HAZARD ANALYSIS AND CRITICAL CONTROL POINTS OF MEAT PIE PRODUCED IN SOME RETAIL OUTLETS IN ZARIA, KADUNA STATE, NIGERIA

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**DEPARTMENT OF VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE,
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DECLARATION

I declare that the work in this project entitled "HAZARD ANALYSIS AND CRITICAL CONTROL POINTS OF MEAT PIE PRODUCED IN SOME RETAIL OUTLETS IN ZARIA, KADUNA STATE, NIGERIA" has been carried out by me in the Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, under the supervision of Prof. E.C. Okolocha and Dr. M. Bello. The information derived from literature has been duly acknowledged in the text and list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

Dolapo Tosin AINA	.....	.....
Name of student	Signature	Date

CERTIFICATION

This thesis entitled **"HAZARD ANALYSIS AND CRITICAL CONTROL POINTS OF MEAT PIE PRODUCED IN SOME RETAIL OUTLETS IN ZARIA, KADUNA STATE, NIGERIA"** by Dolapo Tosin AINA meets the regulation governing the award of the degree of MSc Veterinary Public Health and Preventive Medicine of the Ahmadu Bello University, Zaria, and is approved for contribution to knowledge and literary presentation.

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DEDICATION

To God Almighty, my adorable Parents, my very own Rev. Barnabas Arastus, siblings and well wishers.

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TABLE OF CONTENTS

Title **page**

LIST OF FIGURES

Figure **Title**

LIST OF PLATE

Plate **Title**

LIST OF TABLES

Table **Title**

LIST OF APPENDIX

LIST OF ABBREVIATION

CCP- critical control points

CDC- centre for disease control

CFU-colony forming unit

CM- centimeter

DNA- deoxyribonucleic acid

ELISA- enzyme linked immunosorbent assay

GHP- good hygiene practices

GMP- good manufacturing practices

HACCP- hazard analysis and critical control points

*Inv*A- invasion

ISO- international standard organization

MR-VP- methyl red voges proskaeur

ML- milimetres

PCR- polymerase chain reaction

RT-PCR- real time polymerase chain reaction

TAPC- total aerobic plate count

Taq – Thermus aquaticus

TDA- tryptophan deaminase

TCC-total coliform count

TSI- triple sugar iron

Abstract

Salmonella is among the most important food borne pathogens worldwide contaminating wide range of animal products including meat pie. Hazard analyses of meat pie production line and meat pie produced in retail outlets in Zaria was conducted to determine hazard associated with the production line, raw materials and meat pie produced and also the critical control points. These analyses consisted of watching all the steps involved from raw materials, mixing process to collection of swab samples. A total of 480 samples were collected from meat pie production and were tested for the presence of *Salmonella* spps and also the level of bacterial contamination were determined. Sample type included raw meat flour, egg,

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

The hazard analysis critical control point (HACCP) concept is becoming an increasingly important aspect of Good Manufacturing Practices (GMPs) in safe food production. It is a systematic means of controlling any microbiological hazard that may arise in a food processing or handling operations and aims to identify problems before they occur. The first step is to establish the hazardous organisms associated with a particular food product. It is based on a list of all those bacteria that are known to cause food borne disease in man, following an evaluation of raw materials, the production process, possibilities for contamination etc. More evaluation of the hazards will be made during the identification of critical control points (CCPs) and the setting of control criteria at each CCP. For all types of food business, management awareness and commitment is necessary for implementation of an effective HACCP system (Notermans *et al* 1994).The effectiveness will also rely upon management and employees having the appropriate HACCP knowledge and skills. During hazard identification, evaluation, and subsequent operations in designing and applying HACCP systems, consideration must be given to the impact of raw materials, ingredients, food manufacturing practices, role of manufacturing processes to control hazards and likely end-use of the product.To enable risks involved to be estimated and appropriate measures to be taken, analysis of food production has to be implemented by collection of food samples-specific microbiological monitoring data, in accordance with Hazard Analysis Critical Control Point (HACCP) principles (Zweifel *et al*,

2005). The microbiological control includes testing of the whole production chain; with samples taken from production and consumption (Hatakka, 2000).

The intent of the HACCP system is to focus control at CCPs. Redesign of the operation should be considered if a hazard which must be controlled is identified but no CCPs are found. Operations shall comply with government regulations that includes adequate HACCP, hazard control and quality assurance for receiving, inspecting, transporting, segregating, preparing, manufacturing, packaging, labeling and storing food. Quality assurance operations shall be employed to ensure that food is suitable for human consumption and that food packaging materials are safe and suitable. The efficacy of any HACCP system will nevertheless rely on management and employees having the appropriate HACCP knowledge and skills, therefore ongoing training is necessary for all levels of employees and managers, as appropriate.

