APPLICATION OF HAZARD ANALYSIS CRITICAL CONTROL POINT ON SOME FOOD SERVICE CENTRES IN ZARIA, NIGERIA

BY

BINTA SAMBO
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A THESIS

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DECLARATION

I hereby declare that the work reported in this thesis was carried out by me under the careful supervision of Dr. (Mrs) V. J. Umoh and Dr. C. M. Z. Whong of Ahmadu Bello University Zaria. No part of this work was reproduced from any previous work or had been presented for an award of higher degree. The works of other researchers cited were duly acknowledged.

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Binta Sambo
Ahmadu Bello University Zaria

CERTIFICATION

This Thesis entitled "Application of hazard analysis critical control point on some food service centres in Zaria, Nigeria" carried out by Binta Sambo meets the regulations governing the award of degree of Master of Science, Ahmadu Bello University Zaria and is approved for its scientific and literary contributions.

…….. ……..……..……

Dr. (Mrs) V. J. Umoh Date
Chairman,
Supervisory Committee

…….. ……..

Dr. C. M. Z. Whong Date
Member,
Supervisory Committee

…… ……..

Head of Microbiology Department Date
Ahmadu Bello University Zaria

……. ……..

Dean of Postgraduate School Date
Ahmadu Bello University Zaria

DEDICATION

To my husband, Abdullahi Mukhtar Muhammad who had sacrificed immensely towards the realization of this work and the faith and confidence he placed on me.

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I am most grateful to the Almighty Creator for His infinite mercy, guidance and protection throughout the course of this study.

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ABSTRACT

A survey of the safety of some selected foods sold in some food service centres in Zaria was carried out by applying the hazard analysis critical control point (HACCP) strategy. The analyses consisted of observing the raw materials and environment, watching all steps of the food preparation and handling, recording temperatures and pH before cooking, immediately after cooking and during holding and collecting samples of foods at these points and testing for total viable, coliform, staphylococcal and *Bacillus* counts and presence of *Salmonella*. Structured questionnaire was used to collect information on personal characteristics and hygiene and food handling techniques. All Microbiological analyses were performed using standard procedures. Out of 90 respondents, 53(58.9%) were less than 30 years, 53(58.9%) were males, 37(41.1%) females and 83(84.4%) had formal education and exhibited good hygiene behaviour. Analyses of variance of the mean counts of the organisms from the three categories of food service centres were not significantly different ($p > 0.05$). However, mean counts ranging from 5.76 - 7.91 $\log_{10}$ cfu/g for *B.cereus* and 5.99 – 7.91 $\log_{10}$ cfu/g for *S.aureus* obtained in the foods were above the standard acceptable limits of less than 5.0 $\log_{10}$ cfu/g for *B.cereus* and less than 6.0 $\log_{10}$ cfu/g for *S.aureus.* Out of 90 food and 18 swab samples tested, 82 (75.9%) were contaminated with *S. aureus,* 68 (62.9%) with coliforms and 57 (52.7%) with *B. cereus.* No *Salmonella* was isolated from all the samples. *E. coli* was isolated from pounded yam and vegetable soup in “Bukas” and Cafeterias. The antibiotic-sensitivity test of the isolates to eight antimicrobial agents revealed that 82 out of the 92 isolates tested were resistant to the common drug used in the treatment of infections.

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CHAPTER ONE

Introduction

The foods that we eat are rarely if ever sterile, they contain micro-organisms whose composition depends upon which organisms gain access and how they grow, survive and interact in the food over time. The micro-organisms present will originate from the natural micro flora of the raw materials and those organisms introduced in the course of harvesting, slaughter, processing, storage and distribution (Adams and Moss, 1999). This is not surprising as bacteria and fungi are ubiquitous and are especially plentiful in soil and the environment. From the microbial perspective, food could be viewed as a fertile ecosystem in which these organisms vie for the rich nutrients. The successful micro-organisms earn the right to multiply and predominate (Nester *et al.,* 2001).

In addition to its undoubted value, food has a long association with the transmission of disease. Regulations governing food hygiene can be found in numerous early sources such as the Old Testament, and the writings of Confucius, Hinduism and Islam (Adams and Moss, 1999). Such early writers had at best only a vague conception of the true causes of food-borne illness and many of their prescriptions probably had only a slight effect on its incidence. Even today, despite our increased knowledge, food-borne disease is perhaps the most widespread health problems in the contemporary world and an important cause of reduced economic productivity (WHO 1992). More than two hundred known diseases are transmitted through food (Bryan, 1988). The causes of food-borne illnesses include viruses, bacteria, parasites, toxins, metal, prions, and the symptoms range from mild gastroenteritis to life threatening neurological, hepatic, and renal syndromes (Mead *et*

al., 1999). The available evidence clearly indicates that biological contaminants are the major causes of food borne diseases (WHO, 1992). The problems of food safety in the industrialized world differ considerably from those faced by developing countries. Whereas traditional methods are used for marketing fresh produce in the latter countries, food processing and packaging are the norm in industrialized countries (Mensah *et al.,* 2002). In many areas of the developing countries including Kaduna, Nigeria, untreated waste water flow through channels and rivers to adjacent areas where it is diverted by subsistence farmers to small plots of vegetable crops grown for nearby urban markets. Such vegetables include; carrots, lettuce, tomatoes, cabbage, spinach, okra, garden eggs, and a host of others (Okafo *et al.,* 2003). The public health risks in such uncontrolled use of raw waste water for irrigation are obvious. *Salmonella, E. coli* (Tauxe *et al.,* 1997), *Vibrio* (Islam *et al.,* 1990) and the Helminths (Shuval *et al.,* 1984) have been implicated in fresh produce associated with food-borne disease outbreaks.

Food service establishment refers to a place in which foods are prepared and either served and eaten or taken or sent out to be eaten elsewhere. It includes institutional facilities, catering operations and restaurants (Bryan *et al.,* 1997). In Nigeria, like other developing countries, food service operations are means of income generation by the highly and lowly educated, especially women and their families. Most of such foods are prepared daily from a variety of ingredients to suit both local and continental taste and demand. Food service establishments provide an essential service to people of all walks of life by selling complete ready to eat meals, refreshing drinks and snacks to them. However, such practices as frequent handling of food with bare hands, addition of contaminated raw ingredients, use of contaminated water and preparation of foods in a polluted environment can expose the food to contamination

with pathogens which could result in health hazards (Ehiri *et al.,* 2001). Although there are no surveillance activities in Nigeria, laboratory studies have shown the presence in some street foods of food-borne pathogens and high microbial load (Umoh and Odoba, 1999).

To develop a better understanding of the microbiological problems associated with food preparation and handling in these food service establishments, it became extremely necessary to apply the hazard analysis critical control point (HACCP) strategy. The Hazard analysis critical control point strategy identifies hazards associated with different stages of food preparation and handling, assesses the relative risks and identifies points where control measures would be effective (Bryan, 1988, Ehiri *et al.,* 2001). There has been minimal research into food safety and food handling practices in food service establishments in Nigeria, although similar studies have been conducted in the United Kingdom and United States of America (Taylor, 2001 and Walker *et al.,* 2003). The fact cannot be overemphasized that ready-to-consume foods pose a direct health hazard to consumers if they contain an infective dose of pathogens or toxic levels of their toxins (Adesiyun, 1995). Attempts to control these diseases by chemotherapy through the use of antimicrobial agents particularly antibiotics have resulted in increased prevalence of resistance to these agents. The situation is made more difficult in developing countries including Nigeria where antimicrobial drugs are readily available to consumers across the counter with or without prescription from a medical practitioner (Chinwe and Ezeronye, 2003).

It is against this backdrop that this study was undertaken with the following objectives;

1. To study the level of knowledge of hygiene, sanitation, and food safety in some food service centres, using standard questionnaire forms.
2. To study the safety of some selected foods in some of the centres by the application of Hazard Analysis Critical Control Point (HACCP) system.
3. To characterize the isolates using biochemical tests.
4. To study the antibiotic susceptibility pattern of the confirmed isolates.

CHAPTER TWO

Literature Review

2.1 Health Risks Associated With Food Service Establishments

When food poisoning out breaks are investigated, it has been established that small and medium sized businesses are often important locations in the transmission of food-borne illness (Walker *et al*., 2003). Health risks are associated with initial contamination of raw foods with pathogenic bacteria and subsequent contamination by food handlers during preparation from cross-contamination, survival of pathogens during preparation and microbial proliferation during display (Bryan *et al*., 1988). The use of bare hands to serve food increases the level of contamination as entero-pathogens can survive on the hands for three hours or longer (Mensah *et al*., 2002). The container in which food are served is an important risk factor and the use of paper and leaves increased the risk of contamination. The paper used for holding food is usually newsprint of questionable origin. Leaves are wiped with a piece of cloth without disinfecting. Clearly, the possibility exists that micro-flora on leaves and microbes acquired through poor handling are transferred to foods (Mensah *et al*., 2002). Handling of food at ground level also increases the risks of contamination because dust could easily be blown on to food thus handled. There is also a risk of contamination associated with the exposure of food to flies (Mensah *et al.,* 2002).The preparation of "fufu" involves pounding the staple after cooking by means of a mortar and pestle and turning the stiff dough with the bare hands, which were occasionally washed in a container of water (Mensah *et al.,* 2002).

The fact cannot be overemphasized that ready-to-consume foods pose a direct health hazards to consumer if they contain an infective dose of pathogens or toxic levels of their toxins (Adesiyun, 1995).

2.2 Hazard Analysis Critical Control Point System

Hazard analysis critical control point or HACCP is a quality assurance tool that has been specifically developed for the food industry to enable management to achieve food safety all the way from “the farm to the fork”. It ensures food safety through biological, chemical and physical hazard identification and control in the production process (Buchanan, 1995).It is a powerful food safety management technique that is logical, systematic and preventive in nature. A better approach compared to the traditional inspection and end product methods. It is cost effective and it also complements other Quality Management Systems (QMS) such as ISO 9000. The hazard analysis critical control point system is a risk- reduction system originally developed in the 1960s for foods used in space travel (“Space Foods”), but not applied to the food industry until the 1990s.(Alcamo, 2001).

Hazard analysis critical control points (HACCP) concepts can be applied throughout the food chain, including food- packaging materials, from the primary producer to the end consumer. This would include food and beverage manufacturers, centralized kitchens in the restaurants, hotels and hospitals, catering businesses, pharmaceuticals, and medical device. Both multi-national companies as well as small family-run businesses will benefit from this system (Buchanan, 1995).

Hazard Analysis: - Is the identification of sensitive ingredients, sensitive areas in the processing of food or ingredients, people control etc, from which we can identify the critical points that must be monitored to assure safety of the product.

Critical Control Points (CCP): Are those areas in the chain of food production, from raw materials to finished product(s), where the loss of control can result to an unacceptable food safety risk (Bauman, 1990). A condition is a hazard if its elimination or reduction to acceptable level is essential for the production of safe food.

Hazards are classified according to WHO, (1999) as; Physical, Biological and Chemical.

Physical Hazards: Includes, glasses, metals, wood fragments and other objects that may cause physical damage to the consumer (mouth wound, broken teeth, and others requiring surgical removal). Of the three main types of hazards, the microbiological hazard is the greatest threat to food safety (WHO, 1999).

Biological Hazards: Include Bacteria, Fungi Viruses, and their toxins and pathogenic parasites. These organisms are commonly associated with both contamination from handlers and raw products entering the food establishment. Most of them are killed or inactivated by cooking, and many can be minimized by adequate control of handling and storage practices (hygiene, temperature and time).

Chemical Hazards: Comprise pesticides, herbicides, toxic contaminants, toxic food additives, lubricants, and disinfectants.

2.2.1 Principles of HACCP

There are seven principles of HACCP on which a food safety management system is based. A food safety management system based on the principles of HACCP will enable hazards to be identified and controlled before they threaten the safety of food (Adams &Moss1999).

1. **Identify the Hazards;** Look at each step (e.g. purchasing, delivery, storage, preparation, cooking, chilling etc.). In an operation and identify what can go wrong e.g. ***Salmonella*** in a chicken product (biological hazard), detergent in a chicken product (chemical hazard) or a piece of glass in a salad (physical hazard).
2. **Determine the Critical Control Points (CCPs):** Identify the points in an operation that ensures control of the hazards e.g. cooking burgers to a minimum of 70^{o}C for 2 minutes will kill *E. coli* 0157 and other pathogens.
3. **Establish Critical Limit(s):** Set limits to enable the identification of a CCP when it is out of control e.g. the temperature at the centre of a beef product following cooking must reach a minimum of 70^{o}C for 2 minutes.
4. **Establish a System to Monitor Control of the CCP:** When CCPs and critical limits have been identified it is important to have a way to monitor and record what is happening at each CCP. Typically monitoring will involve measuring parameters such as temperature and time. Monitoring should in all cases be simple, clear and easy to use e.g. recording the final cooking temperature and time for a cooked chicken product.
5. **Establish the Corrective action to be taken when monitoring indicates that a particular CCP is not under control:** When monitoring indicates that a CCP is not under control, corrective action must be taken e.g. the temperature of the food in a refrigerator rises to 10^{o}C due to a technical fault. Discard the food and repair

the refrigerator using the manufacturer's instructions to ensure the correct temperature of 5^{o}C is achieved.

6. **Establish Procedures for Verification to confirm the HACCP system is Working Effectively:** Review and correct the system periodically and whenever changes are made to an operation e.g. microbiological analysis of a chicken product to verify that it is free of ***Salmonella*** before and after cooking.
7. **Establish documentation concerning all procedures and records appropriate to these principles and their application:** For the successful implementation of HACCP, appropriate documentation and records must be kept and be readily available. It is unrealistic to operate HACCP or to demonstrate compliance with the current legislation without providing evidence such as written records. As with the HACCP itself, the complexity of the record keeping will very much depend on the nature and complexity of the business. The aim should be to ensure control is maintained without generating excessive paperwork e.g. cooking temperatures, delivery or cleaning records.

2.2.2 Food Safety Practices

A failure in implementing food safety practices can predispose a prospective consumer to food poisoning risks and can as well put out a food business. A Hazard Analysis Critical Control Point (HACCP) programme is the best way to protect a food business from the risk. (WHO, 1999 and Panisello *et al.,* 1999).

The chart below takes a look at how food is handled from delivery to table, and the temperature safety standards defined by current government standards.

1. **Receiving:** Before accepting deliveries, make sure the temperature is within prescribed limits. Check both surface and interior temperatures. Use either an instant read or a pocket digital thermometer for a quick interior check and a laser infrared thermometer for surface temperatures. Refrigerated foods not higher than 41^{o}C (5^{o}C), and frozen foods not higher than 0^{o}F (-17.8^{o}C). Use a thermometer for quick, easy inspection of frozen food deliveries. Discard dry goods if there is any dampness, discoloration or damaged package.
2. **Storing:** All foods must be properly stored to minimize the risk of bacterial growth or contamination. Refrigerated foods must be stored at 41^{o}F (5^{o}C) or lower, use separate refrigerator thermometer for different food storage temperature. Frozen foods need an air temperature below 0^{o}F (-17.8^{o}C).Use two freezer thermometers, one at the warmest point, the other at the coldest to monitor air temperature.
3. **Preparing/Cooking:** Always use a good quality cutting board. Choose plastic cutting boards over wooden ones. Bacteria can hide in the cracks of wooden cutting boards and utensils. Be sure to wash hands in warm soapy water before preparing food and after using the bathroom, changing diapers and handling pets. Kitchen towels, sponges and cloths can harbour bacteria. Wash them often and replace sponges every few weeks. Food borne pathogens are killed by safe cooking temperatures. Use a digital or laser thermometer to check foods often, to help prevent bacteria from multiplying on foods too quickly and to ensure foods, grills, fryers and ovens maintain the right temperature for uniform cooking.

Salmonella can grow inside fresh, unbroken eggs. Be sure to cook eggs until the yolk and white are firm, not runny. Scramble eggs to a firm mixture. Avoid recipes in which eggs remain raw or only partially cooked (for example, Mouse, egg drinks, Caesar salad, etc). Pasteurized eggs or egg substitute can be used instead. This is particularly important for children, pregnant women, the elderly and those whose immune systems are compromised by illness or by medical treatment (e.g. chemotherapy). If food is prepared and cooked ahead of time, divide large portions into small, shallow containers and refrigerate. This ensures rapid, safe cooling.

4. **Serving/Holding:** Hold foods out of the danger zone, hot foods above 140^{o}F (60^{o}) and cold foods below 41^{o}F (5^{o}C), to inhibit bacteria. Always use thermometers to check air temperature in holding cabinets, hot boxes, walk in coolers, freezers, steam tables and under heat lamp warmers. Always use clean dishes and utensils to serve food, not those used to prepare the food.
5. **Reheating:** Some bacteria survive cooking and multiply to dangerous levels during chill- down. Food must be reheated to an internal temperature of 165^{o}F (74^{o}C) to eliminate new pathogens. Use an instant read, laser or hand-held digital thermometer to ensure all foods are reheated adequately. Reheating kills pathogens, it won't eliminate toxins, such as that produced by *Staphylococcus aureus*.

Table 2.1 U.S. FOOD AND DRUG ADMINISTRATION CONSUMER GUIDELINES FOR SAFE INTERNAL TEMPERATURES OF SOME FOODS, 2001

Fresh Beef		
	Fahrenheit	**Celsius**
Medium Rare	145	63
Medium	160	71
Well done	170	77
Ground Beef	160	71
Fresh Veal		
	Fahrenheit	**Celsius**
Medium Rare	145	63
Medium	160	71
Well Done	170	77
Fresh Lamb		
	Fahrenheit	**Celsius**
Medium Rare	145	63
Medium	160	71
Well Done	170	77
Game		
	Fahrenheit	**Celsius**
Deer	165	74
Rabbit	180	82
Duck	180	82
Goose	180	82
Ratites		
	Fahrenheit	**Celsius**
Ostrich	160	71
Rhea	160	71
Emu	160	71
Eggs		
Fried Poached	(Cook until yolk and white are firm)	
Casseroles	160	71
Sauces, Custards	160	71
Poultry		
	Fahrenheit	**Celsius**
Chicken	180	82
Turkey	180	82
Turkey Roast (boneless)	170	77
Stuffing (inside of outside bird)	165	74
Fresh Pork		
Chops, Roast, Ribs		
Medium	160	71
Well Done	170	77
Cured Pork		
	Fahrenheit	**Celsius**
Ham, Fresh	160	71
Sausage, Fresh	160	71

(Alcamo, 2001)

The standard regulations of U.S. Food and Drug Administration (FDA) require food processors to monitor and control eight key sanitation areas (Alcamo, 2001).

1. The safety of water that contacts food or used to make ice.
2. The condition and cleanliness of utensils, gloves, outer garments, and other food contact surfaces.
3. The prevention of cross-contamination from raw products and unsanitary objects to foods.
4. The maintenance of hand-washing and toilet facilities.
5. The protection of foods and food surfaces from adulteration with lubricants, fuel, pesticides, sanitizing agents and other contaminants.
6. The proper labelling, storage, and use of toxic compounds.
7. The control of employee health conditions that could result in food contamination.
8. The exclusion of insects and pests from the food plant.

The hazard analysis critical control point system is now the food safety management system of choice for most countries throughout the world and pressure for its adoption world wide is growing stronger (Buchanan,1995).

2.2.3 Risk Assessment in HACCP

Historically, efforts to improve the safety of foods through control or inspection of processing and handling have been based on subjective qualitative assessments (Hathaway, 1993). The need for more systematic quantitative methods has been identified (Hathaway, 1993). Hazard analysis and critical control point (HACCP) and risk assessment provide tools that may help (Mcnab, 1998). Risk assessment can be viewed as a method of systematically identifying and assessing factors that

influence the probability and consequences of a negative event occurring. Components of this may include a wide range of specific efforts such as studies investigating the carcinogenicity of a new chemical or the virulence of an emerging pathogen, studies investigating acceptable levels of exposure to a chemical or the thermal destruction of bacterial spores, biological dose-response studies, or economic impact studies (Mcnab, 1998). A complete risk assessment might require linking information from quantitative risk assessments, microbial predictive modelling, and dose-response modeling can assist on the design and refinement of HACCP programmes for microbial food safety (Buchanan 1995, Kindred, 1996 and McMeekin and Ross, 1996). If we are uncertain of the factors that influence the probability and impact of food poisoning, then we are less certain of the efficacy and efficiency of strategies for presenting microbiologically safer foods for consumption (Mcnab, 1998). Therefore understanding uncertainty is important in quantitative risk and policy analysis (Morgan *et al*., 1992). The risk assessment helps analyze the entire food process, identify the critical points, and set critical limits. It also evaluates alternative processes and out of process events such as a breakdown in a refrigeration system (Whiting and Buchanan, 2001). The risk assessment provides the underlying support to the HACCP plan and is the means to quantitatively link the food process to public health (Buchanan, 1995).

2.2.4 HACCP of Common Complementary Foods

During cooking all foods attained temperatures capable of destroying vegetative forms of food-borne pathogens. However, the risk of contamination increases by storage of food at ambient temperature, by using insufficiently high temperatures to reheat the food, and by adding contaminated ingredients such as

dried ground Cray fish and soy beans powder at stages where no further heat treatment was applied. The purchase of contaminated raw foodstuffs and ingredients, particularly raw “akamu” from vendors in open markets is also a critical control point (CCP) (Ehiri *et al.,* 2001). In a comprehensive review of studies assessing the contamination of complementary foods, (Ehiri *et al.,* 2001) stressed the need to apply the hazard analysis critical control point strategy. Heat treatment of food (e.g. cooking) not only improves the taste, smell, appearance and digestibility, it also reduces the number of micro-organisms, Improves keeping qualities by inhibiting moulds, yeast and bacteria that promote decay and infection. Thus, heat treatment is a practice aimed at improving overall safety of food. This makes it a CCP (Oranusi *et al*., 2003).

2.2.5 Combinations of Complementary Foods and Storage

Combinations of different foods increase the risk of rapid bacterial growth during storage. While the combinations may increase palatability and nutritionally beneficial to children they typically involve the addition of microbiologically sensitive ingredients, such as soy beans powder, dry ground crayfish and dry ground chillies (pepper) (Ehiri *et al.,*2001).

