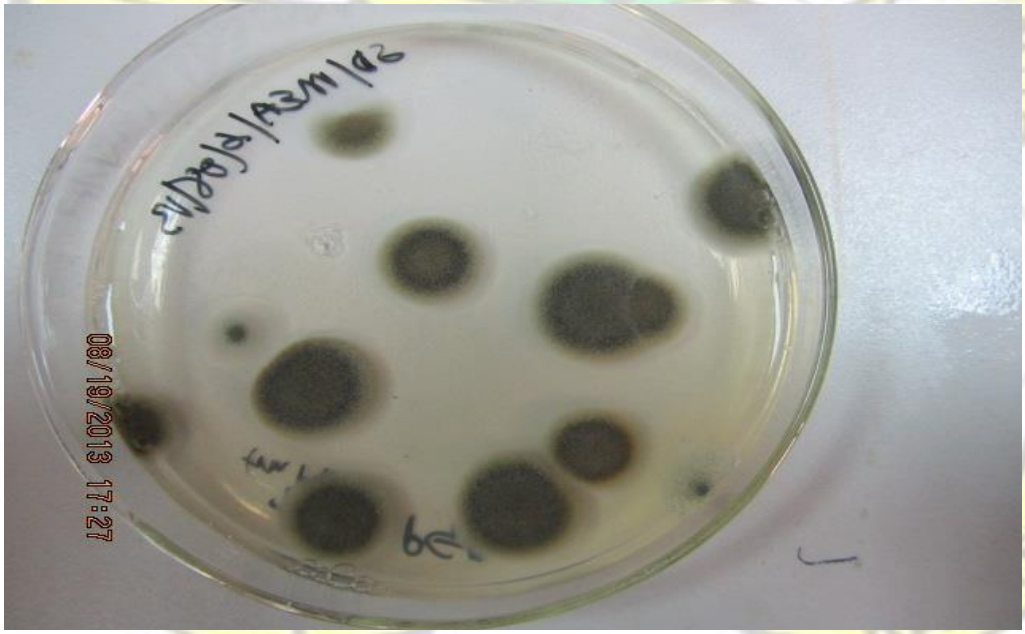


Fungal growth on Malt Extract Agar



Fugal growth on Potato Glucose Agar

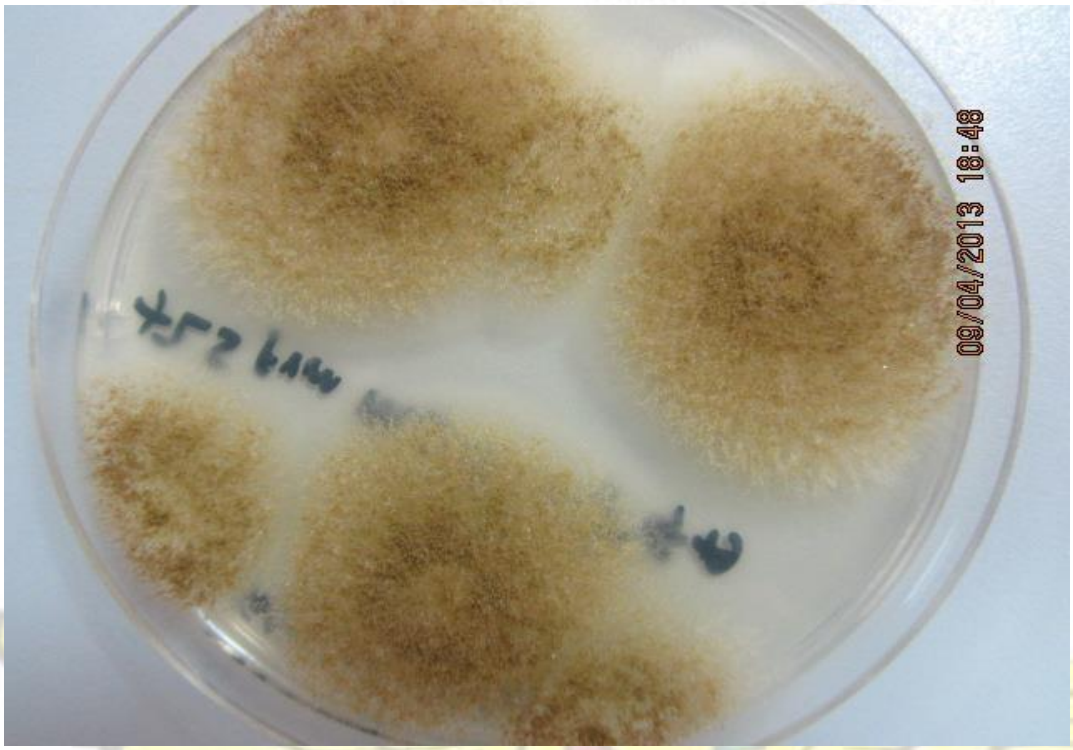
Fungal growth on Potato Glucose Agar



Fungal growth on Malt Extract Agar

Pure colonies of fungal growth on Malt Extract Agar after sub culturing

Pure colonies of fungal growth on Malt Extract Agar after sub culturing



Fungal growth on Potato Glucose Agar

Appendix E: Training Manual for Food Handlers to Ensure Food Safety at all Times

PERSONAL HYGIENE

Hand Washing

All food production and service personnel will follow proper hand washing practices to ensure the safety of food served to customers.

All employees involved in handling food must wash hands using the following steps:

Procedure:

- Wash hands using soap from a soap dispenser. Lather at least 10 seconds.
- Wash hands (including under the fingernails) and forearms vigorously and thoroughly with the soap and warm water (water temperature should be at least 100ºF) for a period of 20 seconds.
- Use a sanitary nail brush to remove dirt from under fingernails.
- Wash between fingers thoroughly.
- Use only hand sinks designated for that purpose. Do not wash hands in sinks in the production area.
- Dry hands with single use towels or a mechanical hot dryer. (Retractable cloth towel dispenser systems are not recommended.) Turn off faucets using a paper towel in order to prevent recontamination of clean hands if foot pedals are not available.

Hand washing should be done at the following times;

- When entering the facility before work begins.
- Immediately before preparing food or handling equipment.
- As often as necessary during food preparation.
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  In the restroom after using the toilet and when you return to your work station.

  When switching between working with raw foods and working with ready-to-eat or cooked foods.

  After touching face, nose, hair, or any other body part, and after sneezing or coughing.

  After cleaning tables.
- ❖ Between each task performed and before wearing disposable gloves.
- ❖ After eating, or drinking.