PRINCIPLES OF THE HACCP SYSTEM

The Codex document on the Hazard Analysis and Critical Control points system and guidelines for its application (CAC, 1997a) list the following seven principles of the HACCP system:

PRINCIPLE 1

Conduct a hazard analysis.

PRINCIPLE 2

Determine the Critical Control Points (CCPs).

PRINCIPLE 3

Establish critical limit(s).

PRINCIPLE 4

Establish a system to monitor control of the CCP.

PRINCIPLE 5

Establish the corrective action to be taken when monitoring indicates that a particular CCP is not under control.

PRINCIPLE 6

Establish procedures for verification to confirm that the HACCP system is working effectively.

PRINCIPLE 7

Establish documentation concerning all procedures and records appropriate to these principles and their applications.

Among the many reasons for using HACCP are the following conditions:

1 HACCP is a systematic, disciplined approach to process control that is based upon science
2 HACCP requires record keeping which provides an audible document trail and a historical perspective of control.
3 HACCP avoids the use of statistically unreliable end product testing to assure food safety.
4 Responsibility and accountability are clearly assigned.
5 The HACCP concept is a logical, common sense approach that can be used to educate employees and the public in safe food handling procedures.
6 Timely adjustments are made to processes which will prevent loss of control and loss of product.

7 If control is lost it will be detected and appropriate actions can be taken to assure food safety and avoid costly products recalls.

8 Successful implementation of HACCP maintains consumer confidence in product safety with no perceptible change in product cost.

9 HACCP is the most effective means to assure food safety.

Ready-to-eat foods can be described as the status of foods being ready for immediate consumption at the point of sale. Ready-to-eat foods could be raw or cooked, hot or chilled and can be consumed without further heating (Tsang, 2002). Different terms have been used to describe such ready to eat foods. These include convenient, ready, instant and fast foods. Examples of such ready to eat foods include pastries, meat pie, sausage, rolls, burger, moimoi, salad or coleslaw, fried meat, fried chicken, milk and milk products (Caserani and Kinston, 1974). Street foods are ready-to-eat foods prepared and sold by vendors especially in streets and similar public places. The preparation and sale of street foods is an old-age activity. It is almost universal in developing countries, and in the industrial world. This activity has reached new dimensions as a result of rapid urbanization. The street foods are served quickly, are tasty and available at reasonable rates. They also attract all the age groups especially the younger generation. The safety and shelf-life of the street foods depend upon the interaction of chemicals, physical and microbial factors. From the health point of view the microbiological quality of foods become important. Street foods are frequently associated with diarrheal diseases which occur due to improper use of additives, the presence of pathogenic bacteria, environmental contaminants and disregard of Good Manufacturing Practices (GMPs) and good hygiene practices (GHPs).

According to Doyle and Evans (1999), food borne diseases are diseases resulting from ingestion of bacteria, toxins produced by microorganisms present in food. Data on issues of food borne diseases

are well documented world-wide (Hazariwala *et al.*,2002). Foodborne illnesses is a major international health problem with consequent economic reduction and death (Duff *et a*l., 2003).

Outbreaks of food borne diseases are caused by foods that are contaminated intrinsically or that become contaminated during harvesting, processing or preparation (Torok *et al*., 1997). In most countries, the most common foodborne illness is *Staphylococcus* food intoxication (Talaro *et al*., 1996). Enterotoxigenic *Staphylococcus* strains and *E.coli* strains have been isolated from foods implicated in illnesses (Adeyiwu, 1995, Firstenberg and Sullivan, 1997, Cencil *et al;* 2003). *E.coli* and *S. aureus* are normal flora in humans and animals, their presence in foods are indications of excessive human handling (Adamolekun and Adamolekun, 1992).