In Nigeria, Ogbadu *et al.,* (2000), associated the regular stomach disorder after consumption of high peppery foods to the presence in such condiments a high numbers of *B. cereus.* While on display in markets, all complementary foods are exposed to dust and flies, and are constantly handled with unwashed hands and serving spoons (Ehiri *et al.,* 2001).

2.2.6 CCP of Some Nigerian Complementary Foods

1. **Akamu:** This is the most widely used complementary food in eastern Nigeria, it is also known as “Ogi” in western Nigeria or “Ugi” in parts of east Africa. It is a complex carbohydrate produced by fermentation of Maize, millet, or sorghum (guinea corn). The major CCPs are; purchasing from unreliable sources, including market vendors, cooking, addition of contaminated ingredients after heat treatment, storage after preparation and reheating; (Ehiri *et al.,* 2001).
2. **Jollof Rice:** This dish is a very common in Nigerian staple diet; it consists of cooked rice mixed with ingredients such as crayfish, palm oil, tomatoes, onions, and seasoning. Meat and fish are normally added, depending on cost. The major CCPs are cooking, storage, and reheating; (Ehiri *et al.,* 2001).
3. **Moimoi:** This food is made from black-eyed beans (cowpeas). Sources of potential hazards in its preparation and serving include the use of contaminated water for processing, dirty utensils, and contamination by food handlers. There is also considerable potential for contamination from the use of empty milk cans for steaming and from unwashed leaves used for wrapping the food. However, most of the risks can be eliminated with adequate cooking. For this reason, the CCPs are cooking, storage, and reheating (Ehiri *et al.,* 2001).
4. **Agidi:** “Agidi” is made from dry ground maize that has been cooked to boiling point. It is relatively safe if prepared under hygienic conditions and served immediately after preparation. The CCPs include cooking, storage and reheating.

The most important faulty practice includes; intermittent assessment of manual grinding, using unwashed hands, using dirty utensils, and using potentially contaminated wrapping materials. Ehiri *et al.,* 2001 used the HACCP strategy to study diarrhoea diseases among children in 120 household in Imo state, Nigeria and in their

conclusion stated that: Although an unsafe environment poses many hazards for children's food, the hygienic quality of prepared food can be assured if basic food safety principle are observed. When many factors contribute to food contamination, identification of CCPs becomes particularly important and can facilitate appropriate targeting of resources and prevention effort. Using HACCP data to promote complementary food hygiene is of paramount importance in situations of extreme poverty and where adequate surveillance of food borne disease may be lacking (Ehiri *et al.,* 2001). The data can be used to inform health and social authorities, train, public health personnel and design culturally appropriate hygiene intervention (Ehiri *et al.,* 2001). Epidemiological evidence shows that undue delay between cooking and consumption of food is a major contributing factor to most outbreaks of food diseases (Ehiri *et al.,* 2001).

2.3 Diarrhoeal Disease and the Developing Countries

Each year, in the developing countries, one out of five children dies of diarrhoea before the age of five years, most of the 5 million individuals who die of diarrhoea each year are infants, but no age group is spared (Nester *et al.,* 2001). The epidemiology of this disease involves transmission by the Faecal-oral route, usually ingesting food or drinking water contaminated with animals or human faeces. Diarrhoea is defined as the passage of loose, liquid or watery stools. These liquid stools are usually passed more than three times a day (Park, 2002). The all too familiar symptoms of lower alimentary system disease include diarrhoea, loss of appetite, nausea and vomiting, and sometimes fever or any combination of these symptoms can occur. Members of the medical profession ascribe the symptoms as gastroenteritis, while other prefer the term "Stomach Flu". The name dysentery is

given to diarrhoea illness when pus and blood are present in the faeces (Nester *et al.,* 2001). Diarrhoea and dysentery rank second to malaria among the ten common types of infection in Nigeria, and rank the highest among the five most common causes of deaths in hospitals (Linus, 2003).A survey on knowledge, attitude and responses of a rural Nigerian community to outbreaks of cholera by Bamidele *et al.,* 2003, revealed that in spite of the people's knowledge of the causes and health consequences of cholera infection this did not translate into proper attitude and practice of appropriate environmental sanitation.

Behaviour related to personal and environmental hygiene and food preparations contribute greatly to the occurrence and severity of out breaks. These out breaks have never been announced publicly and this supports the claim that epidemics are being down played by Government officials in this country (Bamidele *et al.,* 2003). In the spring of 1997, an epidemic of severe diarrhoea broke among 90,000 sick and malnourished refugees in the Democratic Republic of Congo. More than 1,500 deaths occurred over a 3-week period (Nester *et al*., 2001).

The main agents of diarrhoeal diseases are:-

1. Enteroviruses, e.g. rotavirus;
2. *Escherichia coli*
   a. Enterotoxigenic *E. coli* (ETEC)
   b. Localize- adherent *E. coli* (LA-EC)
   c. Diffuse- adherent *E .coli* (DA-EC)
   d. Enteroinvasive *E coli* (EIEC)
   e. Enteroheamorrhagic *E .coli* (EHEC)
3. *Campylobacter* spp.
4. *Shigella*
5. *Vibro cholerae* 01 and 0139
4. *Salmonella* (non-typhoid)

5. *Entamoeba histolytica*
6. *Giardia Lambia*
7. *Cryptosporidium*

(Lucas and Gilles 2003)

Bacillus cereus causes distinct exo-toxin mediated diarrhoea and emetic food poisoning syndromes and a variety of infections (Umoh *et al.,* 1995).

2.3.1 Control of Diarrhoeal Diseases

Programmes for the reduction of diarrhoea morbidity and mortality include;

1. Oral dehydration therapy; highly effective in preventing death from dehydration in acute episode.
2. Promotion of breast- feeding (exclusively for 4 months, continue for 2 years.)
3. Improving weaning practices (soft mashed cereals, pulses: small serving of vegetable oil).
4. Improving water supply and sanitation (safe water, use of latrines and safe disposal of stools).
5. Promoting personal and domestic hygiene (hand washing, prevention of food contamination.
6. Specific chemotherapy for invasive bowel infections or presence of *Helicobacter Pylori*.
7. Zinc supplement.

(Lucas and Gilles, 2003).

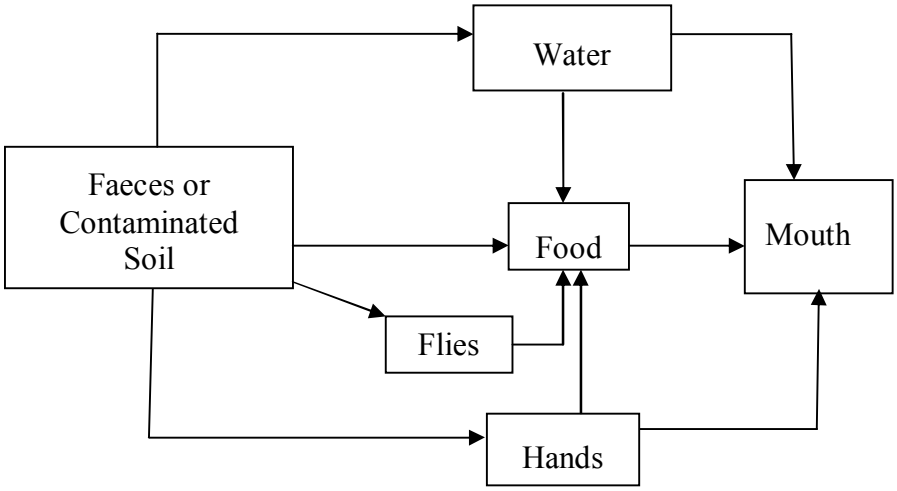
2.3.2 Mechanisms of Microbial Diarrhoea

Among the mechanisms by which an organism may colonise and disrupt intestinal function to cause mal-absorption or diarrhoea are microbial attachments, localised effacement of the epithelium, production of secretory enterotoxins,

production of cell-destroying cytotoxin(s), or direct epithelial cell invasion; (Guerrant *et al.,* 1999). Finlay and Cossart (1997) have elegantly reviewed microbial adhesion to host cells, pathogen up take, bacterial survival, and replication in mammalian cells and cell intoxication and death caused by bacterial products.

2.4 The Faeco-Oral Route

The direct ingestion of gross amounts of faeces is uncommon except in young children and mentally disturbed persons. Faeco-oral transmission occurs mostly through in apparent faecal contamination of food, water and hands, the three main items that regularly make contact with the mouth. (Lucas and Gilles, 2003).



Pathways of faeco-oral transmission. Adapted from Lucas and Gilles, (2003).

As shown on the above diagram, food occupies a central and important position. Not only can it be contaminated directly by faeces, but it can also be contaminated indirectly through polluted water, dirty hands, contaminated soil and filth flies. Water may be polluted directly by faeces but also indirectly from the polluted soil on the river bank. There are many opportunities for the contamination of hands; the person may contaminate hands on cleaning after defaecation or in touching or handling contaminated objects including soil. Filthy flies in particular the common

housefly, spread faecal material and play a role in the transmission of gastro-intestinal infections. The housefly mechanically transfers faecal pollution by;

a. Carrying faeces on its hairy limbs.
b. Regurgitating the contents of its stomach on to solid food as a means of liquefying it (vomit drop).
c. Defaecation on the food; its faeces may contain surviving organisms derived from human faeces.

It should be noted that minute quantities of faeces can carry the infective dose of various pathogens. Thus, dangerously polluted water may appear sparkling clear, contaminated food may be free of objectionable odour or taste, and apparently clean hands may carry and transmit disease (Lucas and Gilles, 2003). As of the year 2000 an estimated 2.4 billion people still lack access to an adequate means of excreta disposal (WHO, 2000). Most urban centres in Africa and Asia have no sewage system at all including many cities with a million or more inhabitants (WHO, 1992). Human excreta are an important source of pathogenic organisms, especially the causative agents of diarrhoea diseases. In addition, faeces are attractive to flies who, not only spread pathogenic organisms contained in the faeces but also breed in them. Therefore, the disposal of human excreta is of major public health significance (Lucas and Gilles, 2003).

2.5 Food Borne Diseases

Food-borne diseases, i.e. illnesses due to contaminated food are one of the most wide spread health problems of the contemporary world and an important cause of reduced economic productivity (WHO, 1984). Food-borne illnesses in human beings due to bacterial pathogens and their toxins are well-documented worldwide (Todd, 1983).

The spectrum of food and water borne infections is wide including cholera, campylobacteriosis, cryptosporidiosis, *E coli* infections, salmonellosis, Shigellosis, enteric fevers, brucellosis, hepatitis, amoebiasis and *trematode* infections (Cartwright,2003). Food poisoning is an acute gastroenteritis caused by the ingestion of food or drink contaminated with either living bacteria or their toxins or inorganic chemical substances and poisons derived from plants and animals. The condition is characterized by

i. History of ingestion of a common food.

ii Attack of many persons at the same time and

iii. Similarity of signs and symptoms in the majority of cases (Park, 2002).

Food borne illnesses are problems that threaten developing as well as developed countries (WHO, 1991). Although reports on food borne diseases are often inadequate and outbreak incidents are usually underreported. Many large out breaks have been documented which resulted in great losses and suffering for victims, societies, and countries (Ali and Spencer, 1995). Food poisoning may involve an infection of the gut (food-borne infection) and /or the actions of toxins (Food-borne intoxication). The risk of food poisoning involves the following factors;

1. **The initial level of contamination on or in the food:** In the production of food, the greater the initial contamination the less likely it is to be reduced to safe levels by routine processing. In prepared foods, contamination may be above or below the level needed to cause disease.
2. **The type of processing, storage, distribution and preparation involved in the production of food:** The risk of food poisoning depends on the ease with which a pathogen can gain access to and grow in or on food at each stage of the food chain, and on the success or failure of those processes used to

eliminate pathogens. With meat and poultry, major food poisoning pathogens should ideally be eliminated at farm level, i.e. in livestock, since control of contamination is much more difficult after slaughter and distribution of the products.

3. **The dose/response relationship, particularly the smallest dose (of cells or toxin) able to cause disease:** susceptibility to pathogens varies widely with age, acquired immunity and state of health. 'Special risk' groups, in which the risk is greater and the disease may be far more serious, include infants, the very old, pregnant women, and the immuno-compromised (including AIDS patients). Because 'dose/response' can vary, acceptable (i.e. safe) levels of contamination are often based on epidemiological studies (Singleton, 1997).

Microbes that cause food-borne illness commonly enter food through cross-contamination and grow in temperatures favourable to them (Wardlaw and Insel, 1996).

Overall, it is important to practice sanitary food-handling procedures when preparing any food. And as one final precaution, watch for safe food-handling techniques when you eat out. Check that foods in a salad bar are iced, custard and pudding pies are chilled, hot food bar are hot, and vending machines are checked regularly, especially those containing sandwiches and milk. Send back any meat, poultry, seafood, or fish that does not appear thoroughly cooked. Keep track of how long cooked foods or salads have been sitting on the buffet table and avoid anything there for 2 hours or more. Food stored and served in dormitory and cafeterias should be properly handled (Wardlaw and Insel, 1996). As the eating habits of our society change, so does the pattern of food borne illnesses. Twenty years ago, food borne disease outbreaks were primarily local occurrences that

followed a family gathering or church picnic at which undercooked meat or eggs or improperly stored potato salad, were served. Today a greater variety of foods are being implicated including more fresh fruits and vegetables, and the outbreaks are often wide spread, some times involving hundreds or thousands of people (Nester *et al.,* 2001). Substantial increase in 'eating out' and 'partying', catering is a major contributory factor to food transmitted diseases (Mossel *et al.,* 1995). Diseases of microbial origin that are transmitted by food are usually divided into two main categories (Mossel *et al.,* 1995).The first contains illnesses resulting from the presence in a food of micro-organisms infective upon ingestion. These organisms are able to cause disease either by invasion of the host or by the release of toxic substances (toxins), resulting from the growth in the intestinal tract of some other organ. Such diseases are generally termed "Food - borne infections". The second category of food transmitted illness results from intestinal absorption of toxins that have been synthesized in a food during proliferation and metabolism of certain micro organisms. Such diseases are called food-borne intoxications (Shier and Mebs, 1991). For this to occur, relatively large numbers of viable cells, i.e. much greater than $10^5 g^{-1}$ or ml^{-1} must have developed in a food prior to consumption; (Mossel *et al.,* 1995).Food-borne infectious diseases are frequently divided into two further groups, on the basis of two related parameters. These are;

i. The minimum infective dose (MID), i.e. the lowest number of cells which will initiate infection and cause disease symptoms in previously healthy individuals.
ii. The 50% infective dose (ID_{50}) defined as the number of cells that provokes disease symptoms in about 50% of the population at risk. (Mossel *et al.,* 1995).

2.5.1 Factors Affecting Infection Doses of Pathogenic Organisms in Food

The interaction between an infective organism and potential host cannot be evaluated without taking into account pasteur's 'Seed and Soil' model. The seed in this case includes the infectious organism, with its highly variable attributes of entero virulence, and the accompanying members of the microbial community structure of the food of which it is part. The soil comprises the defence mechanisms in the various body systems of the person at risk, particularly those associated with the gastrointestinal tract which determine whether clinical manifestations will arise from infection (Mcfarland *et al.,* 1991).

Intrinsic Properties of the Organisms

The ability of a particular strain of a given pathogenic organism to initiate infection is affected by a number of well-established factors (Sparling, 1983, and Nelles *et al.,*1985). These include genotypic and phenotypic properties ('Virulence') and the conditions to which the organism has been exposed just before encounter with the host (Matin and Katz, 1991).

The Co-Infection Phenomenon

An investigation of an out break of Listeriosis in Philadelphia prompted Schwartz *et al.,* (1989) to suggest that a second pathogenic organism may change previously asymptomatic carriage of a primary organism to clinical disease. This could arise from modification of the balance of the normal enteric flora, damaging the intestinal mucosa or altering the motility of particular areas of the enteric tract. An extremely low dose of an entero-pathogen might hence trigger disease, if ingested in combination with another pathogenic agent.

The co-infection phenomenon shows a striking similarity with so called multiple chemical sensitivity in human toxicology, a chemical that is not acutely toxic at a

given level may exert adverse effects at that same concentration, when ingested in combination with other compounds (Ashford and Miller, 1991)

Type of Food in Which the Pathogen is Ingested

The food in which an organism is ingested can markedly affect the number of microbial cells required to initiate infectious disease, for example, numbers of enteric pathogens in excess of 10^4 are normally required to cause infectious bacterial enteritis (Mossel *et al.,* 1995). However, if such organisms are ingested with a small volume of water or food between meals, they pass almost immediately from the stomach into the duodenum (Mossel *et al.,* 1995) and in fact at markedly lower levels of cfu's. Another important possibility is that enteric pathogens are protected against the marked bactericidal effect of the gastric fluid of healthy individuals when enveloped in lipids in foods (Fontaine *et al.,* 1978). As a consequence of such phenomena the minimum oral infective dose of pathogens ingested with food may be as low as one to ten cells (D'Aoust; 1985).

Host Resistance

Obviously those already clinically ill should not be exposed to an additional pathogenic stress through the ingestion of contaminated food (Mascola *et al.,* 1988). However the response of apparently healthy consumers to an accurately defined dose of an infective agent varies widely (Mintz *et al.,* 1994, Swerdlow *et al.,* 1994). It is markedly affected by:

i. The acidity and hence lethal effect (Gorden and Small, 1993) of the gastric fluid.
ii. Humoral and cellular immunological defence (Rubin and Young, 1994), age (Lew *et al.,* 1991), pregnancy, life style (Plotnikoff *et al.,* 1992; Sheridan *et al.,* 1994) and immuno – incompetence resulting from sub clinical disease, e. g AIDs (Wolfson *et al.,* 1985).

iii. State of nutrition Wiedermann *et al.,* 1993, Semba, 1994).

iv. The ability of the intestinal flora to prevent the establishment and colonization of entero-pathogens on the various critical sites of the intestinal lining ('competitive exclusion') (Gorbach *et al.,* 1988).

v. Previous exposure to a given pathogen giving rise to protective immunity in some instances (Black *et al.,* 1992, Ward and Bernstein, 1994).

Groups at a particular risk in this sense are the segment to the population termed the YOPIs: the young, old, pregnant and immuno-compromised (BairdParker, 1994). Their vulnerability in many respects result from lack of immune-response.A study of infectious intestinal disease (IID) in England, estimated that 20% of the population suffered an IID in a year (Cartwright, 2003). The illnesses can usually be controlled by self medication. The control of food and water borne infections is the responsibility of public health authorities. Most countries have legislation regarding food and water hygiene, but the implementation of the law varies with very understandably, public health priorities being focused on diseases such as acquired immuno-deficiency syndrome and tuberculosis (Cartwright, 2003).

2.5.2 *Salmonella* Food Poisoning

Salmonella constitute a major threat to our health and welfare. They are responsible for 16 million annual cases of typhoid fever and 1.3 billion gastroenteritis, which together results in more than 3 million annual deaths (Groisman and Ochman, 2000). *Salmonella* food poisoning is an extremely common form of food poisoning. Five reasons have been attributed for its increase in recent years:

a) An increase in communal feeding.
b) Increase in international trade in human food.
c) A higher incidence of salmonellosis in farm animals.
d) Widespread use of house-hold detergents interfering with sewage treatment.
e) Wide distribution of prepared foods (Park, 2002).

Agent(s): The species of most often incriminated in human outbreaks are *S. typhimurium S. cholera-suis* and *S. enteritidis.*

Source: Salmonellosis is primarily a disease of animals. Man gets the infection from farm animals and poultry through contaminated meat, milk and milk products, sausages, custards, egg and egg products. Rats and mice are another source; they are often heavily infected and contaminate food stuff by their urine and faeces. Temporary human carriers can also contribute to the problem. Incubation period is 12-24 hours (Park, 2002).

Mechanism of Food Poisoning: The causative organisms, on ingestion, multiply in the intestine and give rise to acute enteritis and colitis. The onset is generally sudden with chills, fever, nausea, vomiting, and profuse watery diarrhoea which usually last 2-3 days. Mortality is about one percent (Wardlaw and Insel, 1996). Most outbreaks of *Salmonella* infection can be traced to mistakes in food handling (Wardlaw and Insel. 1996). Food-borne typhoid usually results from the direct contamination of a food by a human carrier. Good hygiene is therefore the most effective means of preventing both cases and spread of typhoid and paratyphoid (Mossel *et al.,* 1995). Meticulous hand

hygiene and avoidance of touching food by hand is the effective preventive strategy.

The Organism and its Characteristics

Salmonella are members of the Enterobacteriaceae. They are Gram-negative, non-spore forming rods which are facultatively anaerobic, catalase-positive, oxidase– negative, and are generally motile with peritrichous flagella. Growth has been recorded from temperatures just above 5^{o}C up to 47^{o}C with an optimum at 37^{o}C. *Salmonella* are heat sensitive and are readily destroyed by pasteurization temperatures (Adams and Moss, 1995).Methods for the isolation and identification of *Salmonella* in foods has arguably received more attention than those for any other food borne pathogen. Pre- enrichment in a non- selective medium (e.g. peptone water) increases the recovery rate of *Salmonella* by allowing the repair of cells which have been sub-lethally damaged. Such damage can result from any exposure to adverse conditions that might occur during food processing, such as chilling, freezing or drying. The selective enrichment stage is intended to increase the proportion of *Salmonella* cells in the total micro flora by allowing them to proliferate while restricting growth of other micro-organisms present. To this end, a number of different media have proposed employing selective agents such as bile, brilliant green, malachite green, tetrathionate and selenite. The most widely used are selenite-cystine broth. From the selective enrichment broths, cultures are streaked on to selective and differential solid media. The selective agents used are bile salts or desoxycholate and/or brilliant green and the diagnostic reaction is usually provided by the inability of most *Salmonella* to ferment lactose and/or the production of hydrogen sulfide (Adams and Moss, 1999). *Salmonella* produce non-lactose fermenting pale coloured colonies which on Desoxycholate Citrate Acid), (DCA)

have black centres (H_2S–producing *Salmonella*) (Cheesbrough, 2000).*Salmonella* are primarily inhabitants of the gastrointestinal tract. They are carried by a wide range of food animals, wild animals, rodents, pets, birds, reptiles, and insects, usually without the display of any apparent illness. They can be disseminated via faeces to soil, water, foods and feeds, and hence to other animals including humans (Adams and Moss, 1995).