- ❖ Any other time an unsanitary task has been performed – i.e. taking out garbage, handling cleaning chemicals and picking up a dropped food item or any other item.
- ❖ Wash hands only in hand sinks designated for that purpose.
- ❖ Dry hands with single use towels. Turn off faucets using a paper towel in order to prevent recontamination of clean hands.
- ❖ Change disposable gloves as often as hand washing is required.
- ❖ Wash hands before donning and after discarding gloves.

Work Attire:

- ❖ Wear appropriate and clean uniform with sleeves.
- ❖ Wear clean non-skid, close-toed work shoes (or leather tennis shoes) that are comfortable for standing and working on floors that can be slippery. ❖ Wear clean and appropriate apron on site, ❖ Do not wear apron to and from work.
- ❖ Take off apron before using the restroom.
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- ❖ Change apron if it becomes soiled or stained.

Hair Restraints and Jewelry:

- ❖ Wear a hair net or cap in any food production area that completely covers all hair.
- ❖ Keep beards and moustaches neat and trimmed.

  Refrain from wearing jewelry in the food production area.

  Only a plain wedding band is permitted.

  No necklaces, bracelets, or dangling jewelry are permitted.
- ❖ No earrings or very small ones are permitted.

Cuts, Abrasions, and Burns:

- ❖ Bandage any cut, abrasion, or burn that has broken the skin.
- ❖ Cover bandages on hands with gloves and finger cots as appropriate.

EATING, DRINKING AND GUM CHEWING AT WORK

Restaurant employees will eat and drink in designated areas

Procedure:

- ❖ All restaurant employees must never eat in the work area.
- ❖ Eating (with the exception of cooks tasting foods to ensure quality) is NOT allowed in the production and service areas.
- ❖ Drinking from a closed beverage container or glass of water is permitted in production area, when placed out of sight.
- ❖ Refrain from chewing gum or eating candy during work in a food production area.
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GLOVE AND UTENSILS USE

Gloves or utensils will be used for handling all ready-to-eat foods and also when there are cuts, sores, burns, or lesions on the hands of food handlers.