In Nigeria and many other developing countries, there are inadequate diagnostic facilities, leading to inadequate investigation of outbreaks and the subsequent gross under-reporting of food-borne illnesses. Bacteria are the causative agents of food borne illnesses in 60% of cases requiring hospitalization and 5000 deaths each year in United States of America (Mead *et al.,* 1999). In France it is estimated that these pathogens cause 10,200-17,800 hospitilizations yearly (Vaillant et al., 2005).The international impact of food borne illness is difficult to estimate. However, 2.1 million children in developing countries die due to diarrheal-related illnesses annually. It is suspected that food or water is the vehicle for many of these illnesses (WHO, 2002). Since food is biological in nature and is capable of supplying consumers with nutrients, it is equally capable of supporting the growth of contaminating microorganisms. Water supplies may contain pathogens when contaminated with feacal matter. In Nigeria a number of foods have been reported to have high incidence of bacteria (Adesiyun, 1995; Okonko *et al*., 2009; Bello *et al.,* 2013; Adesetan*et al*., 2013). Despite increasing insight into the contamination routes, pathogenic microorganisms found in food is not declining(Anon, 1992). The number of registered cases of foodborne

intoxications and infections rises each year. It was estimated that there are about 4.5 million cases in the Netherlands (Hoogenboom-Verdegaal *et al.,* 1989). Epidemiological research revealed that most cases resulted from meals prepared at home (80%) (Hoogenboom-Verdegaal *et al.*,1992). However, many foodborne diseases where large numbers of people were involved have been reported. Most of these cases were traceable to catering enterprises producing fresh meals. Consumption of the contaminated food components usually occurred 24hours or more after production. It is very important that measures are taken to prevent food borne diseases; not only can sickness and discomfort be averted in this way, but also economic losses will be limited (Todd, 1989). As the level of primary contamination with pathogens is generally low, mainly spoilage organisms will be present at the expiry data. Research by Roberts and Gilbert (1986) confirms this report. They examined meal components served to airline passengers in London. Total counts were sometimes high, but examination for the presence of *Salmonella, Bacillus cereus, Clostridium perfringes and Staphylococcus aureus* resulted in only a few positive cases. Many researchers have realized that adequate protection of the consumer from foodborne illnesses cannot be achieved by microbiological quality controls of final products, but rather by controlling the whole production process (Boberg and David, 1974; Bryan and Lyon, 1984; Bryan, 1990). In order to achieve this, HACCP is recommended (ICI&F, 1989).

However, meat pies have the tendency to get spoilt quickly. This is because of its fillings, which are mainly meat, potatoes and little quantity of vegetables. Due to the high nutrient content available in the fillings of the meat pie, this makes microorganisms to thrive easily in meat pie. Meat pie are preferred taken hot and in an attempt to maintain it hot, high electric bulb voltage or ban-marie are normally used. The possibility of contaminating the meat pie with spores of thermophilic bacteria such as *Clostridium perfringes* is not rare.

1.2 Statement of Research Problems

The presence of *Salmonella* in ready-to-eat food (meatpie) depicts a deplorable state of poor hygiene and sanitary practices employed in the processing and packaging of these food products (El-Gohany 1994). As a result of this, meat pie sold in kiosk, eateries, shops and on the streets are exposed to high contamination thereby resulting in food-borne illnesses (Tjos *et al.,* 1977; Epstein and Havter, 2011).

This unhygienic and unsanitary preparation, processing, storage, distribution and sales of meat pie by food vendors in addition to exposure to the bad environmental conditions pose great hazards to public health (Williams, 1984).

There have also been cases of spoilt meat pie fillings at the point of purchase. Therefore there is need to evaluate the microbial quality of meat pies consumed by the ever increasing population especially when they are purchased from local points of sales.

1.3 Justification

Salmonella is of significant public health concern, because humans and animals can become infected from consumption of food/feed and drinking water contaminated with *Salmonella* spp from faeces of infected animals (Jackson *et al.,* 2007).

This type of surveillance work provides an important baseline measurement for the microbiological quality of foods available to consumer within a locality and threats to human health that are associated with consumption of these foods.

Also, there is paucity of information on the bacteriological quality and isolation of *Salmonella* spp from meat pies sold in Zaria. This, therefore, shows the importance of this study. Hence the

study is aimed at determining the wholesomeness of meat pies sold in Zaria Metropolis with a view to ascertain their wholesomeness and make relevant recommendations for the promotion of public health.

1.4 Aim and Objectives

1.4.1 Aim of the study

The aim of this work was to assess the bacteriologic quality of meat pie sold in some retail outlets in Zaria, Nigeria

1.4.2 Objectives of the study

1. Determine the critical control points in the production of meat pie sold in retail outlets in Zaria, Nigeria.
2. Determine the level of bacteriological contamination of meat pie sold in retail outlets in Zaria.
3. Isolate and identify *Salmonella* spp at different stages of meat pie production, using phenotypic and serologic characters
4. Determine the virulence by detecting *inv*A gene using PCR.