2.5.3 Staphylococcal Food Poisoning:

An estimated 30% of all cases of food borne illness worldwide are caused by *Staphylococci* (Shebuski *et al.,* 2000). The bacterium *Staphylococcus aureus* causes 20% to 40% of cases of food-borne illness each year. While growing in food, this microbe produces an endo-toxin that once ingested causes nausea, vomiting, diarrhoea, headaches, and abdominal cramps. Thus, illness caused by the bacterium is classified as bacterial intoxication (Wardlaw and Insel 1996). Staphylococcal food poisoning (SFP) is an intoxication resulting from the ingestion of food or beverages containing preformed enterotoxins (SEs) (Mclauchlin *et al.,* 2000).

Agent: Enterotoxins of certain strains of coagulase positive *Staphylococcus aureus.* Toxins can be formed at optimum temperatures of 35 - 37^{o}C. These toxins are relatively heat stable and resist boiling for 30 minutes or more (Park, 2002).

Source: *Staphylococci* are ubiquitous in nature, and are found on the skin and in the nose and throat of man and animals. They are common agents of boils and pyogenic infections of man and animals. Foods involved are salads, custards, milk and milk products. The incubation period is short because of “preformed” toxin, usually 1-6 hours (Wardlaw and Insel, 1996). The main human reservoirs of these

organisms are the skin and nasal cavity, and about 40-44% of healthy humans carry *Staphylococci* in the nose (Su and Wong, 1996).

Mechanism of Food Poisoning: Food poisoning results from ingestion of toxins preformed in the food in which bacteria have grown ("Intradietetic" toxins). Since the toxin is heat-resistant, it can remain in food after the organisms have died. The toxins act directly on the intestines and Cerebro-neuro System (CNS). The illness becomes manifest by the sudden onset of vomiting, abdominal cramp and diarrhoea. In severe cases, blood and mucus may appear. Death is uncommon, and no immunity develops against future attacks (Wardlaw and Insel, 1996).The Staphylococcal enterotoxins are heat stable and highly resistant to inactivation by gamma irradiation, freezing and dehydration. The enterotoxins, at a pH greater than 2, are also resistant of degradation by proteolytic enzymes such as trypsin, chymotrypsin, rennin, and papain present in the small intestine (Hazariwala *et al.,* 2002).Staphylococcal intoxication is a leading cause of food-borne intoxication and enterotoxigenic *Staphylococcus aureus* strains have been isolated from foods implicated in illnesses and from market foods including those ready to eat (Picard *et al.,* 1987).

The Organism and its Characteristics

The genus *Staphylococcus* is placed in the family micrococcaceae*, Staphylococcus aureus* is the most significant human pathogen among the staphylococci (Sandel *et al.,* 2003). *Staphylococcus aureus* is a gram-positive coccus forming spherical to ovoid cells. They are catalase positive, oxidase-negative, facultative anaerobes. *Staphylococcus aureus* is a typical mesophile with a growth temperature range between 8-48^{o}C and an optimum at 37^{o}C. The range of temperature over which enterotoxin is produced is narrower by a few degrees and

has an optimum at 35- 40^{o}C. Growth occurs optimally at pH values of 6-7 with minimum and maximum limits of 4.0 and 9.8-10.0 respectively. The pH range over which enterotoxin production occurs is narrower with little toxin production below pH 6.0. A characteristic of *Staph. aureus* which is particularly important consideration in some foods is its tolerance of salt and reduced water activity (a_w) (Adams and Moss, 1995).Foods contaminated with *S. aureus* are potential source of enterotoxins if the foods are held at an inappropriate temperature allowing growth and synthesis of enterotoxins by the micro-organisms (Nelles, *et al.,* 1985).*Staphylococcus aureus* belongs to the normal flora found on the skin and mucous membranes of mammals and birds. This bacterium can be disseminated in the host environment and can survive for long periods of time (Hennekine, *et al.,* 2003).The most successful and widely used selective plating medium for *Staphylococcus aureus* is the one devised by Baird-Parker in the early 1960s. It combines the virtues of a high degree of selectivity, a characteristic diagnostic reaction, and the ability to recover stressed cells. Lithium chloride and tellurite act as selective agents while egg yolk and pyruvate assist in the recovery of damaged cells. Reduction of the tellurite by *S. aureus* gives characteristic shiny, jet-black colonies which are surrounded by a zone of clearing resulting from hydrolysis of the egg-yolk protein Lipovitellenin. Colonial appearance on Baird-Parker agar (BPA) gives presumptive identification of *S. aureus* which is often confirmed by tests for the production of coagulase and thermostable nuclease (Adams and Moss,1999).

Foods involved in *S. aureus* food poisoning are usually hand prepared foods, such as salad, pounded yam, “eba”, “kunu” (pudding), fried meat, bush meat, fried chicken, Bread, cake, “chin- chin” and “moi-moi; (Nduka and Yakubu, 1990). Staphylococcal food poisoning is a major cause of food- borne disease in almost

every country of the world (Lopes *et al.,* 1993). Coagulase is still recognized as a typical characteristic of *S. aureus* and is routinely used for identification of the bacterium (Chang and Huang, 1995). Although coagulase tests may have false-positive results and coagulase reactions less than 4+ determined (Chaung and Huang 1995), Sperber and Tatini (1975), found that almost all strains of *S. aureus* produce a 4+ coagulase reaction, whereas the plasma clot caused by other bacteria is usually 2+ or 3+. However, Chang and Huang (1995), proposed that any degree of plasma clotting could be considered a positive reaction. Thermostable nuclease (TNase) is also a characteristic enzyme of *S. aureus*. There is considerable interest in the use of TNase for detection of Staphylococcal growth and the likely presence of enterotoxins on foods (Chang and Huang (1995). Another specific product of *S. aureus* is protein A, protein A is a cell wall constituent of *S. aureus* and is mainly covalently linked to the peptidoglycan structure, but some 8 to 30 % of the protein is secreted into the medium during the exponential growth phase. This property has been used to develop a latex agglutination test and an enzyme liked immuno-sorbent assay (ELISA) for the identification and detection of the micro organism from foods (Chang and Huang, 1995).

2.5.4 *Bacillus cereus* Food Poisoning

Outbreaks of *B. cereus* food-poisoning have been reported from many countries. Of the foods implicated, the most common are carbohydrate-based foods prepared and stored at ambient temperature for a reasonable time before consumption. In Nigeria, limited research has been done on *B. cereus* in foods, but it has been isolated from dried food condiments (Yusuf *et al.,* 1992). *Bacillus cereus* sporulates readily in foods. Consequently *B. cereus* is much more likely than *Clostridium perfringens* to survive a heating process and subsequently germinate,

multiply and cause illness. Indeed *B. cereus* is more generally involved in food incidents than *Clostridium perfringens* (Mossel *et al*., 1995).

Agent(s):. Two different toxins are responsible for two types of *B. cereus* food-borne poisoning. The emetic toxin which causes vomiting is a peptide, exceptionally resistant to heat (126^{o}C for over 90 minutes), acid, alkali and proteolytic enzymes, while the toxin which causes diarrhoea is a protein that is sensitive to all these, the emetic toxin is thus more likely to cause diseases (Sakurai *et al.,* 1994).

Source: *Bacillus cereus* is widely distributed in the soil and the atmosphere (Yusuf *et al.,* 1992). Foods mostly implicated include, meat, soup, vegetables, pudding farinaceous foods, boiled rice. The emetic intoxication mostly occurs upon ingestion of boiled or fried rice that has been allowed to stand at elevated temperature for several hours after cooking. This type of *B. cereus* poisoning is common in the UK and in the Netherlands, where fried-rice products are eaten frequently. Incubation period is between 8-16 hours (Ginsbery *et al.,* 1994).The mechanism of food poisoning is similar to that of staphylococcal enterotoxin. The increasing frequency with which *B. cereus* infections are encountered is in part due to host predisposition by suppressed or compromised immunity, metabolic disorder, or malignant diseases (Hassan and Nabbut, 1995). Human cases of *B. cereus* poisoning are generally associated with foods containing a high number of colony forming units (cfu>10^6 g or ml) (WHO, 1984), although some cases involving lower presumptive enumeration (10^1/g or ml) have also been reported (Vasconcellos and Rabinovitch, 1995).

The Organism and its Characteristics

Bacillus cereus is a facultative aerobic bacterium that produces spores and enterotoxin. Given its wide distribution in nature, the bacterium is a common cause of food poisoning, inducing diarrhoea and/or vomiting (Vasconcellos and Rabinovitch 1995).*Bacillus cereus* is a Gram positive facultatively anaerobic with large vegetative cells. It grows over a temperature range from 8 to 55^{o}C, optimally around 28-35^{o}C and does not have any marked tolerance for pH (min 5.0-6.0) depending on the acidulant or water activity (min 0.95). Spores are central, ellipsoidal in shape and do not cause swelling in the sporangium. *B. cereus* is widely distributed in the environment and be can be isolated from soil, water and vegetation. This ubiquity means that it is a common component of the transient gut flora in humans. The spores show a variable heat resistance, recorded D values at 90^{o}C in phosphate buffer range between around 1 min up to 36 min. Resistance appears to vary with serovar (Adams and Moss, 1995). Enumeration of *B. cereus* is generally carried out using selective media, or media that, in addition to being selective is used to demonstrate phospholipolytic activity, *B. cereus* produces at least one phospholipase “C” also known as lecithinase. Of the most widely used media, two are especially notable, the medium described by Mossel Koopman and Jongenius (Mossel *et al.,* 1967), containing Mannitol, egg yolk, phenol red, polymyxin G and agar (MYP also MYPA), and LG medium version of MYP described by KIM and Goepart 1971, also containing polymyxin and useful for enumeration of *B. cereus* cfu at low concentrations. Vasconcellos and Rabinovitch, (1995), formulated a new selective and indicator medium that enables the quantification of *B. cereus* for the purposes of food quality control. The medium which has been designed to keep inhibition of this bacterium to a minimum and at the

same time to ensure adequate suppression of a wide range of contaminants without resorting to the usual expediency of polymyxin, The Vasconcellos, Rabinovitch medium (VRM) is composed of Tris (hydroxymethtyl) aminomethan, sodium chloride, magnesium sulphate, resazurin, powdered agar-agar fresh egg yolk and tryptone. The VRM medium is simple to prepare and the formula is less costly than those used to make KG or MYP medium.

2.5.5 *Escherichia coli* Food Poisoning

Escherichia coli are commonly found in the intestinal tract of humans and other animals. Since 1982 at least six strains, most notably *E. coli* 0157: H7 have been shown to cause food-borne illness. It is estimated that *E. coli* 0157: H7 causes 10,000 to 20,000 cases of diarrhoea and about 250 deaths annually. It is quickly becoming a major threat for food-borne illness. Children and the elderly are most susceptible (Wardlaw and Insel, 1996). *E. coli* have been involved in food-borne illness and have been recovered from various food types, processed or raw (Bonita and Stevens, 1980). Several well documented outbreaks, such as that in Scotland in 1996-97, have heightened public awareness of food as a vehicle for transmission of this pathogen (Maher *et al.,* 2001).

Agents: *E. coli* Strains that cause diarrhoea fall into four groups with different pathogenic mechanisms. These include the enterotoxigenic, enteroinvasive, enteropathogenic and enterohaemorrhagic groups (Gross, 1992). Some researchers have further added two more categories of *E.coli* to the above group. These include the enteroaggregative, and the diffusively adherent groups (Buchanan and Doyle, 1997).

Source: The bacterium is transmitted primarily via contaminated ground beef and roast beef, un-pasteurised milk, salad greens grown in cow manure, and contaminated water, incubation period is 2-4 days (Wardlaw and Insel, 1996).

Mechanism of Food Poisoning: The causative organisms, on ingestion, multiply in the intestine causing severe abdominal cramps, bloody diarrhoea and kidney failure.

In 1993 at least 4 people died and 700 became ill in Washington and surrounding Western states after eating at a chain of quick-service restaurants. The source of problem was undercooked hamburger contaminated with *E. coli* 0517: H7 (Wardlaw and Insel, 1996). Cooking meat until it is grey or brown in colour, and juices run yellow to clear (no pink colour left), and then avoiding recontamination, are important ways to prevent this type of food–borne illness, (Wardlaw and Insel, 1996). Faecal contamination of water supplies and contamination from food handlers have been most frequently implicated in out breaks of *E. coli* (Adams and Moss, 1999).

The Organism and its Characteristics

Escherichia is the type genus of the Enterobacteriaceae family and *E. coli* is the type species of the genus. It is catalase negative, oxidase negative, fermentative, Gram negative, non-sporing rod (Adams and Moss, 1995), *E. coli* is a typical mesophile growing from 7-10^{o}C up to 50^{o}c with an optimum around 37^{o}c. It shows no marked heat resistance, with a D-value at 60^{o}C of the order of 0.1 min, and can survive refrigerated or frozen storage for extended periods. Some strains are more heat resistant than other members of the family and will survive heating to 60^{o}C for 15 minutes or 55^{o}C for 60 minutes (Gross, 1992). A near neutral pH is optimal for growth, but growth is possible down to pH 4.4 under otherwise optimal conditions (Adams and Moss, 1995). An important property of microbial pathogen associated

with faeco-oral routes or transmission is the ability to survive extremely acidic environments as well as moderately acidic environments containing weak acids (Eklund, 1983). Resistance to low pH can be important to food-borne pathogens for survival in specific foods and gastrointestinal tract (Lin *et al*., 1996). *E. coli* grow well on non-selective media forming smooth, colourless colonies of 2-3 mm in diameter in 18 hour on nutrient agar, large red colonies on McConkey agar, and greenish metallic sheen on Eosin Methylen blue Agar (EMB). *E.coli* can be differentiated from other members of the Enterobacteriaceae on the basis of a number of sugar fermentation and other biochemical tests. Classically, an important group of tests used for this purpose are known by the acronym IMVIC (Adams and Moss, 1999). These tests for the ability to produce:

- Indole from tryptophan (I)
- Sufficient acid to reduce the medium pH below 4.4, the breakpoint of the indicator Methyl red (M)
- Acetoin (acetylmethyl Carbinol)V and the ability to utilize citrate (C).

Escherichia coli strains that cause diarrhoea fall into four groups with different pathogenic mechanisms. These include the enterotoxigenic, enteroinvasive, enteropathogenic and enterohaemorrhagic groups (Gross, 1992). Some researchers have further added two more categories of *E. coli* to the above group. These include the enteroaggregative, and the diffusively adherent groups (Buchanan and Doyle 1997). In a recent study of street foods in Accra, Ghana by Mensah *et al.,* 2002, Enteroaggregative *E.coli* was isolated from macaroni, rice and tomato stew. A study on foods sold in Nigerian schools showed an unacceptable level of bacteria. In addition, *E.coli* and *Shigella Flexneri* were isolated from

samples (Olukoya *et al.*,1991). *E. coli* proliferates on warm cooked foods such as "moi-moi", "agidi", meat products such as sausage, snail, fried meat and fish during processing. Their presence in foods therefore, indicates the level of sanitary standard practiced during food processing (Nduka and Yakubu 1990). A great many strains of *E.coli* produce heat-labile (LT) and/ or heat stable (ST) enterotoxins that act on the small intestine (Mossel *et al.,* 1995). coliform bacteria belong to the systematically defined family Enterobacteriaceae but are further defined by functional characteristics rather than systematic genus and species (Sloat and Ziel. 1992). The WHO has defined coliform*s* as any rod- shaped, non-spore forming, gram-negative bacteria capable of growth in the presence of bile salts or other surface active agents. They are cytochrome-oxidase negative and able to ferment lactose at either 35^{o} or 37^{o}C with the production of acid, gas and aldehyde within 24 - 48 hours (Sloat and Ziel, 1992). The total coliform groups comprising of *Escherichia*, *Citrobacter*, *Klebsiella, enterobacter* and indicator organisms. Contamination indicated by the presence of total coliform*s* is indicative of inadequate disinfecting of drinking water (Sloat, and Ziel 1992).

2.5.6 Prevention and Control of Food Poisoning

a) Food Sanitation:

i. Meat inspection: The food animals must be free from infection. This can be ensured by their examination by veterinary staff both before and after slaughter.

ii. Personal hygiene: A high standard of personal hygiene among individuals engaged in the handling, preparation and cooking of food is needed.

iii. Food handlers: Those suffering from infected wounds, boils, diarrhoea, dysentery, throat infection, etc. should be excluded from food handling.

The medical inspection of food handlers is required in many countries, this is of limited value in the detection of carriers, although it will remove some sources of infection.

iv. Food handling techniques: The handling of ready to eat foods with bare hands should be avoided. The importance of rapid cooling and cold storage must be stressed. Milk, milk products and egg products should be pasteurized. Food must be thoroughly cooked. The heat must penetrate the centre of the food leaving thereby no cool spots. Most food poisoning organisms are killed at temperatures over $60^{o}C$.

v. Sanitary improvements: Sanitization of all work surfaces, utensils and equipment must be ensured. Food premises should be kept free form rats, mice, flies and dust.

vi. Health education: Food handlers should be educated in matters of clean habits and personal hygiene such as frequent and thorough hand washing.

b) **Refrigeration:** In the prevention of bacterial food poisoning, emphasis must be placed on proper temperature control. Food should not be left in warm pantries, a few germs can multiply to millions by the next morning, foods not eaten immediately should be kept in cold storage to prevent bacterial multiplication and toxin production. “Cook and eat the same day” is a golden rule. When foods are held between $10^{o}c$ - $49^{o}C$. They are in the danger zone for bacterial growth. Cold is bacteriostatic at temperature below $4^{o}C$ and refrigeration temperature should no exceed this level.

c) **Surveillance:** Food samples must be obtained from the food establishments periodically and subjected to laboratory analysis if they are unsatisfactory. Continuing surveillance is necessary to avoid outbreaks of food-borne diseases (Park, 2002).

2.6 Food Hygiene Awareness among Food Handlers

Food handlers have a major role in the prevention of food poisoning during food preparation and distribution. Foods handlers may cross-contaminate raw and processed food stuff as well as inadequately cooked and stored foods (Walker *et al*., 2003). They can also be asymptomatic carriers of food poisoning organisms (Cruickshank, 1990). Food borne illness has been associated with improper storage or reheating of cooked food and cross contamination (Walker *et al.,* 2003). These contributory factors are due to lack of food hygiene awareness or implementation. Nevertheless it is not only the ignorance of food hygiene that causes food poisoning, but also the lack of applying the acquired knowledge (Bryan, 1988, Ehiri and Morris, 1994). A number of studies have demonstrated a lack of correlation between food hygiene training and improvements in food hygiene behaviour (Walker *et al*., 2003). A previous study in Italy of 411 food handlers interviewed on a face-to-face basis determined that whilst they had a positive attitude towards food safety this was not supported by observed practices (Angelillo *et al.,* 2003). This confirmed that knowledge alone does not lead to changes in food handling practices. There are many proposed reasons for the lack of impact of training initiatives. These include; recruitment from lower socio- economic classes with low educational levels, rapid staff turn over, high level of seasonal staff, literacy and language problems and poor motivation due to low pay and job status (Taylor, 2001).

It is plausible that the lack of continuous training and reinforcement contributed to the lack of food hygiene knowledge concerning a number of key aspects in safe food production (Walker *et al.,* 2003). A study conducted on "Food handler's hygiene knowledge in small food businesses by Walker *et al.,* (2003) revealed that 60% of 440 food handlers did not know that food poisoning was caused by food that looked, smelt and taste normal. These results clearly revealed that the majority of food handlers did not understand that organoleptic assessment of food was insufficient to identify food contaminated by pathogenic bacteria. Therefore they were relying on incorrect physical attributes for food safety control. Forty-two percent of handlers did not recognize the microbial risks associated with cooked rice. In another study (Tebutt, 1992) 29% of 72 food handlers did not know that disinfectants were used for reducing bacteria to a safe level and similarly the staff at the butchers that caused the *E. coli*: 0157 outbreak in LanarKshire, Scotland thought biodegradable cleaners were bactericidal (Walker *et al.,* 2003). Although, food handlers may be aware of the need for personal hygiene, they do not comprehend crucial aspects of hygiene such as cleaning of work-surfaces (Walker *et al.,* 2003). The basic lack of hygiene knowledge and understanding could prove to be a major barrier to the effective implementation of HACCP in small food businesses. Therefore a major challenge in the food industry is to motivate food handlers to apply what they have learnt regarding food hygiene (Ehiri and Morris, 1994). Food handlers need to be able to identify high-risk foods that support the survival and multiplication of pathogens and are intended for consumption without further treatment, (Walker *et al.,* 2003).

The hygienic aspects of vending operations are a major source of concern, for example, stands are often crude structures, and running water may not be readily

available. Also toilets and adequate washing facilities are rarely available. The washing of hands, utensils, and dishes is often done in buckets or bowls. Disinfection is not usually carried out, and insects and rodents may be attracted to sites where there is no organised sewage disposal. Finally food is not adequately protected from flies and refrigeration is usually unavailable (Mensah *et al.,* 2002).

2.7 Antimicrobial Medications

The development of Salvarsan by Paul Ehrlich in 1910 was the first documented example of a chemical used successfully as an antimicrobial medication (Nester *et al.,* 2001). Antimicrobials are chemotherapeutic agents that are effective in treating microbial infections. Antibiotics are antimicrobial chemicals naturally produced by particular micro-organisms. Most modern antibiotics come from micro- organisms that normally reside in the soil, these include species of the bacteria *Streptomyces* and *Bacillus* and the eukaryotic fungi *penicillium* and *cephalosporium* Nester *et al.,* (2001).

Antimicrobial Activity

Antibacterial agents can be grouped by their mode of action, i.e. their ability to inhibit the synthesis of the cell wall, cell membrane, proteins and nucleic acids of bacteria Cheesbrough (2000).

Cheesbrough (2000) describes the Mode of Action of Antimicrobial Agents as;

1. ***Inhibitors of Bacterial Cell Wall Synthesis***

a. Penicillins (Beta-Lactam agents). Examples are Benzylpenicillin (penicillin G). Phenoxymethyl (penicillin V), Ampicillin, Amoxycillin, Flucloxacillin, Cloxacillin Carbenicillin Tricarcillin, Azlocillin, Piperacillin and Mecilllinam.

b. Cephalosporins (Beta-Lactam agents); examples are cepharadine, cefuroxime, ceftazidime, cefotaxime.

c. Glycopeptides: examples are Vancomycin and Teicoplanin.