Procedure:

All employees handling food or utensils must:

- Wash hands thoroughly prior to putting on gloves and when gloves are changed.

Change gloves when:

- Beginning each new task.

  They become soiled or torn.

  They are in continual use for four hours.

  Finished handling raw meat and before handling cooked or ready-to-eat foods.

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- ❖ Use utensils, such as spatulas or tongs, as an alternative to gloves.

  Cover cuts and sores on hands, including fingernails, with clean bandages. If hands are bandaged, clean gloves or finger cots (protective coverings) should be worn at all times to protect the bandage and to prevent it from falling into food.
- ❖ Inform kitchen supervisor of all wounds.

HYGIENE STANDARDS FOR SERVICE

All food will be served in a manner to ensure food safety.

Procedure: Employees involved in the service of food must observe the following procedures:

Cleaning and sanitation:

- ❖ Before food is placed in service area clean on around the service area, using warm soapy water and designated clean cloths. Thoroughly rinse after washing.
- ❖ Sanitize on and around the service area, using an approved chemical sanitizer at proper concentration.
- ❖ Wipe down area as needed throughout service with cloth stored in sanitizing solution away from food.
- ❖ Cloths used for cleaning food spills should not be used for anything else

Service utensils/service ware:

- ❖ Store utensils properly on a clean, sanitized food-contact surface.
- ❖ Keep hands away from the food item.
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- ❖ Clean and sanitize utensils before using.
- ❖ Use separate utensils for each food item.

  Handle glassware and dishes properly; so hands are not in contact with surfaces that will be touched by food or patron's mouth.

  Hold flatware and utensils by the handles.

HOLDING OF FOOD

All hot food will be held hot (above 57º Celsius) and cold food will be held cold (below 5º Celsius). Temperatures of food will be taken routinely to ensure that proper temperatures are maintained through holding to ensure the safety of the food served to customers.

When in doubt about the safety of food, throw it out.

Procedure: Employees involved in the production or service of food must:

Holding Hot Food:

- ❖ Prepare and cook only as much food as is needed. Batch cooking is ideal for maintaining food temperature and quality.
- ❖ Use hot-holding equipment that can keep hot food at 57º Celsius or higher.
- ❖ Follow manufacturer's instructions in using hot-holding equipment. [NOTE: Customize your SOP by including instructions. For example, you may need to indicate that the steam table wells need to be filled with hot water and at what level.]
- ❖ Keep foods covered to retain heat and to keep contaminants from falling into
- ➢ Measuring internal food temperatures once an hour using a calibrated thermometer.
- ➢ Record temperatures in the Holding Temperature Log. If temperatures are below 57º Celsius, then reheat to 74º Celsius.

- Discard hot potentially hazardous food after four hours if they have not been properly held at or above 57º Celsius.
- Do not mix freshly prepared food with food being held for service.

Holding Cold Food:

- Use cold-holding equipment that can keep cold foods below 5º Celsius.
- Measure internal food temperatures once an hour using a calibrated thermometer and record temperatures in the Holding Temperature Log.

  If temperatures are above 5º Celsius, then refrigerate.

  Protect cold food from contaminants with covers or food shields.
- Discard cold potentially hazardous foods after four hours if they have not been properly held below 5º Celsius.
- If there are no temperature controls, cold food held for longer then six hours must be discarded.
- Place cold food in pans or on plates first, never directly on ice.
- Wipe the clean and sanitize thermometer stem with alcohol wipes prior to taking the temperatures of each food. Open the sanitizer package with clean hands.

Preparing cold foods:

- Pre-chill ingredients for food served cold (sandwiches and salads) to below 5°C before combining.

REHEATING FOOD

All food will be reheated to an internal temperature of 74° Celsius and held at least 15 seconds to assure the safety of food.

Procedure: Employees reheating food should:

- Remove leftover food from the freezer/refrigerator.

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- ❖ Check the temperature of the food to make sure it is lower than 5° Celsius using a calibrated thermometer. Record on the Reheating Log.
- ❖ Reheat the food product to 74° Celsius for 15 seconds using an oven, stove, or steamer. The goal is to take the food through the temperature danger zone (5° Celsius - 57° Celsius) as quickly as possible. Discard food that has not reach this temperature within two hours.
- ❖ Serve the food immediately, or place the food in a steam table or a pre-heated hot cart and recheck temperature to make sure temperature is held at or above 57° Celsius.