2. Inhibitors of Protein Synthesis

a. Aminoglycosides: Gentamicin, Tobramycin, Kanamycin, Streptomycin, Amikacin and Netilmicin

b. Macrolides; Erythromycin

c. Lincosamides; clindamycin

d. Chloramphenicol

e. Tetracyclines; Tetracycline and Doxycycline

3. Inhibitors of Bacterial Nucleic Acid synthesis

a. Quinolones; Nalidixic acid and fluroquinolones (ciprofloxacin, Norfloxacin.

b. Sulphonamides and Trimethoprim; Co-trimoxazole, Sulphadimidine, Trimethoprim, and Sulphadoxine.

c. Metronidazole.

The *β*-Lactam drugs irreversibly inhibit enzymes involved in the final steps of cell wall synthesis. They have been extensively studied because they are such important antimicrobial agents, having very few side effects other than allergic reactions. Their properties such as acid stability, spectrum of activity, and half-life can be modified by chemically altering their structure. The different *β*-lactam drugs vary in their spectrum of activity. Some are more active against Gram-positive bacteria, where as others are more active against Gram-negative bacteria (Nester *et al.,* 2001). Some bacteria can resist the effects of certain *β*-lactam drugs by synthesizing an enzyme called a *β*-lactamase that breaks the critical *β*-lactam ring, destroying the activity of the antibiotic. Just as there are many *β*-lactam drugs, there are various *β*-lactamases, these differ in the range of drugs they destroy. A *β*-lactamase that

was originally detected in some strains of staphylococci only inactivates members of the penicillin family to reflect this fact, it is often called a penicillinase, In contrast some of the *β*-lactamases produced by Gram-negative organisms inactivate a wide variety of *β*-lactam drugs. The extended spectrum *β*-lactam inactivates on both the penicillins and the cephalosporins. As a whole, Gram-negative bacteria can produce a much more extensive array of *β*-lactamases than can Gram-positive organisms (Nester *et al.,* 2001).

2.8 Antimicrobial Resistance

Antimicrobial resistance has now become a serious public health concern. Up to 90% cases of *Salmonella dysenterae* are resistant to co-trimoxazole and naladixic acid, Multi-resistant *Salmonella typhi* is widespread in endemic countries (Lucas and Gilles, 2003). Several investigations have been conducted to study the antimicrobial resistance pattern of *S.aureus* and it has been shown that the organism is resistant to β-lactam antibiotics, aminoglycoside and macrolides. *S.aureus* strains carry a wide variety of multi-drug resistant genes on plasmids, which can be exchanged and spread among different species of *Staphylococci* (Chinwe and Ezeronye, 2003). The development of resistance is multifactorial namely:

i. Overuse and misuse of antimicrobials by doctors and health personnel
ii. Poor compliance
iii. Self medication
iv. Counterfeit drugs
v. Poor control of antimicrobials in hospitals
vi. Poor standards of hygiene (personal and environment) in hospitals
vii. Use of antibiotics in animal husbandry, horticulture and aquaculture
viii. International travel and trade.

(Lucas and Gilles 2003)

The situation is made more difficult in developing countries such as Nigeria where antimicrobial drugs are readily available to consumers across the counter with or without prescription from a medical practitioner. Such a practice has led to misuse of antimicrobial drugs with the associated high prevalence of drug resistance among the Staphylococci (Chigbu and Ezeronye, 2003). *B. cereus* isolated from dairy products where resistant to carbenicillin, ampicillin, penicillin and cephalothrin. The resistance was attributed to frequent use of antibiotic in animal feeds and chemotherapy. *B. cereus* isolated from rice was also resistant to ampicillin, carbenicillin, cephalothrin, polymyxin B and penicillin G. (Umoh *et al.,* 1995). Antimicrobial resistance is increasing in many species of Enterobacteriaceae as well as in other Gram-negative, Gram-positive, and anaerobic bacteria. Current antimicrobial resistance issues for Enterobacteriaceae include the emergence and proliferation of extended spectrum B- lactamases, B-lactamase inhibitor resistant TEM enzymes, stably depressed and plasmid-encoded Amp (cephalosporinases, Fluoroquinolone resistance) and the dissemination of multidrug-resistant (MDR) strains multidrug-resistant strains have arisen in a multitude of bacterial species including most species of Enterobacteriaceae and are of particular concern because of their potential for widespread dissemination, acquisition of additional resistance element, and complications in therapeutic management of infected patients, particularly in seriously ill patients (Karlowsky, *et al.,* 2003). The incidence of MDR *Salmonella* strains is on the rise in developing countries prompting the investigation of broad-spectrum cephalosporins and fluoroquinolones as alternative treatments. Although these antibiotics have been used successfully in the treatment of organisms

responsible for this disease, resistance has been documented. Genes responsible for the majority of the resistance to chloramphenicol, ampicillin, trimethoprim / sulfamethoxazole, and tetracycline are often encoded by conjugative plasmids and are likely to have evolved because of antibiotic misuse (Robertson *et al.,* 2003). Like disinfectants, antibiotics may be either bactericidal or bacteriostatic, and one which is bactericidal at one concentration may be bacteriostatic at a lower concentration (Singleton, 1997).

2.9 Surveillance of Food Borne Disease

Surveillance of food borne disease is an important component of food safety programmes at all levels of government and within each type of food industry (Bean and Griffin 1990). The word surveillance is derived from the French word "Surveiller," which means to watch over (a person or area) presumably for the purpose of directions, supervision, or control (Guzewich *et al.,* 1997). According to Guzewich *et al.,* (1997), surveillance when applied to a disease means the continued watchfulness over the distribution and trends of incidence through systematic collection, consolidation and evaluation of morbidity and mortality reports and other relevant data. Intrinsic to this definition is the regular dissemination of the basic data and interpretations to all who contributed and to all others who need to know (Bean and Griffin, 1990). The final link of the surveillance chain is the application of these data to prevention and control. More specifically, surveillance of food borne disease is a continuous and systematic process that consist of:

a) Receiving notification of illnesses
b) Investigating incidents and reporting findings
c) Collating and Interpreting data

d) Disseminating information to affect control of current problems and to provide guidance for preventing the disease in the future.

(Guzewich *et al.,* 1997).

2.9.1 Reasons for Food Borne Disease Surveillance

According to Guzewich *et al*., (1997), there are several reasons for food borne disease surveillance which includes;

a) Preventing further spread of disease, including controlling outbreaks as they occur.
b) Gathering data on the incidence of food borne diseases and prevalence of the etiologic agents, vehicles, and reservoirs, including identifying factors that contributed to the outbreaks on which preventive measures and educational activities ought to be based, and by so doing, providing a data bank for designing HACCP systems and preparing risk assessments.
c) Estimating health and economic impacts of food borne diseases.
d) Anticipating problems and focusing research in areas of high risk.
e) Evaluating the effectiveness of current preventive and control practices.
f) Providing information upon which base rational food safety programmes goals and priorities.

There are at least four types of food borne disease surveillance (Guzewich *et al.,*1997):

a) Reports of incidents by the agency responsible for disease surveillance.
b) Laboratory isolations of food borne pathogens from human beings
c) Sentinel community studies.

d) Hazard surveillance and food borne disease surveillance activities are crucial part of food safety programmes. The information developed through these activities can lead to resolving imminent hazards and to changing programmes to make them more effective and responsive (Todd *et al.,* 1997). The fact that food borne diseases are not diminishing means that present preventive and control programmes are not effective precise focusing of preventive measures awaits substantial improvements in investigation and reporting outbreaks of food borne diseases and careful epidemiologic study of them.(Todd *et al.,* 1997). Hence, surveillance serves as an early alert of disease in a community, region, or nation and should trigger prompt action for control and preventive activities. Therefore without an effective Surveillance system, an early alert of an outbreak may not be possible, and control measures will not be implemented, or implementations of such measures will be delayed significantly (Guzewich *et al.,* 1997).

CHAPTER THREE

Materials and Methods

3.1 Questionnaire Interview of *Food Handlers and Consumers*

The questionnaire forms were designed to obtain information on hygiene practices, food-safety practices, sanitation in food preparation areas, waste disposal practices, sanitary facilities in food service centres and food-handlers personal hygiene practices (questionnaire form one[Appendix 1]). Knowledge of diarrhoea and food-poisoning (questionnaire form two [Appendix 2]). Other information collected included, whether or not food-handlers are aware of proper food preparation procedures, right cooking methods, correct food-handling methods after cooking, sources of food contamination so as to avoid them (questionnaire form three [Appendix 3]). The study area was divided into three zones namely: Samaru–Hanwa Zone, Sabongari-Congo and Zaria City–Wusasa zone. The questionnaire forms were used to collect information from 45 food-service centres from the three zones. For each zone, a total of fifteen centres were visited comprising of five "Bukas", five Cafeterias and five Restaurants. The selection of food-service centres for the study was based on consent given by the proprietors of the food-service centres after presenting a letter of introduction from the Head of the Microbiology Department (Appendix iv and v). The proprietors were assured that all information provided during the interview will be kept in total confidentiality and used solely and exclusively for research purpose.

3.2 Selection of Food-Service Centres for HACCP

The selection of food-service centres for HACCP was also based on consent given by the individuals running the affairs of the centres. A lot of the centres refused to allow their food samples to be collected due to mistrust, thinking that

the researcher was acting on behalf of an agency despite the letter of introduction from Microbiology Department, Ahmadu Bello University Samaru Zaria, presented to them. As such the selection of food-service centres for the HACCP centred on only those that gave their consent.

3.3 Description of Food-Preparation and Handling in the Food Service Centres Selected

Restaurants

Restaurant A is located within Ahmadu Bello University main campus, it has a well equipped kitchen comprising of gas/electric cooker, Kerosene stoves, deep freezers, sinks for washing plates, storage room and a dinning room for serving consumers. Food-preparation and cooking were done in the kitchen. The food-service centre had five employees, three were involved in cooking and two attended to consumers. Sink in the dinning room was for consumers hand washing. Cooked food are held in food warmers and kept on serving tables in the kitchen.

Restaurant B is located close to the North gate of Ahmadu Bello University Main Campus. It also had a well equipped kitchen and a storage room. There were sinks for washing plates and ingredients and a dinning room for serving meals. The food centre had fifteen employees engaged in food preparation and serving. Cooked food were held in food warmers and kept on kitchen tables.

In Restaurant C, located at Kofan Doka (Zaria City), food-preparation and cooking was done outside the main kitchen in a semi covered surrounding using fire-wood. Cooked food was packed into food warmers and kept inside the kitchen for serving. Washing of plates and ingredient were done under running tap close to the cooking area. Toilets and urinary facilities were available in all the three restaurants.

Cafeterias

Cafeteria A is situated at the Ahmadu Bello University Main Campus, it had a kitchen within the cafeteria building but food-preparation and cooking were done outside in a temporary sheltered kitchen, with running tap water located near the kitchen was used for the washing of raw foods and other cooking utensils. The waste water passes through a drainage pipe into a soak away. Cooked foods were dished into food warmers and served in the dinning room. Refrigerators and deep freezers were used to keep drinks cold. The cafeteria had about eleven employees.

Cafeteria B is also located at the Ahmadu Bello University Main Campus and shared identical features with cafeteria A.

Cafeteria C is situated at Ahmadu Bello University Congo Campus. It is a small cafeteria with a kitchen and dinning room. The kitchen is equipped with electric and gas cookers, kerosene stoves, sinks and tables on which food preparation was done. Cooked foods were kept in food warmers in the kitchen.

In all the three cafeterias toilet and urinary facilities were available.

“Bukas”

“Buka” A is located at the Samaru market not far from the central mosque. It is a one room shop arranged with two tables and four chairs around each table with hand washing bowls and drinking water jugs on it. Food preparation and cooking were done in the open air in front of the shop with kerosine stoves and fire wood. The “Buka” had a running tap very close to the building where washing and cleaning were done. Waste water was discarded into gutters in close proximity with the cooking area, the “bukas” had two employees.

“Buka” B is also a one room shop with one large table and chairs, and two hand washing bowls and water jug on the table. Food preparation and cooking were done

in front of the shop with kerosene stoves and fire wood, pipe borne water was used for cooking and other purposes. Waste water was discarded on the ground to be absorbed into the soil. The “Buka” is located amidst other houses and close to the Sabongari Market, it had three employees.

“Buka” C is located in Kofan Doka Motor park (Zaria-City). It is a one room shop, also arranged with two tables and chairs with washing bowls and water jugs. Cooking was also done in front of the shop with kerosine stoves and firewood. Washing of utensils was done in plastic buckets and bowls and the waste water was thrown into nearby gutters. A tap with running water for cooking was at the rare end of the shop, it had three employees.

Toilet and urinary facilities were not available at all the three “Bukas” visited, public toilets at the market and Motor Park sites were patronized by the food handlers.

3.4 Hazard Analysis Critical Control Points (HACCP)

The hazard analysis critical control point (HACCP) approach was used to investigate processes and procedures that could contribute to microbial contamination, survival and growth and to identify critical points where controls could be applied to prevent or eliminate microbiological hazards or to reduce them to acceptable level as described by Ehiri *et al.,* (2001). The hazard analysis consisted of observing the raw materials used, the environment and holding practices, and to identify sources and modes of actual or potential contamination. Samples were taken at sequential stages of preparation and handling using sterile wide-mouth bottles with tight-fitting lids. Hot samples were cooled immediately in an insulated plastic box containing ice blocks. Samples were subsequently tested for total aerobic plate count,

S. aureus, B. cereus and *E. coli* counts or presence of *Salmonella*. The critical control point consisted of measuring temperature and pH of the samples at three points; raw food, immediately after cooking and after holding at room temperature for an average of six hours. Sterile thermometer which was washed, cleaned with 70% alcohol and allowed to dry was used for measuring a large portion of dished out foods representing the temperature of the whole food. The pH of the samples was also measured using a digital pH meter (Model H198107 Hanna instrument ISO 9001 certified company).Water samples, swab of working surfaces and worker's hand were taken by first dipping a sterile swab stick in sterile sample bottles containing 10ml normal saline. Each surface were then swabbed several times after which the swab sticks were rinsed in separate bottles of sterile saline, this involved soaking the swabs in the solution and then pressing them against the side of the bottles to squeeze out the solution from the swabs. These were placed in ice-packed container and transported to the laboratory for analysis.

Based on the observations and discussions made during the food preparation, a detailed schematic flow chart of the food preparation and handling were made. Potential sources of contamination from raw food materials, equipments, utensils and persons preparing the foods as well as likelihood of microbial survival or destruction and likelihood of microbial multiplication were noted on the charts. Critical control points were monitored and indicated at appropriate steps (figs. 1, 2, 3, 4 and 5).

3.5 Selection of Food Samples for Enumeration

Jollof rice: This dish is the most widely consumed food in all the categories of the food service centres studied. Preparation involved, parboiled rice mixed with

ingredients such as tomatoes, chillies, onions, meat and meat stock, groundnut oil and seasoning. In all the categories of food centres, the method of parboiling raw rice, washed and strained in a colander (a big plastic sieve) was practiced, while tomatoes, chillies and onions were ground in public grinders. The method of cooking in all the food centres was the same with a slight variation, with the addition of diced carrots and peas in restaurants and cafeterias. Carrots and peas were not included in the “bukas” (fig 1, 2).

Pounded yam: This is also a widely consumed food. The preparation of pounded yam involved pounding the staple after cooking by means of a mortar and pestle and turning the stiff dough with the serving plate in restaurants and cafeteria and with bare hand in “bukas”. The stiffed dough were scooped in small proportions and wrapped in polythene bags which were rift open with a knife. The general method of cooking and handling pounded yam was the same in all the categories of food service centres.

Vegetable soup: This soup is made from a mixture of ground tomatoes, chillies and onions, ground melon seeds, spinach and seasoning. Shredded spinach, washed and drained in a colander (observed in Restaurants and Cafeterias) was the last ingredient added to the soup which was allowed to simmer for a very short time. “Bukas” do not drain the washed spinach (fig. 4,5).

3.6 Media preparation and sterilization

Media was prepared according to manufacturer’s instructions and sterilized by autoclaving for 15 minutes at 121^{o}C.

3.7 Enumeration of Micro-organisms

The enumeration of micro-organisms from different foods was conducted by the technique described by Umoh and Odoba (1999). Ten grams of each sample was aseptically transferred into 90 millilitres of 0.1% sterile peptone water and thoroughly shaken.

Serial dilution: Each sample was ten-fold diluted in 0.1% sterile peptone water and 0.1 ml of dilutions 10^{-2} and 10^{-4} were spread onto Nutrient agar (NA), Baird Parker agar (BPA), Vasconcellos, Rabinovitch medium (VRM), Eosine Methylene Blue (EMB) agar, and Selenite F broth and incubated at 37^{o}C for 24hours.

Total Aerobic Plate Count: Total Aerobic Plate Count was carried out by surface plating 0.1 ml of 10^{-2} and 10^{-4} of the samples on Nutrient Agar (Oxoid) plate and spread with a sterile hockey stick shaped glass spreader. Following plating, the surface of the medium was allowed to dry after which the plates were inverted and incubated at 37^{o}C for 24 hours. Counts of microorganisms were done after 24 hours using a colony counter and were recorded.

Bacillus, E. coli **and Staphylococcal Counts**

Inoculations were performed as for total aerobic plate count using Vasconcellos, Rabinovitch medium (vasconcellos and Rabinovitch, 1995), eosine methylene blue agar (Oxoid) and Baird Parker Agar (Merck) respectively. Creamy or pale yellow colonies on VRM were counted as *Bacillus cereus,* greenish metallic sheen on EMB was counted as *E. coli* and black shiny colonies on BPA were counted as *S.aureus.*

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Salmonella:

The peptone water pre-enriched samples were cultured onto selenite F broth (Oxoid) for selective enrichment, incubated at 37^{o}C for 24hours and subsequently streaked on Deoxycholate citrate acid (Lab M) for *Salmonella* isolation.

Purification and storage of Isolates

Typical colonies of *S.aureus, E. coli* and *B.cereus* from BPA, EMB and VRM plates respectively were purified by repeated streaking on nutrient agar plates, Gram stained and stored on nutrient agar slants at 4^{o}C until characterized.

3.8 Biochemical Characterization of Isolates

The biochemical characterization of isolates stored on nutrient agar slants was carried out as described by Cheesebrough, (1984), Jones *et al.,* (1994) and Singleton (1997). Briefly, the tests were carried out as follows:

Motility Test

All Gram positive and Gram negative isolates on nutrient agar slants were tested for motility. Tubes of motility indole urea (MIU) medium (Bacto) were stab-inoculated with test organisms and incubated at 37^{o}C for 24 to 48 hours. Development of growth from the line of stabbing towards the rest of the entire medium and cloudiness of the medium was recorded as positive for motility, while restriction of growth to the line of stabbing indicate a negative result .

Oxidase Test

Both Gram positive and negative Isolates on slants were smeared on a filter paper soaked with a few drops of 1% freshly prepared oxidase reagent

(Tetramethyl-p-phenylene diamine dihydrochloride), using a sterile toothpick stick. The development of an intense purple colour within 30 seconds indicated a positive test; failure of colour development (purple) within 30 seconds indicated a negative result.

Catalase Test

All Gram positive cocci and rods stored on nutrient agar slants were tested for catalase production. Tubes containing 2-3 ml of 3% hydrogen peroxide solution were inoculated with 24 hours culture using a sterile glass rod. A positive test was indicated by active bubbling while the absence of bubble indicated a negative result.

Citrate Utilization

Isolates were inoculated onto Simmon's citrate agar slants in bijou bottles and incubated at 32^{o}C for 24 to 72 hours. A positive result was indicated by the development of a deep blue colour of the slants.

Indole Test

Presumptive *E. coli* and *S.aureus* Isolates were grown in 5 ml of peptone water for 48 hours, to the culture was then added Kovac's indole reagent (0.5 ml per 5 ml culture) and shaken gently. In the positive test, indole (present in the culture) dissolves in the reagent which then becomes red and forms a layer at the surface of the medium.

Methyl- red (MR)/ Voges-Proskauer (VP) Test

All Gram positive and Gram negative organisms on slants were tested for acid production. 4 ml of MR –VP broth in tubes were inoculated with isolates and incubated at 37^{o}C for 48-72 hours. 1 ml of the incubated broth

was transferred into a tube and 2-3 drops of methyl red added to the tube. The appearance of a red colour on the addition of the indicator indicated a positive methyl red test. To the rest of the broth in the original tube, 0.2 ml of 40% potassium hydroxide solution was added followed by 0.6 ml of an ethanolic solution of 5% alpha-naphthol. The tube was then vigorously shaken and placed in a sloping position (for maximum exposure of the cultures to air), and examined after 30 and 60 minutes. The development of a red colour from the top of the broth indicated a positive VP test.

Urease Test

Isolates were inoculated onto slants of Christensen's urea agar and incubated at 32^{o}C for one week. The slants were observed daily within the incubation period for colour change from yellow to red which is an indication of a positive result.

Gelatin Liquefaction Test

Presumptive *Bacillus* Isolates were inoculated onto tubes of plain gelatine (Oxoid) and incubated at 37^{o}C and observed for gelatine liquefaction for a period of 3-7 days. Before observation, the tubes were refrigerated for 2 hours to enable solidification of gelatine. Tubes that solidified during refrigeration period were indication of negative results while those that did not solidify indicated positive results.

Nitrate Reduction

About 0.5 ml of sterile nitrate broth was inoculated with a heavy growth of the test isolates and incubated at 37^{o}C for 4 hours. One drop of sulphanilic acid reagent and one drop of alpha–naphthylamine reagent were added and shaken. Development of a red colour in the tubes indicated a positive result. In

the absence of red colour development, a pinch of zinc oxide was added and colour development around the granules indicated the presence of nitrate i.e. nitrate had not been reduced to nitrite hence indicating a negative result. The absence of colour development upon addition of the zinc oxide after two minutes indicated that nitrate was no longer in the broth i.e. it had been reduced to nitrite and hence indicated a positive result.

Phenylalanine Deaminase Test and Litmus Milk Reaction

The Gram positive Isolates were grown overnight on phenylalanine agar, 0.2 ml of a 10% solution of ferric chloride was added over the growth on the culture slants. A positive enzyme production was indicated by the formation of a green colour on the surface of the culture slant. The isolates were also inoculated into tubes of litmus milk (BACTO) and incubated at 37^{o}C for 3 -7 days. The tubes were observed within this period. For colour change from mauve to white or pale yellow and curd formation.

Starch Hydrolysis

Gram positive isolates on slants were inoculated onto plates of nutrient agar incorporated with 10% potato starch suspension. The plates were then incubated at 37^{o}C for 48 hours. After the incubation period, the plates were flooded with iodine solution. Starch hydrolysis was indicated by the appearance of zones of clearance around growth colonies.

Coagulase Test

The tube coagulase test was adopted in testing for the production of coagulase from presumptive *S. aureus* isolates using freshly prepared human and sheep plasma. One in ten dilution (0.2 ml of plasma and 1.8 ml of

physiological saline) were made and inoculated with test organism, which were then incubated at 37^{o}C for 6 hours. The tubes were checked every 30 minutes for 6 hours for evidence of the production of clot, gelling or formation of a loose web of fibrin all of which indicated a positive coagulase test.

Haemolysis of Human and Sheep Erythrocytes (Red Blood Cells)

All coccal isolates were inoculated on blood agar base (Oxiod) containing 10% human and sheep blood respectively. The plates were incubated at 37^{o}C for 24 hours. Clear heamolysis (discolouration of the blood medium to pale yellow) indicated a positive haemolysis test.

Growth in 7% Nacl

Presumptive *Staphylococcus* and *Bacillus* Isolates were inoculated into tubes of nutrient broth containing 7% Nacl and incubated at 37^{o}C. The tubes were then observed for growth for a period of 7-14 days.

Fermentation of Sorbitol McConkey Agar

Presemptive *E. coli* isolates were inoculated onto sorbitol McConkey agar and incubated at 37^{o}C for 24 hours. The appearance of pink colonies on the medium indicated sorbitol fermentation (positive result).

Utilization of Sugar

Screw capped test tubes containing 5 ml aliquots of sterile basal medium made up of 0.5% (w/v) yeast extract, 0.75% (w/v) peptone and 0.1% (w/v) bromothymol blue were incorporated with either 6% (w/v) glucose,

mannitol and lactose. The sugar solutions were added at a rate of 2 ml to the 5 ml basal medium into which Durham tubes were inverted and sterilised. Each tube was then inoculated with 0.1 ml of 24 hour old cultures of the isolates, incubated at 37^{o}C and observed daily for 2 weeks for fermentation and gas formation. Fermentation was positive with change in colour from green to orange in the tubes, while gas formation was indicated by air bubbles in the inverted Durham tube.

Spore Stain

A smear of the presumptive *Bacillus* were made on the slide, dried and heat fixed. The entire slide was flooded with 5% aqueous malachite green and steamed for 3-6 minutes, rinsed under running water and was counter stained with 0.5% aqueous Safranin. Spores should stain green while vegetative cells are coloured pink or red.

3.9 Antibiotic Sensitivity Test

Confirmed *E. coli B.cereus* ***and*** *S.aureus* isolates were tested for antibiotic sensitivity. Sensitivity of isolates to antibiotics was determined by the Disc-diffusion technique, method of (Kirby-Bauer) as recommended by the National committee for clinical laboratory standards (NCCLS, 1997) on Mueller Hinton agar (Oxoid) and Multo-disc (HABdiscs ABTEK) containing Ampicillin (10µg), Chloramphenicol (10µg), Cloxacillin (5µg), Erythromycin(5µg), Gentamicin (10µg), Penicillin (1i.u), Streptomycin (10µg) and Tetracycline (10µg), for gram-positive microorganisms and Ampicillin (25µg), Colistin (25µg), Gentamicin (10µg), Nalidixic acid (30µg), Nitrofurantoin (200µg), Co-trimoxazole (25µg), Streptomycin (25µg) and tetracycline

(25µg) for gram-negative organisms. A 24 hour culture of the isolates was emulsified in 3-4 ml of 0.85% physiological saline and standardized by visual comparison with McFarland 0.5 turbidity standard (0.6 ml of 1% w/v of barium chloride to 99.4 ml of 1%v/v of sulphuric acid) as described by Cheesbrough (2000). Using a sterile swab the plates were streaked evenly on the surface and allowed to dry for about 3-5 minutes, antibiotic paper discs were placed about the centre of the plates using sterile forcep. All plates were incubated at 35^{o}C for 24 hours. Using a ruler on the underside of the plates, the diameter of each zone of inhibition in millimetres was measured. The zones sizes of each antimicrobial was interpreted using the interpretative chart, reporting the organisms as 'Resistant', 'Intermediate' and 'Susceptible'. The standard positive and negative control strains used to test the performance of the method are:

i. *Staphylococcus aureus* ATCC021001

ii. *Escherichia coli* ATCC11775

The standard strains were obtained from Pharmaceutical Microbiology Department, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria. The control isolates were tested for purity and sensitivity pattern before use in the experiment.

3.10 Statistical Analysis:

The Turkey's Test, a multiple (or pair wise) comparison Test obtained under the ONE-WAY Analysis of variance Menu of the SPSS Statistical Package was used to test for differences in organism counts of the different samples, first within the same eatery type (Restaurants, Cafeterias and "Bukas") and then between the grouping, at the 0.05 level of significance.

CHAPTER FOUR

Results

4.1 Hygiene and Food Safety Practices and Knowledge of Diarrhoea

A total of 45 food centres comprising (15 restaurants, 15 cafeterias and 15 “bukas”) in Zaria were visited and each centre was presented with questionnaire forms. Questionnaire form one (Appendix 1) was filled based on the researcher’s observation of the various hygiene/ food safety practices and sanitation of the food centres. There were significant differences ($p < 0.05$) among the three categories in hygiene practices, 14 (93.3%) of restaurants had adequate water for cleaning utensils, all 15 cafeterias had adequate water for cleaning utensils while only 7(46.7) of “bukas” had adequate water for cleaning utensils this is shown in (Table 1). Water for washing raw materials and cleaning utensils were not adequate in “bukas” as they were in restaurants and cafeterias (Table 1). Refrigeration facility was available in only two out of the fifteen “bukas” visited while the availability of this facility was similar in restaurants and cafeterias. Diarrhoea was defined by 72 (80%) respondents as the passage of more than three liquids stools in a day. 18 (20%) associated diarrhoea with mucoid stools (Table 2). A total of 84 respondents (93.3 %) believed that diarrhoea was caused by germs while 6 (6.7 %) associated diarrhoea with chemicals in food and water (table 2). 84 (93.3%) of respondents agree that diarrhoeal pathogens were transmitted through dirty food and water while 6 (6.7%) did not agree, 78 (86.7%) associated transmission of diarrhoeal pathogens with dirty hands and 74 (82.2%) with dirty utensils while 12(13.3%) and 16 (17.8%) did not associate transmission of diarrhoeal pathogens with dirty hands or utensils respectively (Table 2).

4.2 Personal Characteristics of Food Handlers

Table 3 shows responses to questionnaire on personal characteristics and hygiene of food handlers (Questionnaire two [Appendix 2]). 53(58.9%) of the respondents were less than 30 years indicating the active participation of youths in food preparation and handling in these centres, while 9 (10.0%) were above 50 years. A total of 7(7.8%) of the respondents had no formal education. 44(97.8%) of the food handlers exhibited good hygiene behaviour as they were dressed in clean cloth, 33(73.3%) wore hair cover and 41(91.1%) kept cut nails. Frequent hand washing was not observed in any of the centres.

4.2.1 Hazard analyses of Selected Foods (Jollof rice, Vegetable soup and pounded yam)

Figures 1, 2, 3, 4 and 5 are flow charts illustrating the steps involved in the preparation of jollof rice, vegetable soup and pounded yam as was observed in the three categories of food service centres. The method of jollof rice preparation in all the centres was the same with a slight variation in the addition of diced carrots, cabbage and peas in the restaurants and cafeterias, carrots and peas were not added to the jollof rice in the “bukas”. A slight variation was also observed in the preparation and handling of vegetable soup in the three centres, washed vegetables were drained in colanders before added to soup in the restaurants and cafeterias while “bukas” do not drain the vegetables. No variation was observed in pounded yam preparation except in handling, restaurant and cafeterias used serving plates in turning the stiff dough of pounded yam, the “bukas” used bare hands. Hazards were assessed and pointed at the various steps of preparation and handling and critical control points established. Table 4 shows some of the foods prepared by the food service centres during the study. A large variety of

foods were prepared by all the three categories of food service centres. With the restaurants and cafeterias cooking similar foods. Cooking methods was sufficient to kill all forms of vegetative microbes but potential for post-cooking contamination existed as talking while serving food and reheating of food was not observed.

4.3 Microbiological Analysis

A total of 108 samples were collected during food preparation and handling from three food centres (Restaurants, Cafeterias and "Bukas"). Eighty- two (75.9%) were contaminated with *S.aureus,* 68(62.9%) were contaminated with coliforms and 57(52.7%) were contaminated with *B.cereus*. The microbiological analysis revealed that aerobic count for food from the three restaurants were not significantly different from each other ($P > 0.05$). Similarly staphylococcal, *Bacillus* and coliform counts, were not different for the three food centres. *Salmonella* was not isolated from any of the samples. The mean counts of the organisms isolated from the three restaurants are shown in (Table 7). Table 8 shows the mean counts of all the organisms isolated from the three cafeterias. There were significant differences ($P < 0.05$) in aerobic plate counts and Staphylococcal counts for the three cafeterias, there were no significant differences in coliform and *Bacillus* counts. Similarly there were no significant differences within the "Bukas" in all the isolates. Table 9 shows the mean counts of the organisms isolated from all the samples. There were no significant differences ($P > 0.05$) in all the organism counts of samples drawn from Restaurants and Cafeterias and "Bukas" and Restaurants and "Bukas". Tables 10, 11 and 12 shows the mean counts, processing temperatures and pH at various stages of food preparation and handling in the three categories of eatery (Restaurants, Cafeterias and

"Bukas") respectively. However most of the counts were above the permissible level of counts (< 5.0 $\log_{10}$/g and < 6.0 $\log_{10}$/g) for *Bacillus* and Staphylococcal counts respectively (ICMSF, 1996).

4.4 Biochemical Characterization of Isolates

Table 13 shows the results of the biochemical characterization of stored presumptive isolates form raw rice, held Jollof rice, raw yam, pounded yam, held pounded yam, ground vegetable paste, held vegetable soup, hands and working surface swab and water samples. Based on the results obtained, 6 isolates were confirmed as *E. coli,* where in 3 were isolated from pounded yam, 2 from held vegetable soup and 1 from raw yam. Table 13 also shows the result of the biochemical characterization of presumptive *B. cereus* isolates, a total of 57 isolates were confirmed as *B. cereus* isolated from raw yam, pounded yam, held Jollof rice, raw rice, raw ground vegetable paste, held vegetable soup, hand and working surface swab samples. Similarly, (Table 13) shows the result of the biochemical characterization of presumptive *S.aureus*, a total of 82 isolates were confirmed as *S.aureus.* Eighty-one percent of the 82 *S.aureus* isolates were of the Alpha (() haemolytic pattern and coagulated human and sheep plasma, while 15 (18%) gave beta haemolysis. Salmonella was not isolated from any of the samples.

4.5 Antibiotic-Sensitivity Test

Table 14 presents the result of the antimicrobial susceptibility test. Of the 92 isolates tested, many were resistant to the commonly used antibiotics. Out of the 6 E.coli tested, 6(100.0%) were resistant to ampicillin, 4 (66.6%) were

resistant to tetracycline and 2 (33.3%) were resistant to co-trimoxazole. All the isolates were sensitive to nitrofurantoin and gentamycin, 5 (83.3%) to nalidixic acid, and streptomycin. Of the 54 *S.aureus* isolates tested, 92% were resistant to ampicillin, 59.2% to tetracycline, 83.3% penicillin. 90.7% were sensitive to gentamycin and streptomycin, and 44.4% to cloxacillin. Out of 32 the *B. cereus* isolates tested, 81.2 % were resistant to ampicillin, 90.6% to cloxacillin and 100% to penicillin, 90.6% were sensitive to streptomycin, 78.1% to tetracycline and 62.5% to chloramphenicol.

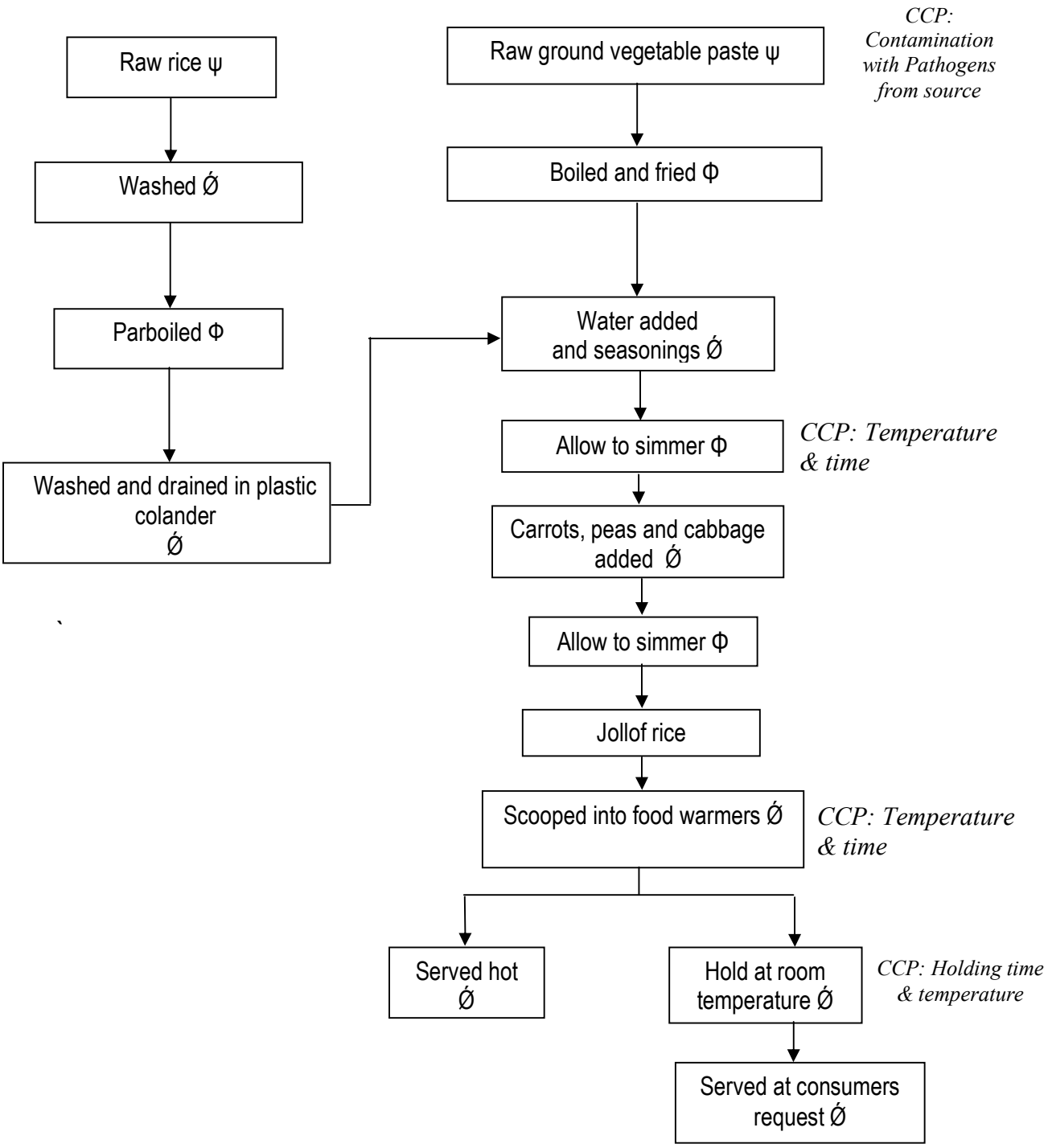


Figure 1: Flow Chart of Jollof rice preparation and handling in restaurants & cafeterias

Key:

CCP = *Critical Control Point*

Ψ = *Initial Contamination Likely*

Ǿ = *Cross contamination possible*

Φ = *Reduction in vegetative cells but not the spores*

Ю = *Microbial growth likely*

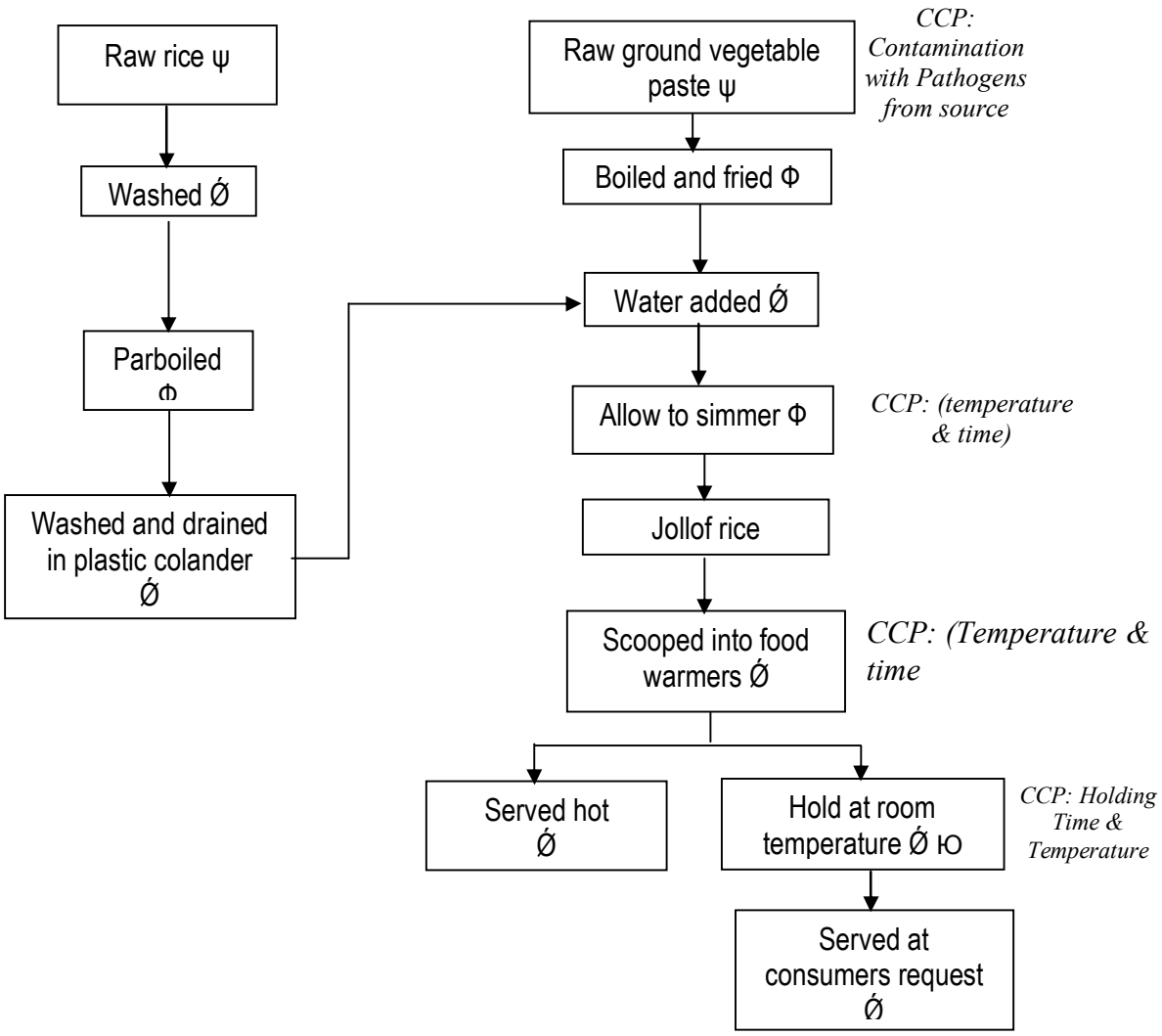


Figure 2: Flow chart of Jollof rice preparation and handling in "bukas"

Key:

CCP = *Critical Control Point*

Ψ = *Initial Contamination Likely*

Ø = *Cross contamination possible*

Φ = *Reduction in vegetative cells but not the spores*

Ю = *Microbial growth likely*

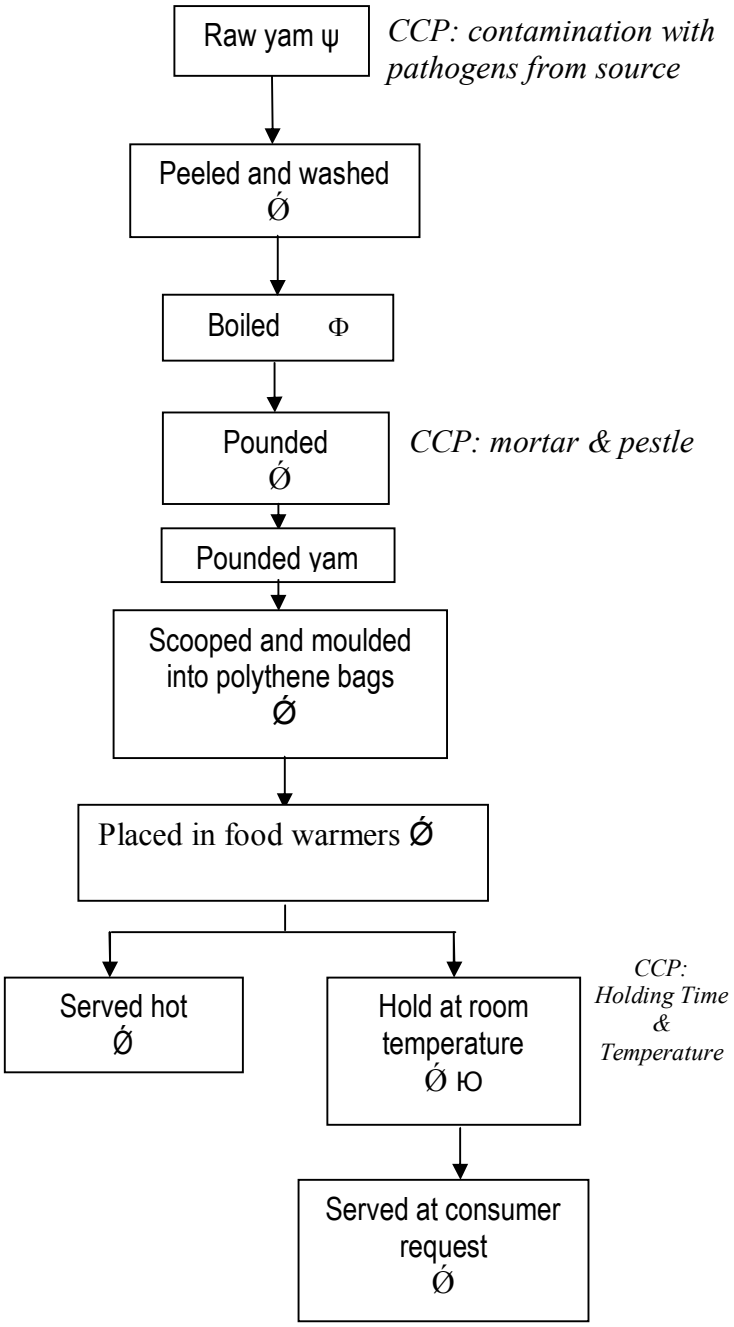


Figure 3: Flow chart of pounded yam preparation and handling in restaurants, cafeterias and "bukas"