- Check the temperature of the food before serving if the food has been held.
- Discard any potentially hazardous foods held in the temperature danger zone (5° Celsius to 57° Celsius) for more than four hours.

COOKING FOOD

All foods will be cooked using appropriate practices and procedures to ensure safety. This includes cooking foods to required internal temperatures and taking and recording temperatures.

THAWING FOOD

All foods will be thawed using appropriate practices to ensure food safety.

Procedure: Steps for thawing food include:

- Use one of the three acceptable methods for thawing food:
- Thaw food in the refrigerator at 5° Celsius or below. NEVER thaw food at room temperature.
- Thaw food needed for immediate service under potable running water at 21° Celsius or lower. Prepare the product within 4 hours of thawing.
- Thaw the product in the microwave if product will be cooked immediately.
- Use the lowest shelf in the cooler for thawing raw meat to prevent cross-contamination and separate raw products from cooked and ready-to-eat products.
- Do not refreeze thawed food, unless they are first cooked or processed.

Food contact surfaces:

- When possible use color-coded cutting boards for all products. Red for raw meat, green for vegetables or fruits, and yellow for raw poultry.
- Food contact surfaces should be smooth, easily cleaned and sanitized, with appropriate material. Clean and sanitize all food contact surfaces prior to and after use. Cleaning and sanitizing steps need to be done separately in order to be effective.

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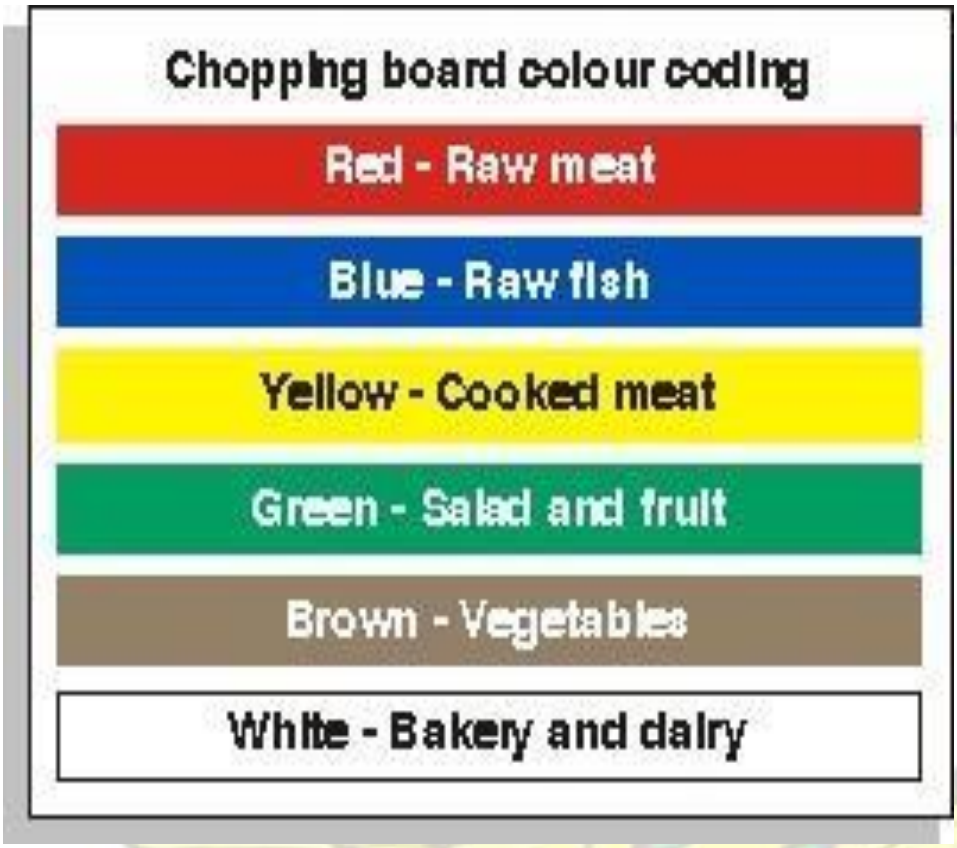
Chopping board colour coding
Red - Raw meat
Blue - Raw fish
Yellow - Cooked meat
Green - Salad and fruit
Brown - Vegetables
White - Bakery and dairy

Colour Coded Chopping Boards

Eliminate the risk of bacterial cross contamination during food preparation!