Key:

CCP = *Critical Control Point*

Ψ = *Initial Contamination Likely*

Ø = Cross *Contamination Possible*

Ю = *Microbial Growth Likely*

Φ = *Reduction in vegetative cells but not the spores*

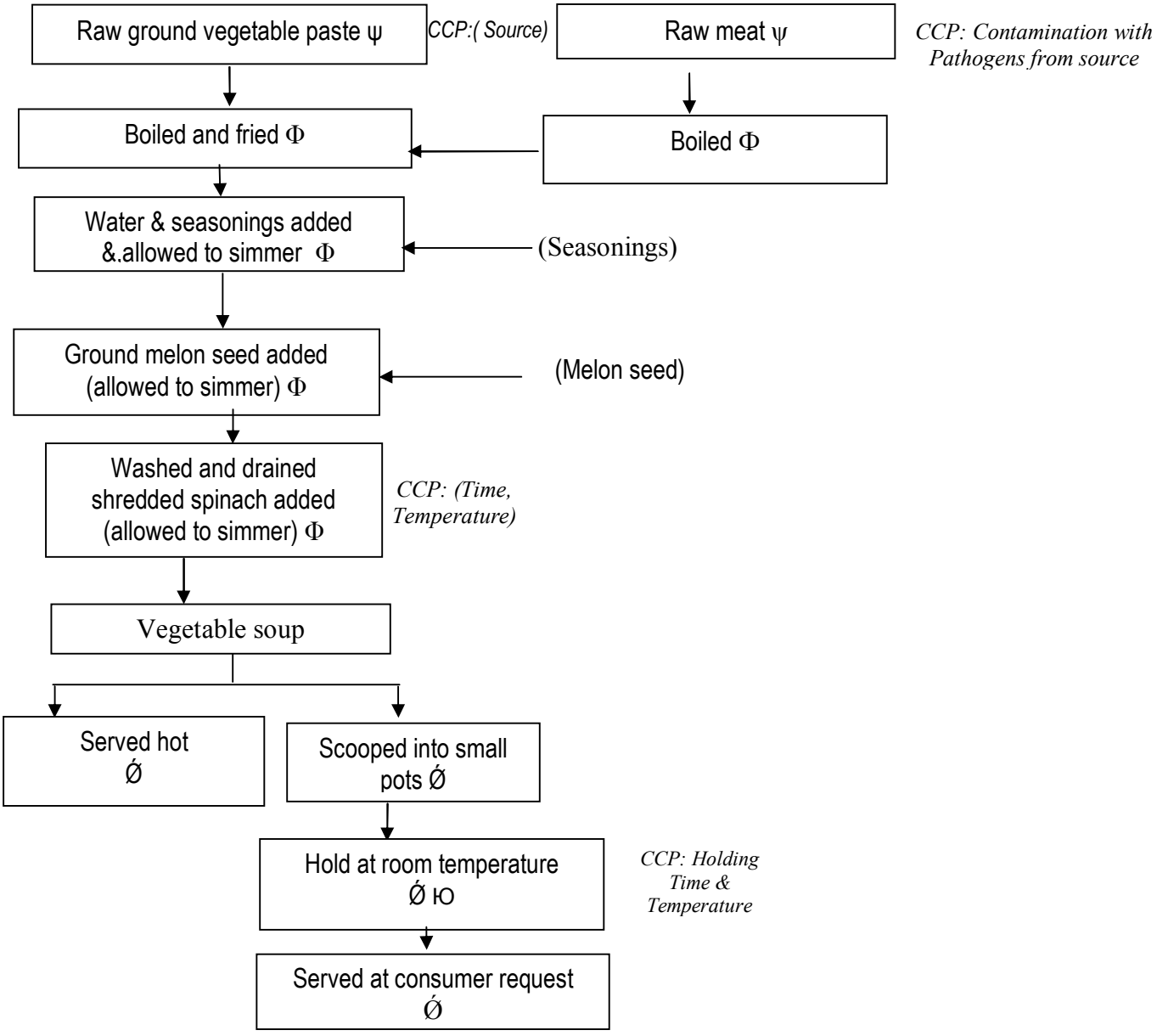


Figure 4: Flow chart of vegetable soup preparation and handling in restaurants and cafeterias

Key:

CCP = *Critical Control Point*

Ψ = *Initial Contamination Likely*

Ø = *Cross Contamination Possible*

Φ = *Reduction in Vegetative cells but the spores*

Ю = *Microbial Growth Likely*

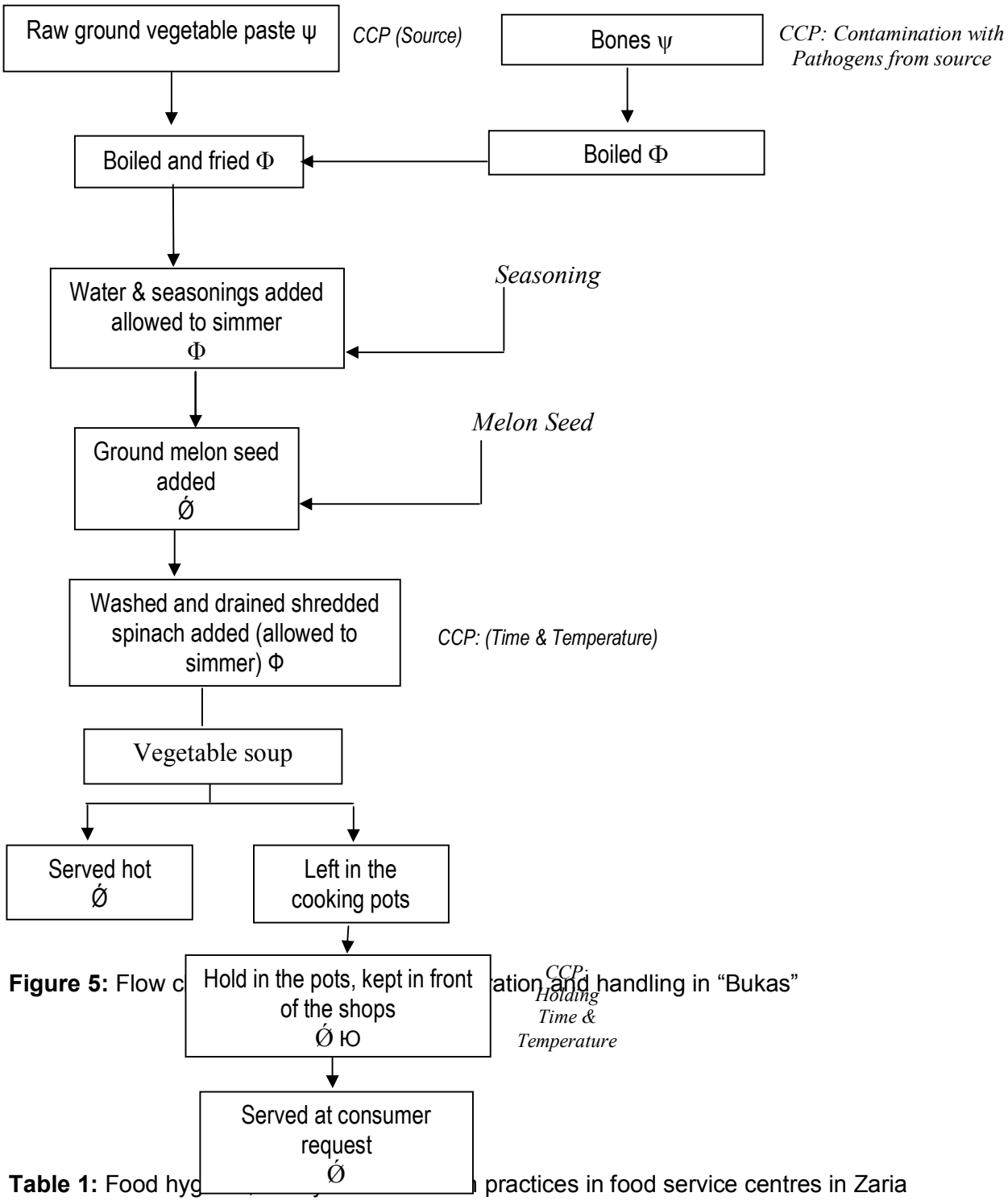


Figure 5: Flow c ration and handling in "Bukas"

Table 1: Food hyg practices in food service centres in Zaria

Parameters	Frequencies		
Hygiene Practices	**R (n= 15)**	**C (n= 15)**	**B (n= 15)**
Clean water is provided for drinking	15(100.0)a	15(100.0)a	15(100.0)
Water for washing raw material is adequate	14 (93.3)	15 (100.0)	9 (60.0)
Water for cleaning utensils for consumers and equipment is adequate	14 (93.3)	15(100.0)	7 (46.7)
Hand washing facility is adequate	15 (100.0)	15 (100.0)	15 (100.0)

Key:
CCP = *Critical Control Point*
Ψ = *Initial Contamination Likely*
Ø = *Cross Contamination Possible*
Φ = *Reduction in vegetative cells but not the spores*
Ю = *Microbial Growth Likely*

Facilities for holding ready to eat food are adequate	15 (100.0)	15 (100.0)	15(100.0)
Food Safety Practices			
Refrigeration facility available	15 (100.0)	12 (80.0)	2 (13.3)
Holding cooked food exposed to flies, dust, insects, rodents etc.	0	0	0
Holding food sale covered	15 (100.0)	15(100.0)	15(100.0)
Holding food at ground level	0	0	0
Food is cooked well in advance of consumption	15 (100.0)	15 (100.0)	15 (100.)
Food is served with spoon/fork	15(100.0)	15(100.0)	15(100.0)
Sanitation in Food Preparation Area			
Food area is swept daily	15 (100.0)	15 (100.0)	15 (100.0)
Food area is clean with soap and water	15 (100.0)	15 (100.0)	5 (33.3)
Food area is rarely clean	0	0	2 (13.3)
Waste Disposal Practices			
Waste water disposal is adequate	15 (100.0)	14 (93.3)	0
Garbage/refuse disposal is adequate	15 (100.0)	13 (86.7)	13(86.7)
General cleanliness of vicinity adequate	15 (100.0)	13(86.7)	11(73.3)
Evidence of sewage in vicinity	0	0	2(13.3)
Evidence of human waste in vicinity	0	0	1(6.7)
Evidence of animals/animal waste in vicinity	0	0	3(20)
Evidence of flies in vicinity	15 (100.0)	15 (100.0)	15 (100.0)
Numbers of workers	6-25	8-20	2-4

R = Restaurants

C = Cafeterias

B = “Bukas”

a = Figures in brackets represent percentages

Table 2: Knowledge of the causes of diarrhoea and sources of contamination of food by germs in food service centres in Zaria

Parameter	Response (n=90)	
Definition of Diarrhoea		
Passage of more than three liquid stools in a day	72 (80.0)a	
Passage of Mucoid Stools	18 (20.0)	
Transmission of Diarrhoeal Pathogens		
Dirty Food	84 (93.3)	
Dirty Water	84 (93.3)	
Dirty Hand	78 (86.7)	
Dirty Utensils	74 (82.2)	
Cause of Diarrhoea		
Chemicals in Water	6 (6.7)	
Germs	84 (93.3)	
Contamination of Food is Detected Through:		
Smell of food	17 (18. 9)	
Sight of food	8 (8. 9)	
Taste of food	65 (72.2)	
Germs Multiply in Food		
Kept at room temperature	86 (95.6)	
Kept at refrigeration temperature	4 (4 .4)	
Sources of Contamination of Food by Food Poisoning Germs		
	Yes	**No**
Raw food materials	68 (75.6)	22 (24.4)
Food ingredients	65(72.2)	25 (27.8)
Dirty Water	84 (93.3)	6 (6.7)
Dirty Utensils	80 (88.9)	10 (11.1)
Dirty Equipment	74(82.2)	16 (17.8)
Human Hands	82 (91.1)	8 (8. 9)
Dirty Environment	81 (90.0)	9 (10.0)
Dust	53 (58.9)	37 (41.1)
Packaging with paper/leaves	83 (92.2)	7(7.8)
Sewage in food vicinity	80 (88.9)	10 (11.1)
Garbage/ refuse dump in vicinity	84 (93.3)	6 (6.7)
Presence of rodents in vicinity	82 (91. 1)	8 (8. 9)
Human waste in food vicinity	84 (90.0)	6 (6.7)
Toilet/Urinary in close proximity to food area	81(90.0)	9 (10.0)
Dirty habit of food handlers	84 (93.3)	6 (6.7)

a = Figures in brackets represent percentages

Table 3: Personal characteristics and personal hygiene of respondents in food service centres in Zaria, Nigeria

Parameter	Frequency (n = 90)
AGE (YEARS)	
<30	53(58.9)a
30-39	15 (16.7)
40-49	13 (14.4)
>50	9 (10.0)
SEX	
Female	37 (41.1)
Male	53 (58.9)
EDUCATIONAL ATTAINMENT	
Primary School	6 (6.7)
Secondary School	24 (26.7)
Post Secondary School	44.(48.9)
Arabic School	9 (10.0)
No Education	7 (7.8)
PERSONAL HYGIENE OF FOOD HANDLERS	**(n = 45)**
Dressed in clean cloth	44 (97.8)
Wore hair cover	33 (73.3)
Wore covering/Apron on personal cloth	8 (17.8)
Keeps cut Nails	41 (91.1)
Appears Sickly	1 (2.2)
Frequent hand washing habit	0 (0.00)
Has dirty habit(s)	1(2.2)

a = Figures in brackets are percentages

Table 4: Some of the foods prepared by the restaurants studied in Zaria

Food	Ingredients for Food preparation	Cooking method	Handling after Cooking	Sources of contamination
Moimoi	Beans, Pepper, Onions, Spices, Oil, Chillies	Steaming	Placed in Warmers	-Polythene bags -Fork use in removing Moimoi from bag
Jollof rice	Rice, Chillies, Onions, Oil, Meat, Peppers, Tomatoes and spice	Boiling, Frying	Scooped into food warmers	-Serving spoon - Talking
Pounded Yam	Yam + water	Boiling	-Pounding -Wrapping	-Mortar and pestle -Dipping pestle in cold water -Polythene bags
Vegetable soup	Egusi, Spinach, Chillies, Onions, Tomatoes, Peppers, Palm Oil, and Spices	Frying and boiling	Scooped into serving pots	-Vegetables -Not reheating
Fried rice	Rice, oil, onion, spices, carrots, peas	Boiling & Frying	Transferred into warmers	- Serving spoon - Talking
Semovita	Semovita flour and water	Boiling	- Wrapping	- Polythene bag - Scooping plate
Plantain/ Potato chips	Plantain, Potatoes ,Oil	Frying	Sieved into a colander	- Perforated spoon - Colander - Serving utensils
Tuwo Rice	Rice & water	Boiling	- Boiled rice mashed with wooden spoon - Tuwo moulded in polythene bags	- Use of dirty polythenes - Dipping scooping plates at intervals into water - Moulding tuwo in a dirty calabash
Jollof Spaghetti	Spaghetti, spices, Tomatoes, Chillies, Peppers, Onions, Maggi, Oil Meat, Salt	Frying & Boiling	Cooked Spaghetti is placed into food Warmers using serving spoon	- Talking and sneezing while serving
Salad	Lettuce, Salad cream, Tomatoes, Onions, carrots			- Allowed to stay long - Vegs not adequately cleaned - use of bare hands in shredding
Pepper soup	Cowleg/Head/Tail Intestines	Boiling	Transferred to smaller pots	- Not reheating soup - Inadequate washing of spinach
Rice & Beans	Rice, Beans, Salt	Boiling	Placed in food warmers	- Talking while serving - Inadequately cleaned utensils - Rice, Beans may contain spores of B. cereus

Table 5: Some of the foods prepared by the Cafeterias studied in Zaria

Food	Ingredients for Food preparation	Cooking method	Handling after Cooking	Sources of contamination
Moimoi	Beans, Onions, Pepper, oil, Spices	Steaming	Placed in Warmers	-Polythene bags -Fork used for removing from polythene bags
Jollof rice	Rice, Chillies, Onions, Oil, Meat Cabbage, Spices	Boiling, Frying	Scooped in Food warmers	-Serving Spoon -Talking while serving
Eba	Gari and water	Boiling	Wrapped in Polythene bags	- Polythene bags - Scooping plate
Pounded Yam	Yam and water	Boiling	Pounding Wrapping	-Mortar and pestle -Dipping pestle in cold water -Polythene bags
Vegetable soup	Spinach, Chillies, Egusi, Peppers, Oil, Spices	Frying and boiling	Scooped into Smaller serving pots	-Not reheating -Serving spoons -Vegetable
Semovita	Semovita flour and water	Boiling	- Wrapping in polythene bags	- Polythene bag - Flat plate - Wooden spoon
Spaghetti	Spaghetti, spices, Tomatoes, Chillies, Onions	Frying & Boiling	Placed into food Warmers	- Talking while serving
Salad	Lettuce, Salad cream, Tomatoes, Onions, carrots			- Vegs not adequately cleaned - use of bare hands in shredding
Okra	Okra, pepper, palm oil, Onion, Spices	Boiling	Scooped into smaller serving pots	-Not reheating - Serving spoon -Vegetable
Chips/plantain	Potatoes/Plantain, oil and salt	Frying	Drained in a colander and served	-Perforated spoon - Coander
Tuwon Rice	Rice and water	Boiling	-Boiled rice mashed with wooden spoon - Tuwon moulded in polythene bags	-Use of unclean wooden spoon -Dipping of scooping plate at intervals in water and placing tuwon in unclean calabash for moulding
Amala	Yam flour & water	Boiling	-Scooped from pot and placed in polythene bags	- Flour may contain sports of B. cereus -Use of dirty wooden spoon

Table 6: Some of the foods prepared by the "Bukas" studied in Zaria

Food	Ingredients for Food preparation	Cooking method	Handling after Cooking	Sources of contamination
Jollof rice	Rice, Chillies, onions, Spices, Meat, Tomatoes, pepper	Boiling, Frying	Scooped into Food warmers	-Use of unclean serving spoon -Talking
Pounded Yam	Yam and water	Boiling	- Pounding - wrapping into polythene bags	-Mortar and pestle -water used for dipping pestle at intervals of pounding -Polythene bags
Vegetable soup	Spinach, Chillies, Tomatoes, Peppers, Palm Oil, Spices	Frying and boiling	Scooped into Smaller pots	-Not reheating -Vegetable
Semovita	Semovita and water	Boiling	- Wrapping	- Gari may contain spores of B. cereus - Insufficient heat treatment of Semovita
Tuwo Rice	Rice & water	Boiling	- Mashed with wooden spoon - Moulding in calabash	- Wooden spoon & calabash - Flat plate for scooping & dipping in water
Rice & Beans	Rice, Beans and water	Boiling	Scooped from pot to warmers	- Unclean serving spoon - Plates -Talking while serving
Eba	Gari and water	Boiling	Wrapped into polythene bags	-Scooping plate -Polythene bags
Stew	Tomatoes, Chillies, Onions, Oil, Meat. Pepper, and Spices	Frying and Boiling	Scooped into smaller pots	-Not reheating -Serving spoon
Okra	Okra, Chillies. Onions, Peppers, Palm oil. Spices	Frying and Boiling	Scooped into smaller pots	-Not reheating -Okra

Parameter **Counts Log_{10} cfu/g***

	APC	SC	BC	CC
Jollof rice (n = 9)				
RF	6.73±1.09	5.95±0.30	5.70±0.15	<l
IAC	<l	<l	<l	<l
H	6.12±0.14	6.69±1.13	5.80±6.20	5.90±0.11
Vegetable Soup (n=9)				
RF	7.38±0.39	6.61±0.92	6.30±0.75	6.1±0.25
IAC	<l	<l	<l	<l
H	6.03±4.88	6.66±1.08	5.58±0.48	5.76±2.11
Pounded Yam (n=9)				
RF	7.59±0.45	6.72±0.88	7.15±0.98	5.89±1.28
IAP	5.77±0.54	5.55±0.45	<l	5.58±0.47
H	6.74±1.07	6.80±0.87	6.64±1.08	6.57±1.08
Water (n=9)	<l	<l	<l	<l
Hand Swab (n=3)	6.07±0.19	7.56±0.40	<l	<l
Working Surface Swab (n=3)	5.93±3.85	6.57±1.19	<l	5.54±0.0

Table 7: Microbial quality of foods at different stages of preparation and handling in the restaurants

APC = Aerobic Plate Count
SC = Staphylococcal Count
BC = *Bacillus* Count
CC = Coliform Count
RF = Raw Food
H = Holding for an average of six hours
IAC = Immediately after Cooking
IAP = Immediately after Pounding
* Counts are means of three Restaurants
± = Standard deviation
<l = Organism not isolated

Table 8: Microbial quality of foods at different stages of preparation and handling in the cafeterias

Parameter	**Counts Log_{10} cfu/g***			
Jollof Rice (n = 9)	**APC**	**SC**	**BC**	**CC**
RF	6.44±0.38	6.33±0.57	6.33±0.62	6.84±0.10
IAC	<l	<l	<l	<l
H	6.14±0.48	6.41±0.44	5.96±0.84	6.86±0.10
Vegetable Soup (n=9)				
RF	5.56±0.45	6.39±0.51	6.33±0.51	6.11±0.61
IAC	<l	<l	<l	<l
H	6.23±0.4	6.48±1.50	6.09±0.55	5.88±6.27
Pounded Yam (n=9)				
RF	6.05±0.12	5.86±0.12	0.46±0.55	5.92±6.27
IAP	6.36±0.46	6.18±0.54	5.60±0.45	6.38±0.46
H	6.97±0.27	6.77±0.20	6.87±0.32	6.66±0.11
Water (n=3)	5.94±0.15	6.04±0.55	5.91±0.10	<l
Hand Swab (n=3)	6.95±0.21	6.91±2.9	5.78±0.12	6.85±0.20
Working Surface Swab (n=3)	6.23±0.0	5.63±0.0	<l	6.28±0.82

APC = Aerobic Plate Count
SC = Staphylococcal Count
BC = *Bacillus* Count
CC = Coliform Count
RF = Raw Food
H = Holding for an average of six hours
IAC = Immediately after Cooking
IAP = Immediately after Pounding
* Counts are means of three Cafeterias
± = Standard deviation
<l = Organism not isolated

Table 9: Microbial quality of foods at different stages of preparation and Handling in the "Bukas"

Parameter	Counts Log_{10} cfu/g*			
	APC	**SC**	**BC**	**CC**
Jollof Rice (n = 9)				
RF	5.72±0.63	7.69±0.63	5.91±3.70	<l
IAC	<l	<l	<l	<l
H	7.25±1.03	6.54±0.93	7.83±0.20	5.87±6.41
Vegetable Soup (n=9)				
RF	7.28±0.92	6.27±1.33	6.62±1.17	6.29±1.35
IAC	<l	<l	<l	<l
H	6.89±1.52	7.03±0.98	6.07±1.00	6.28±1.17
Pounded Yam (n=9)				
RF	6.33±0.64	6.36±1.38	6.33±1.20	5.37±0.47
IAP	5.63±0.47	5.72±0.17	5.98±2.60	5.93±4.60
H	7.76±0.12	6.67±0.76	6.22±0.18	5.92±5.72
Water (n=3)	5.94±0.0	5.60±0.4	<l	5.76±0.25
Hand Swab (n=3)	5.41±0.43	7.34±1.17	<l	<l
Working Surface Swab (n=3)	7.04±1.02	5.66±0.53	5.78±0.14	<l

APC = Aerobic Plate Count

SC = Staphylococcal Count

BC = *Bacillus* Count

CC = Coliform Count

RF = Raw Food

H = Holding for an average of six hours

IAC = Immediately after Cooking

IAP = Immediately after Pounding

* Counts are means of three Bukas

± = Standard deviation

<l = Organism not isolated

Table 10: Bacterial counts*, processing temperature and pH at various stages of Jollof rice preparation and handling for the restaurants, cafeterias and "bukas"

Procedure and Period of Sampling	**R_A**	**R_B**	**R_C**	**C_A**	**C_B**	**C_C**	**B_A**	**B_B**	**B_C**
Before Cooking									
pH	7.1	7.0	7.0	7.1	7.0	7.0	6.9	7.1	7.0
Temperature	-	-	-	-	-	-	-	-	-
APC	6.00	6.20	7.99	5.04	6.10	6.64	5.99	5.27	6.17
CC	<l	<l	<l	<l	<l	<l	<l	<l	<l
SC	5.59	6.04	6.14	6.71	6.27	6.93	7.62	7.57	7.80
BC	5.77	<l	5.56	6.64	6.30	5.73	6.27	5.94	5.89
Immediately after Cooking									
pH	6.9	6.9	6.9	6.9	6.9	6.9	6.8	6.9	6.8
Temperature	73	83	80	61	69	73	81	73	80
APC	<l	<l	<l	<l	<l	<l	<l	<l	<l
CC	<l	<l	<l	<l	<l	<l	<l	<l	<l
SC	<l	<l	<l	<l	<l	<l	<l	<l	<l
BC	<l	<l	<l	<l	<l	<l	<l	<l	<l
Holding for Service (For an average of 6 hours)									
pH	6.8	6.8	6.9	6.9	6.8	6.6	6.7	6.7	6.7
Temperature	39	43	43	40	33	45	35	32	29
APC	6.04	6.00	6.27	6.73	5.96	5.77	6.07	7.99	7.69
CC	5.83	5.84	<l	5.89	<l	<l	5.86	5.80	5.93
SC	5.98	7.90	5.90	6.80	6.74	5.91	6.00	7.62	7.77
BC	5.73	<l	5.80	5.76	5.80	6.89	7.07	7.72	7.70

-Not determined

APC = Aerobic Plate Count

CC = Coliform Count

SC = Staphylococcal Count

BC = *Bacillus* Count

R = Restaurants

C = Cafeterias

B = "Bukas"

*All counts are means of duplicate plate counts (Log_{10}cfu/g)

<l = Organism not isolated

Table 11: Bacterial counts*, processing temperature and pH at various stages of vegetable soup preparation and handling for restaurants, cafeterias and "bukas"

Procedure and Period Of Sampling	**R_A**	**R_B**	**R_C**	**C_A**	**C_B**	**C_C**	**B_A**	**B_B**	**B_C**
Before Cooking									
pH	4.9	5.8	5.2	4.8	4.9	4.7	4.6	5.4	4.9
Temperature	-	-	-	-	-	-	-	-	-
APC	7.83	7.07	7.23	5.74	6.69	5.90	7.73	7.90	6.23
CC	6.00	5.84	5.80	5.68	6.88	6.55	7.78	5.94	5.14
SC	6.07	6.10	7.60	5.82	5.77	6.67	5.81	5.23	7.77
BC	5.90	7.17	5.84	5.70	6.98	6.61	5.99	7.98	5.91
Immediately after Cooking									
pH	5.2	6.0	5.5	5.0	5.1	5.0	5.0	6.0	5.1
Temperature	63	69	65	59	60	61	60	62	57
APC	<l	<l	<l	<l	<l	<l	<l	<l	<l
CC	<l	<l	<l	<l	<l	<l	<l	<l	<l
SC	<l	<l	<l	<l	<l	<l	<l	<l	<l
BC	<l	<l	<l	<l	<l	<l	<l	<l	<l
Holding for Service (for an Average of 6 Hours)									
pH	5.2	6.0	5.4	4.9	5.0	4.8	4.9	5.8	5.0
Temperature	36	32	29	29	27	30	32	28	30
APC	5.98	6.04	6.07	6.00	6.69	6.96	7.54	7.94	5.14
CC	5.74	5.77	<l	5.81	6.86	5.94(+)	7.60(+)	5.27	5.98
SC	6.07	7.91	5.99	5.91	6.57	6.71	5.90	7.62	7.59
BC	5.55	6.11	6.07	6.73	5.23	5.77	6.07	7.97	5.07

(+) = *E. coli* isolated

APC = Aerobic Plate Count

CC = Coliform Count

SC = Staphylococcal Count

BC = *Bacillus* Count

R = Restaurants

C = Cafeterias

B = "Bukas"

*All counts are means of duplicate plate counts (Log_{10}cfu/g)

<l = Organism not isolated

Table 12: Bacterial counts*, processing temperature and pH at various stages of pounded Yam preparation and handing for restaurants, cafeterias and "bukas"

Procedure and Period of Sampling	R_A	R_B	R_C	C_A	C_B	C_C	B_A	B_B	B_C
Before Cooking									
pH	7.0	7.0	7.1	6.6	7.0	6.9	6.8	6.8	6.9
Temperature	-	-	-	-	-	-	-	-	-
APC	7.80	7.07	7.90	5.91	6.10	6.14	6.00	7.07	5.93
CC	5.88	5.90	5.93	6.57	5.25	5.94	5.07(+)	5.92	5.11
SC	7.74	6.23	6.20	5.6	5.88	6.08	6.00	5.20	7.89
BC	7.53	7.89	6.04	6.80	6.76	5.82	6.07	5.27	7.64
Immediately after pounding									
pH	6.9	6.9	6.9	6.5	6.9	6.7	6.7	6.6	6.8
Temperature	50	52	47	42	45	40	41	43	43
APC	6.07	6.11	5.14	6.11	6.09	6.90	5.92	5.07	5.89
CC	5.80	5.90	5.04	6.00(+)	6.92	6.02	5.90(+)	5.92	5.99(+)
SC	5.62	5.97	5.07	6.81	5.81	5.92	5.74	5.88	5.53
BC	<l	<l	<l	5.07	5.90	5.83	5.90	5.93	5.85
Holding for Service (for an Average of 6 Hours)									
pH	6.9	6.9	6.9	6.5	6.9	6.7	6.5	6.5	6.7
Temperature	35	29	31	29	27	27	29	29	27
APC	6.77	7.98	6.25	7.23	6.69	7.00	7.73	7.91	7.66
CC	5.91	7.85	5.95	6.76	6.53	6.70	5.99	5.89	5.80
SC	7.81	6.27	6.32	7.00	6.70	6.00	6.27	6.20	7.55
BC	5.91	7.89	6.11	7.20	6.77	6.60	6.23	6.41	6.04

(+) = *E. coli* isolated

APC = Aerobic Plate Count

CC = Coliform Count

SC = Staphylococcal Count

BC = *Bacillus* Count

R = Restaurants

C = Cafeterias

B = "Bukas"

*All counts are means of duplicate plate counts (Log_{10}cfu/g)

<l = Organism not isolated

Table 13: Biochemical characterisation of presumptive *E. coli, B. cereus* and *S. aureus* from food and swab samples

Test	No. (%) of isolates positive		
	E.coli	*B. cereus*	*S.aureus*
	n=6	n=57	n=82
Gram Reaction	0(0.0)	57(100)	82(100)
Methyl Red	6(100)	-	-
V-P Reaction	0(0.0)	57(100)	-
Indole	6(100)	0(0.0)	82(100)
Urease	0(0.0)	0(0.0)	0(0.0)
Oxidase	0(0.0)	0(0.0)	51(62)
Catalase	6(100)	57(100)	82(100)
Citrate	0(0.0)	55(96)	73(89)
Motility	6(100)	57(100)	0(0.0)
Anaerobic Growth	6(100)	54(94)	51(62)
Spore Formation	-	53(92)	-
Nitrate Reduction	-	57(100)	82(100)
Gelatin Liquefaction	-	57(100)	-
Growth in 7% Nacl	-	53(92)	82(100)
Reaction with Litmus milk	-	57(100)	-
Starch Hydrolysis	-	57(100)	-
Phenylanine Deaminase	-	0(0.0)	-
Coagulase	-	-	82(100)
Haemolysis	-	-	82(100)
Utilization of:			
Mannitol	6(100)	-	-
Glucose	6(100)	57(100)	82(100)
Lactose	6(100)	-	-
Sorbitol MacConkey Agar	6(100)	-	-

- not applicable

Table 14: Antibiogram of *B. cereus, E. coli*, and *S. aureus* Isolated from food and swab samples

Antimicrobial agents(Concentration)/ Test organism	No. (%) Susceptible	No. (%) Intermediate	No. (%) Resistant
B. cereus **n = 32**			
Ampicillin (10μg)	3(9.3)	3(9.3)	26(81.2)
Chloramphenicol(10μg)	20(62.5)	11(34.3)	1(3.1)
Gentamycin(10μg)	28(87.5)	4(12.5)	0(0.0)
Streptomycin(10μg)	29(90.6)	2(6.2)	1(3.1)
Tetracycline(10μg)	25(78.1)	5(15.8)	2(6.2)
Cloxacillin(5μg)	2(6.2)	1(3.1)	29(90.6)
Erythromycin(5μg)	17(53.1)	14(43.7)	1(3.1)
Penicillin(1 i.u)	0(0.0)	0(0.0)	32(100)
E.coli **n = 6**			
Nitrofurantoin(200μg)	6(100)	0(0.0)	0(0.0)
Nalidixic Acid (30μg)	5(83.3)	1(16.6)	0(0.0)
Tetracycline (25μg)	2(33.3)	0(0.0)	4(66.0)
Ampicillin (25μg)	0(0.0)	0(0.0)	6(100)
Colistin(25μg)	0(0.0)	5(83.3)	1(16.6)
Streptomycin(25μg)	5(83.3)	0(0.0)	1(16.6)
Co-trimoxazole(25μg)	3(50.0)	1(16.6)	2(33.3)
Gentamycin(10μg)	6(100)	0(0.0)	0(0.0)
S. aureus **n = 54**			
Ampicillin (10μg)	0(0.0)	4(7.4)	50(92.5)
Chloramphenico(l0μg)	18(33.3)	16(29.6)	20(37.0)
Gentamycin(10μg)	49(90.7)	4(7.4)	1(1.8)
Streptomycin(10(g)	49(90.7)	2(3.7)	3(5.5)
Tetracycline(10(g)	11(20.3)	10(18.5)	32(59.2)
Cloxacillin(5(g)	24(44.4)	8(14.8)	22(40.7)
Erythromycin(5μg)	7(12.96)	27(50.0)	20(37.0)
Penicillin(1 i.u)	8(14.8)	1(1.8)	45(83.3)
Staphylococcus aureus ATCC021001		n =1	
Ampicillin (10μg)		Resistant	
Chloramphenicol (10μg)		Intermediate	
Gentamycin (10μg)		Susceptible	
Streptomycin (10μg)		Susceptible	
Tetracycline (10μg)		Intermediate	
Cloxacillin (5μg)		Resistant	
Erythromycin (5μg)		Susceptible	
Penicillin (1 i.u)		Resistant	
Escherichia coli ATCC11775		n = 1	
Nitrofurantion (200μg)		Susceptible	
Nalidixic Acid (30μg)		Susceptible	
Tetracycline (25μg)		Resistant	
Ampicillin (25μg)		Resistant	
Colistin (25μg)		Intermediate	
Streptomycin (25μg)		Susceptible	
Co-trimoxazole (25μg)		Resistant	
Gentamycin (10μg)		Susceptible	

CHAPTER FIVE

Discussion

5.1 Hygiene / Food Safety Practices and Sanitation

The results showed poor personal hygiene behaviour of the food handlers, although 44(97.8%) were dressed in clean cloths, 33 (73.3%) wore hair covers, and 41(91.1%) kept short nails, frequent hand washing was not observed in any of the food service centres surveyed, therefore the food handlers lack the basic knowledge of the importance of hand washing during food preparation and handling. A hygienic hand washing for food handling purposes could be defined as requiring warm water, soap or detergent and scrubbing or rubbing action for at least 20 seconds. Washing without detergent or soap is generally regarded to be ineffective or at least significantly less effective in removing transient micro flora present on the skin before handling food or that are transferred to the skin by handling raw food (Synder, 1988). Hand washing even with soap or detergent, for less than 20 seconds is also likely to be less effective hygiene activity (Jay et al., 1999). Effective hand washing was therefore not practiced by a significant proportion of food handlers before or during food preparation. It could be reasonable to conclude therefore, that effective hand washing only occurs on a limited basis in food service centres.

Most food handlers were observed to wipe their hands with either their personal clothing or with any rag. This attitude could lead to cross-contamination of food with pathogens. In a previous study conducted by Jay et al., 1999, 18.0% of 1,203 respondents answered that they do dry their hands on the same towel they use for drying dishes.

The rubbing of nose, adjusting head covers, blowing nose, scratching or touching dirty clothing, sneezing, yawning and coughing and many other habitual actions could

dramatically affect food safety, These dirty habits are very hard to control, but a conscious effort should be made to curtail them while in food processing or preparation area (Onuorah et al., 2003). There is also potential health risks associated with methods used by food handlers to test the adequacy of cooking by touching foods with bare hands. Fingers are normally used to feel food stuffs and ingredients for texture and to ascertain the adequacy of manual grinding or pounding, and this has the potential to contribute to the microbial load of the food (Ehiri et al., 2001). Although the time-temperature exposure during cooking was adequate to kill vegetative cells, the frequency of cross-contamination from the hands of food handlers and utensils was high. The holding of cooked foods for prolonged periods (i.e. several hours) after preparation and at temperatures of 27 to 40oC is a hazard. It is unacceptable by most public health standards because it is a contributing factor to the occurrence of food-borne disease.

Poor sanitary handling of foods is identified as a major contribution to contamination in this study and other studies (Umoh and Odoba, 1999). Sanitation comes from the Latin word santas meaning "health", in food plants and food services situation, sanitation means wholesome food, handled in a hygienic environment by healthy food handlers in a way that the food stays safe (Onuorah et al., 2003). This study revealed the poor sanitary measures taken in the sanitation of food preparation and handling areas. The practice of daily sweeping was observed in the food service centres but adequate scrubbing and mopping of floors and working surfaces was lacking especially in the cafeterias and “Bukas”, disinfectants were not used in cleanings. Most of the food handlers did not know that disinfectants were used for reducing bacteria to a safe level. It is therefore likely that the entire environment would contain high number of contaminants. The lack of facilities for waste water drainage and garbage disposal in the 15 “Bukas” surveyed encourages waste to be thrown into nearby streets and gutters.

Such areas act as habitats for rodents, breeding points for flies and media for growth of micro organisms. Ready-to-eat foods sold in unsanitary locations are susceptible to contamination by flies and dust laden micro-organisms. The linkage between houseflies and diarrhoeal diseases has been documented (Umoh and Odoba, 1999, Ehiri et al., 2001 and Mensah et al., 2002). The evidence of flies in cooking and serving vicinities could not be ruled out.

5.1.1 Knowledge of Diarrhoea /Food Poisoning

Responses to this section of the questionnaire demonstrated that the respondents were aware of the frequency of stools an individual should pass in a day before it is attributed to diarrhoea (84%), they also had the knowledge of diarrhoea being transmitted through dirty food and water, and germs are the primary cause of diarrhoea. In a similar study carried out in Ghana by Mensah *et al.,* (2002), a higher percentage (94%) of respondents associated diarrhoea with the passage of three or more stools per day. But it is of public health concern to note that 70% of the respondents in this study did not know that food poisoning was caused by food that looked, smelt and tested normal. This result clearly indicates that the majority of the respondents did not understand that organoleptic assessment of food was insufficient to identify food contaminated by pathogenic bacteria. Only 78(86.7%) out of the 90 respondents associated diarrhoeal transmission with dirty hands and 82.2% associated diarrhoeal transmission with dirty utensils, while 13.3% and 17.8% did not associate transmission of diarrhoeal pathogens with dirty hands and utensils respectively. In a similar study by Mensah *et al.,* (2002), out of the 177 street vendors interviewed, none was aware that dirty hands were a risk factor for diarrhoea. A

total of 18.6% out of 1,203 respondents in a national Australian food safety telephone survey failed to recognise that washing hands before handling or preparing food was a vitally important food hygiene activity (Jay *et al*., 1999). Only 6(6.7%) out of the ninety respondents in this study associated diarrhoea with chemicals. It is generally impossible by visual inspection to tell whether food is dangerously contaminated or not. It should be remembered also that most food-poisoning bacteria cause symptoms only when eaten in large numbers after multiplication in food, they do not usually alter the appearance, taste or smell of the food. In a survey on knowledge, attitude and responses of a rural Nigerian community in Anambra State to outbreaks of cholera by Bamidele *et al.,* (2003), revealed that in spite of the people's knowledge of the causes and health consequences of cholera infection this did not translate into proper attitude and practice of appropriate environmental behaviour. Behaviour related to personal and environmental hygiene and food preparations contribute greatly to the occurrence and severity of food poisoning outbreaks.

5.2 Prevalence of Organisms in Raw Foods

The results of this study showed a high prevalence rate of *B. cereus*, and *S. aureus* in all the raw samples (rice, yam and ground vegetables paste). This is not surprising because most of these commodities were purchased from the open market where they are displayed in open bowls or sacks and sometimes on the ground. Since micro-organisms are widely distributed in the soil and the atmosphere, contamination of these products during processing and handling is expected. Similar high counts (range 2.4×10^6 to 1.7×10^7 cells/g foods) and frequency was observed in dry food condiments sold in the local markets

(Ogbadu *et al.,* 2000). The high prevalence rate of *S. aureus* could also be attributed to its ubiquity. The clinical significance of this organism is largely due to the ubiquity of this species, being found in a wide variety of habitats and frequently as part of the normal human micro flora (Sandel *et al.,* 2003). *S. aureus* can colonise and survive in a wide variety of environmental niches, eradication of the bacterium is difficult owing to its ubiquity. It can asymptomatically colonise the nasopharynx as well as persist outside the host in dust, soil, water and other environments (Clements and Foster, 1999).

The isolation of *E.coli* from raw yam suggests contamination from the food handler, water or the source of the raw yam and is indicative of faecal contamination. Faecal contamination of water supplies and contamination from food handlers have been frequently implicated in outbreaks caused by *E. coli* (Adams and Moss, 1999). Detection of coliforms in foods is an indication of contamination from human source (Okonkwo *et al*., 1994). Coliforms are of intestinal origin and they get into foods because of poor hygienic conditions of the handlers (Abdullahi *et al*., 2004). The preparation of raw food ingredients at ground level and on inadequately cleaned working surfaces as observed in this study could obviously increase the initial microbial load of these ingredients of which subsequent heat treatment might not kill all the organisms especially *B.cereus.* Thus, a critical control point in food preparation and handling (plate 1 and 2).

5.2.1 Prevalence of Organisms in Cooked Foods

Heat treatment of food (e.g. cooking) not only improves the taste, smell, appearance and digestibility, it also reduces the number of micro-organisms,

improves keeping qualities by inhibiting moulds, yeast, and bacteria that promote decay and infection. Thus heat treatment is a practice aimed at improving the overall safety of food. The cooking temperatures attained boiling points (100^{o}C) which substantially obliterate the high counts of organisms obtained from raw products to zero levels, except pounded yam. *S. aureus, B. cereus* and *E. coli* were isolated from pounded yam. This is obviously from post cooking contamination by food handlers, utensils, mortar, pestle and water used for dipping pestle at intervals of pounding and moulding into shapes and wrapping in polythene bags. Time-temperature exposure during cooking was adequate to kill vegetative microbes and not the spores, but potential for recontamination existed from the hands of food workers, utensils, and cloths and sponges used in wiping as confirmed by other studies (Ali and Spencer; 1995). In a similar study fufu was heavily contaminated because of the excessive handling after cooking (Mensah *et al.,* 2002). The pounding process, moulding and wrapping of pounded yam as was observed in this study entails profuse sweating of which some of the sweats could undeniably gain access into the pounded yam resulting in post cooking contamination (Plates 3 and 4).

5.2.2 Prevalence of Organisms during holding for service.

Most of these foods were generally prepared in bulk and displayed for an average of six hours in pots and food warmers without reheating (plate 5). Therefore, the potentials for bacterial growth and proliferation exist. Although the temperature used in cooking and frying is always high enough to kill most vegetative cells, the resistant spores may survive (Umoh and Odoba, 1999). Therefore, the high mesophilic count in this study is suspected to have arisen

from the surviving spores, cross-contamination resulting from food handlers, utensils, and the environment as observed by Umoh and Odoba, (1999). At the period of sampling (May to August), the ambient temperatures ranged from 25 to 32^0C and relative humidity ranges from 22 to 63% (weather record, farm system research programme). The temperature and relative humidity during this period were ideal for the multiplication of micro-organisms. Earlier studies confirmed that staphylococcal and total aerobic counts in milk production were highest in the wet humid period and lowest in the dry harmattan period (Umoh and Odoba 1999).

Hazard analysis and epidemiological studies have revealed that foods prepared in advance and kept at room temperatures for a long time (4-6 hrs or more) showed a massive increase in bacterial counts, reaching a critical level that may cause food-borne illness (Ali and Spencer, 1995). In a similar situation, when rice and related foods were held at room temperature for 4 hrs or longer particularly overnight, high number of bacteria developed during these intervals (Umoh and Odoba, 1999). In this study most of the counts 5.76 to 7.91 $\log_{10}$cfu/g for *B.cereus* and 5.99 to 7.91 $\log_{10}$cfu/g for *S.aureus* were higher than the permissible levels, of less than 5.0 $\log_{10}$cfu/g for *B. cereus* and less than 6.0 $\log_{10}$cfu/g for *S. aureus* (ICMSF,1996). Susceptibility of individuals to pathogens varies widely with age, acquired immunity and state of health. 'Special risk' groups, in which the risk is greater and disease may be far more serious, include infants, the very old, pregnant women and the immuno-compromised (including AIDS patients). Because 'dose/ response ' can vary, acceptable (i.e. safe), levels of contamination are often based on epidemiological studies (Singleton, 1997).

The mean count for *B. cereus* recorded for pounded yam during holding for the Restaurants, Cafeterias and "Bukas" were 6.64 ± 1.0, 6.87 ± 0.31 and 6.22 ± 0.1 $\log_{10}$cfu/g respectively. The mean count of *B. cereus* obtained from Jollof rice during holding for the Restaurants, Cafeterias and "Bukas" were; 5.80 ± 6.20, 5.96 ± 0.84 and 7.83 ± 0.20, respectively. While the mean count recorded for vegetable soup during holding for the Restaurants, Cafeterias and "Bukas" were 5.58 ± 0.4, 6.09 ± 0.5 and 6.07 ± 1.0, respectively. The mean counts of *B. cereus* from jollof rice in this study was less than that reported by Hassan and Nabbut, (1995) which ranged from 10^6 – 10^8 cfu/g. Similar counts were also recorded for Eba (3.0 x 10^5) and Tuwo (4.6 x 10^4) by Yusuf *et al*., (1992) and from street foods in Accra, Ghana (Mensah *et al*., 2001). The holding temperature range of 26 – 45^{o}C places the food in a favourable condition for the growth and multiplication of these organisms. The presence of *B.cereus* coupled with the holding of these foods at ambient temperatures for several hours and the prevailing relative humidity at the time of sampling indicate that the foods could be hazardous.

The wrapping of pounded yam in polythene bags could be a source of contamination by *B. cereus*, so also the mortar and pestle. Strains of *B. cereus* were isolated form paper board used for packaging foods (Pirttijarvi *et al*., 1996). Furthermore, wrapping foods to maintain the warmth and moisture for extended periods, could allow the germination of spores and growth of vegetative cells (Ali and Spencer, 1995). The isolation of *B. cereus* from the hand and working surface indicates the ubiquity and possible sources of this organism. In a survey in Great Britain, 14% of faecal specimens of healthy individuals contain *B. cereus* (Hassan and Nabbut, 1995). *Bacillus cereus* has a widespread distribution in

nature as reflected by its isolation from different sources. Microbes that cause food-borne illness commonly enter food through cross contamination and grow in temperatures favourable to them (Wardlaw and Insel, 1996).

The mean counts of *S. aureus* obtained from pounded yam for the restaurants, cafeterias and "Bukas" were, 6.80 ± 0.8, 6.77 ± 0.2, and 6.67 ± 0.7, respectively. The mean counts obtained from Jollof rice were, 6.60 ± 1.1, 6.41 ± 0.4 and 6.54 ± 0.9 respectively. While the mean counts recorded for vegetable soup were, 6.66 ± 1.08, 6.48 ± 0.50, and 7.03 ± 0.98 respectively. The contamination of these foods with *S. aureus* could be attributed to post-cooking contamination the major sources are probably the food handlers, flies and dust. The temperature range of 26 – 45^{o}C recorded for foods during holding, makes them favourable for the growth and multiplication of *S. aureus*. The fastest rate of growth of *S. aureus* occurs in the range of 20 to 45^{o}C, an initial population can reproduce four times more rapidly at 45^{o}C than at 20^{o}C (Adams and Moss, 1999). In a near-sterile food system with sufficient nutrients and the absence of competitive natural micro flora, an initial inoculum of 10^{4}cfu of *S. aureus* per gram could replicate to 10^{8}cfu/g in 24 hours held at 40^{o}C (Adams and Moss, 1999). Staphylococci are widespread in nature and can be found in the air, in dust, water and on humans and animals. The main human reservoirs of these organisms are the skin and nasal cavity, about 40 to 44% of healthy humans carry *S. aureus* in the nose (Su and Wong, 1997). The high counts of *S. aureus* obtained in this study could be attributed to the regular opening of the food warmers at intervals of serving, talking or un-stifled sneezing while serving and regular touching of nostrils. Although foods may be contaminated from a cough or sneeze, from a healthy carrier, more often, contamination is from an

individual with an infection on the hands or with a cold or sore throat (Onuorah *et al*., 2003). *S. aureus* inhabits the nostrils of virtually everyone at one time or another each nostril containing as many as 10^8 cells, the organisms are mainly disseminated to other parts of the body and to the environment by the hands (Nester *et al*., 2001). Sweating during pounding yam and the use of inadequately cleaned hands during wrapping obviously increased the contamination of this food. Of the various metabolites produced by *S. aureus*, enterotoxins pose the greatest risk to consumer's health (Sandel *et al*., 2003). The presence of *S. aureus* in food indicates a high potential for the production of enterotoxin that causes food poisoning (Su and Wong, 1997). In this study foods were kept up to 4 to 6 hours therefore, the *S. aureus* in these foods presents a risk of causing food-borne intoxication due to handling contamination and holding for long periods without reheating (Umoh and Odoba, 1999).

Eighty-one percent of the *S. aureus* tested for haemolysin production were of the ∀-haemolytic pattern while 18% were of the β pattern. Earlier studies revealed that (-haemolysin producing strains are predominantly human biotypes while β-haemolysin producing strains are usually associated with the environment (Umoh and Odoba, 1999). This indicates that the high number of S. aureus counts recorded in this study probably resulted from contamination by the food handlers, utensils and working surfaces. In this study S. aureus was isolated from all the hand swabs and working surface samples.

E. coli was isolated from pounded yam and vegetable soup during holding. The detection of E. coli in these foods is an indication of contamination most likely from human source. E. coli was isolated from tomato stew and shito (Mensah et al., 2002) and

if reheating is inadequate, bacterial contaminants can multiply. Entero-pathogens can survive on hands for three hours or longer. (Mensah et al., 2002). Therefore, the use of bare hands during wrapping pounded yam and the opening and closing of warmers containing soups at intervals of serving could lead to cross-contamination. These observations agree with the results of similar works (Umoh and Odoba, 1999 and Ehiri et al., 2001). E. coli was detected in the hand washings of high-income and low income mothers in India at levels of 7.0 ± 4.2 log10 cfu/ml and 9.0 ± 5.7 log10 cfu/ml respectively. A study on foods sold in some Nigerian schools showed unacceptable levels of bacteria, in addition E. coli was isolated from some samples (Olukoya et al., 1991). Defective personal hygiene can facilitate the transmission of these pathogens via food to humans, the serving stage is a critical point in the food industry (Mensah et al., 2002). A study of complementary food preparation and handling in Eastern Nigeria also confirmed the presence of enteric pathogens (Ehiri et al., 2001). There were many opportunities for the contamination of hands, a person may contaminate his/her hands on cleaning after defaecation or in touching or handling contaminated objects including soil. Filthy flies particularly the common housefly, spread faecal material and play a role in the transmission of gastro-intestinal infections. Vegetables, were also possible sources, of bacterial contaminants since vendors often used unwholesome cheap supplies in order to maximise their profits (Mensah et al., 2002).

Statistical analysis of these counts showed no level of significance ($p > 0.05$) in the counts of the three categories of food centres (Restaurants, Cafeterias, and “Bukas”). This indicates that the prevailing conditions were not too different at the various centres.

5.3 Hazards and Critical Control Points of some Selected Foods

The application of HACCP strategy to this study is based on the premise that potential hazards and faulty practices can be identified at an early stage in food preparation and handling, and that their identification may lead to measures that prevent or reduce the risks of food-borne illness. This approach (HACCP) determines quickly and relatively cheaply the points in the food preparation, handling and serving processes that are critical to safety. The hazard and critical control points identified in jollof rice, vegetable soup and pounded yam preparation and handling are;

1. ***Ingredients and Cross Contamination***

In this study, high counts of *B. cereus* and *S. aureus* were recorded. These bacteria are commonly found on raw foods especially cereals, vegetables and spices. Vegetables were also possible sources of bacterial contaminants since vendors often use cheap supplies in order to maximise their profits. The continuous display of raw foods in the markets mostly uncovered as well as regular handling by prospective buyers and traders makes them hazardous. Cross-contamination accounts for the presence of pathogen in these foods. It was observed in this study that frequent hand washing was not practiced and the possibility of cross-contamination was high.

2. ***Cooking***

Cooking is a critical control point relative to vegetative forms of pathogenic bacteria that may be on the raw food or that may reach food during preparation. Heat treatment is a practice aimed at improving the overall safety of food, it is therefore a critical control point. Foods cooked by all the

categories of food service centres attained boiling points (100^{o}C) sufficient to kill large numbers of vegetative cells, but not all spores of pathogenic bacteria. Holding of these foods at ambient temperature in warmers is another critical control point.

3. Handling

The handling of food after cooking is a critical control point. Food from the cooking pots were transferred into food warmers using a flat plate or a large serving spoon. Keeping of serving spoons or plate on top of food warmer covers and serving food with uncovered hair (plate6) was observed, this may allow contamination to occur by dust, flies or the hands of the food handlers. Pounding and wrapping of pounded yam were also critical control points. There is consequently a risk of contamination associated with the exposure of food to flies (Mensah *et al.,* 2002).

4. Holding

Epidemiological data from industrialised countries have shown that the primary factor that contributes to outbreaks of food borne diseases is holding cooked foods at ambient temperature for several hours (Bryan *et al.,* 1992). This situation also ought to be a significant factor contributing to outbreaks of food borne diseases elsewhere, and hence, holding of cooked food is a critical control point. In this study cooked foods were held for 4 to 6 hours and the temperature of the foods (27 - 40^{o}C) were favourable for the growth and multiplication of bacterial cells. Criteria for control are to either hold foods for short duration (i.e. less than 4 hrs) or to keep them at temperatures higher than 54.4^{o}C (Bryan *et al*., 1992). There are two basic control measures to counter

this hazard, is either to eat food promptly after cooking or to hold cooked foods at or above 55^{o}C. Cooking of foods in the opened air as was observed in all the bukas could evidently give way to air-borne pathagens (plate7).

5. Serving

During the interval between preparation and serving of food, there is ample time for pathogenic bacteria to proliferate to numbers or produce amounts of toxins sufficient to overwhelm the resistance threshold of healthy adults if this is coupled with temperature abuse, mishandling of serving utensils and talking while serving. Talking while serving foods and the used of inadequately cleaned serving utensils as was observed in this study indicate that serving is a critical control point.

5.4 Antibiotic-Sensitivity Test

The antibiotic-sensitivity test of some of the isolates to antimicrobial agents revealed that the isolates were resistant to the common drugs of choice used in the treatment of infections. The high resistance of *E.coli, S.aureus* and *B. cereus* to the beta-lactam agents (ampicillin, penicillin and cloxacillin) and tetracycline recorded in this study were similar to that studied by Hassan and Nabut (1995). Many studies have reported *B. cereus* to be resistant to the penicillins through the production of beta-lactamases (Hassan and Nabbut, 1995). *Bacillus cereus* isolated from rice was susceptible to chloramphenicol, erythromycin and streptomycin and resistant to ampicillin and penicillin, this is in agreement with the previous report of Umoh *et al., (*1995). *Staphylococcus aureus* is also a major human pathogen of increasing importance as a result of the spread of antibiotic resistance. Bacteria are increasingly developing resistance to many classes of

antibiotics and strains that were previously considered to be harmless are posing significant health threats. The high resistance could be attributed to the widespread and indiscriminate use of antibiotics. This findings corroborates the findings by other researchers elsewhere on bacterial resistance to antimicrobial agents (Chinwe and Ezeronye, 2003, Hassan and Nabbut, 1995) and in this environment (Umoh *et al.,* 2004).

CHAPTER SIX

Conclusion and Recommendation

This research finding indicates the need for stricter implementation of food sanitation code and the licensing of food service centres by the appropriate food agencies such as the National Agency for Food and Drug Administration and Control (NAFDAC), and public health authorities need to intensify efforts to monitor conditions of sanitation and hygiene in these establishments.

The high total aerobic plate counts and the isolation of *E. coli* and *S. aureus* in some of the food samples were indications of post-cooking contamination from the food handlers, utensils and the environment. The mean counts of the organisms from all the food service centres were found to be above the acceptable limits (less than 5.0 $\log_{10}$cfu/g for *B.cereus* and less than 6.0 $\log_{10}$cfu/g for *S. aureus*) suggested by the International commission on microbiological safety of food (ICMSF, 1996), indicating the potentials of food-borne illness. The high prevalence of these organisms after cooking i.e. during holding is basically through cross-contamination by food handlers and survival of spores. Temperature abuse during holding is also another factor contributing to the growth and proliferation of these bacteria. Statistical analysis of these counts showed no level of significance in the counts from the different categories of food service centres, this indicates that the prevailing conditions were not too different at the various sites, particularly the lack of knowledge of the importance of frequent hand washing observed in all the centres and holding foods for longer periods at inappropriate temperatures favourable for bacterial growth and multiplication.

The fact that higher resistance to common used antibiotics were recorded in this study may point to the increased misuse and abuse of these drugs over the years. It is therefore of great concern since these drugs were frequently used in both medical and veterinary practices. There is therefore the need for a stricter control possibly by restricting the prescription and dispensing of antibiotics and other drugs to only qualified professionals. Education of the public on the dangers of in discriminate purchase and use of drugs especially antibiotics is also important in the control of drug misuse.

Using HACCP data to promote food hygiene is of paramount importance in situations of extreme poverty and where adequate surveillance of food borne diseases may be lacking. The HACCP strategy has the potential to make a significant contribution to food safety and can facilitate a more pragmatic approach to developing messages that assure effective behaviour change.

The Hazard analysis undoubtedly point out high risks associated with eating in these food centres. The remedy is education, education of public health personnel, education of food handlers and service personnel, and education of the public who either purchase these foods or become food handlers themselves. Education in food safety also needs to be incorporated into health education programmes. This will be a considerable task with many barriers to overcome. A HACCP investigation serves no value if the findings are not implemented and used by the public.

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Appendix I

QUESTIONNAIRE; FORM ONE

1. Name of food centre: …….
2. Address: ……..
3. Number of Workers: ……
4. Category of centre: Restaurant Cafeteria "Buka"
5. Category of consumers: School Children_____
   University Student_____
   Workers_____
   Traders_____
   Others_____ (Specify)
6. Source of water for cooking _____
7. Source of water for drinking _____
8. Source of water for cleaning/ washing _____

A. HYGIENE PRACTICES

	PARAMETER	RESPONSE		REMARKS
1	Clean water is provided for drinking	Yes	No	
2	Water for washing raw materials is adequate	Yes	No	
3	Water for cleaning utensils for consumers and equipment is adequate	Yes	No	
4	Clean dry utensils are available for serving consumers	Yes	No	
5	Facilities for holding raw food are adequate	Yes	No	
6	Facilities for holding ready to eat food are adequate	Yes	No	
7	Hand washing facility is adequate	Yes	No	

B. FOOD SAFETY PRACTICES

	PARAMETER	RESPONSE		REMARKS
1	Refrigeration facility available	Yes	No	
2	Holding cooked food exposed to flies, dust, insects, rodents	Yes	No	
3	Holding cooked food in mesh protection	Yes	No	
4	Holding cooked food sale covered	Yes	No	
5	Holding food at ground level	Yes	No	
6	Food is cooked well in advance of consumption	Yes	No	
7	Cooked food on morning of sale	Yes	No	
8	Food cooked during sale	Yes	No	
9	Cooked food is sold from cooking pot	Yes	No	
10	Cooked food is scooped into bowl or polythene bags for sale	Yes	No	
11	Cooked food is kept on cooking fire during sale	Yes	No	
12	Food is reheated before sale	Yes	No	
13	Left over food is sufficiently reheated before sale	Yes	No	
14	Food is served with bare hands	Yes	No	
15	Food is served with spoon/fork	Yes	No	
16	Food is served into cup/plate/calabash	Yes	No	
17	Food is served into papers/leaves	Yes	No	

QUESTIONNAIRE, FORM ONE Continued

C. SANITATION IN FOOD PREPARATION AREA

	PARAMETER	RESPONSE		REMARKS
1	Food area is swept daily	Yes	No	
2	Food area is washed with soap and water	Yes	No	
3	Food area is cleaned with soap, water, disinfectant	Yes	No	
S4	Food area is rarely cleaned	Yes	No	

D. **WASTE DISPOSAL PRACTICES**

	PARAMETER	RESPONSE		REMARKS
1	Waste water disposal is adequate	Yes	No	
2	Garbage/refuse disposal is adequate	Yes	No	
3	General cleanliness of vicinity is adequate	Yes	No	
4	Evidence of sewage in vicinity	Yes	No	
5	Evidence of human waste in vicinity	Yes	No	
6	Evidence of animals/animal waste in vicinity	Yes	No	
7	Evidence of rodents, flies/other insects in vicinity	Yes	No	

E. SANITARY FACILITIES

	PARAMETER	RESPONSE		REMARKS
1	Toilet/urinary facilities	Yes	No	
2	Toilet/urinary is cleaned with water soap and disinfectant	Yes	No	
3	Hand washing facility available	Yes	No	

C. PERSONAL HYGIENE (FOOD HANDLERS ONLY)

	PARAMETER	RESPONSE		REMARKS
1	Dressed in clean cloth	Yes	No	
2	Wore hair cover	Yes	No	
3	Wore covering/Apron over personal cloth	Yes	No	
4	Keeps cut nails	Yes	No	
5	Appears sickly	Yes	No	
6	Frequent hand washing habit	Yes	No	
7	Has dirty habits (s)	Yes	No	

QUESTIONNAIRE; FORM TWO

A. PERSONAL DATA FOR FOOD HANDLERS

1. Name ……Category: Food handler ☐

2. Name of food centre…….
3. Sex M☐ F☐
4. Age <30, 30-39, 40-49, 50->
5 Educational level:
   - Primary School ☐
   - Secondary School ☐
   - Arabic School ☐
   - Post Secondary School ☐
   - No Education ☐

6. Training received in food handling
   - On job training ☐
   - In School ☐
   - Centers' organized training

B. KNOWLEDGE OF DIARRHEA/FOOD POISONING

1. What is diarrhoea?
   - i. Passage of more then three liquid stools in a day Yes ☐ No ☐
   - ii. Passage of Mucoid stools Yes ☐ No ☐
2. Transmission of diarrhoeal pathogens
   - Dirty food Yes ☐ No ☐
   - Dirty water Yes ☐ No ☐
   - Dirty hands Yes ☐ No ☐
   - Dirty utensils Yes ☐ No ☐
3. Causes of diarrhoea
   - i. Germ Yes ☐ No ☐
   - ii. Chemical in food/water Yes ☐ No ☐
   - iii. Do no know Yes ☐ No ☐
4. Contamination of food by food poisoning germs is detected by the:
   - Smell of food Yes ☐ No ☐
   - Sight of food Yes ☐ No ☐
   - Taste of Food Yes ☐ No ☐
5. Food poisoning Germs can multiply very fast in food:
   - Kept at room temperature Yes ☐ No ☐
   - Kept at refrigeration temperature Yes ☐ No ☐
   - Kept at frozen temperature Yes ☐ No ☐
   - Kept on fire Yes ☐ No ☐
6. Source of contamination of food by food poisoning:
   - a. Raw food materials Yes ☐ No ☐
   - b. Ingredients Yes ☐ No ☐
   - c. Dirty water Yes ☐ No ☐
   - d. Dirty Utensils Yes ☐ No ☐
   - e. Dirty equipments Yes ☐ No ☐
   - f. Human hands Yes ☐ No ☐
   - g. Dirty environments Yes ☐ No ☐
   - h. Dust Yes ☐ No ☐
   - i. Packing with paper/leaves Yes ☐ No ☐
   - j. Sewage in food vicinity Yes ☐ No ☐
   - k. Garbage/Refuse dump in food vicinity Yes ☐ No ☐
   - l. Presence of rodents, flies, cockroaches etc. in food vicinity Yes ☐ No ☐
   - m. Human waste in food vicinity Yes ☐ No ☐
   - n. Toilet/urinary in close proximity to food Yes ☐ No ☐
   - o. Dirty habits of food handlers Yes ☐ No ☐

Appendix III

FORM THREE

Food	Ingredients for food preparation	Cooking method	Handling after Cooking	Sources of Contamination