1.5 Research Questions

1. What are the critical control points in the production of meat pies sold in retail outlets in Zaria, Nigeria.
2. What is the level of bacteriologic contamination of meat pie sold in retail outlets in Zaria.
3. Does meat pie sold in Zaria contain *Salmonella* spp as microbial contamination?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Ready-to-eat Foods

Ready-to-eat foods are prepared and/or sold by vendors and hawkers in the streets, around and in market places, shops, eateries and other public places (FAO, 1988a). Ready-to-eat food, being an ancient and global phenomenon has assumed a vital proportion and role in the food supply chain of rapidly growing urban and peri-urban communities of developing countries (Clarence *et al.*,2009; Falola *et al.,* 2011; Oranusi and Braide, 2012).

Ready-to-eat foods are available sources of nourishment at reasonable costs near car parks, door steps, factories, markets, offices, roadsides and even schools (Abdulsalam and Kaferstein, 1992). However these foods are reported to be sources of outbreaks of food poisoning and food borne diseases in unsuspecting communities or countries (Stoots *et al.*,1992; Raji, 2001).

2.2 Meat Pie

Meat pie is a savory pie with a filling of meat and other savory ingredients. Meat pie is a popular snack because it is tasty, moist and does not produce crumbs when it is eaten, if it is well prepared. They are generally sold at selected retailers, kiosks and online shops (Craig, 1982; Penelope, 1982; Todd, 2007) in different countries. Prepackaged as frozen foods, serving sizes are available in most selling points based on request.

The Nigerian meat pie is similar to the Jamaican beef patty. It can be fried or baked and the fillings can be almost anything from corned beef (the most widely used) to just onions and tomatoes

2.2.1 History of meat pie

The beginning of meat pie can be traced back to the Neolithic period, around 9500 BC, in the ancient Egypt, Greeks and the Roman Empires. Meat pie were brought to medieval Europe by the Roman crusaders. This pastry became a staple dish in medieval times, and was eventually called “pyes” or “pies”. The origin of this name comes from the type of meat commonly used as filling. Lamb, beef and duck were employed, but a majority of the time it was magpie that was the main ingredient (Craig, 1982). Magpies in medieval England were originally named pies. Some historians think that the popular usage of ‘pie’ birds led to the pastry dish named pie as well (Penelope,1982).

The first use of the word ‘pie’ as food is referenced in 1303 by the Oxford English Dictionary also stating that the term became popular and widely used by 1362 (Craig, 1982). The French and Italians specialized in redefining the pastry of the pie, making it flakier and tastier by new methods of adding butter, rolling, and folding the dough. In the Paris pastry guild was recognized and started to expand their product and so the modern day crust began to be used. Missionaries and explorers spread the meat-based pie dish across the globe. The English Pilgrims of the North American colonies brought the recipes across the ocean with them today. There are several types and variations of meat pie enjoyed all across the globe.

2.2.2 Bacteriological standards in meat pie preparation

The tremendous growth and acceptance of meat pie in food industry in the past decade has resulted in the adoption of bacteriological standards for their preparation and distribution. There have been several reports in the literature concerning the numbers and species of bacteria which were encountered in meat pies by Larken *et al.,* (1955), Canale and Ordal (1957), Litsky *et al.,* Huber *et al.,* (1958), Ross and Thatcher (1958), Kereluk and Gunderson (1959) and Kereluk and Gunderson(1960).

Studies by Canale and Ordal (1957) on poultry pies have indicated that some of the times and temperatures recommended by the manufactures are not sufficient to eliminate the non-spore forming organism. It was also found that the centre of the pies baked under what the authors assumed to be home conditions, did not reach a temperature high enough to reduce the bacterial count satisfactorily (Kereluk and Gunderson, 1960).

2.2.3 Role of bacteriological standard in food safety

Bacteriological standards have been associated with the question of safety to the consumer. Everyone recognizes that food poisoning bacteria are a potential danger in any food. But many have argued that there is no need for standards; on the other hand, proponents of standards have pointed to the incomplete investigation and reporting of outbreaks, and have argued that there may be more outbreaks than we realize (Kereluk and Gunderson, 1960).

They have pointed to laboratory studies that have shown grossly mishandled pre-cooked frozen foods to be truly dangerous. Some have proposed that pathogens should be absent from foods; but others have questioned that a microbiological standard can accomplish this end. Some pathogens such as *E.coli*, *Salmonella* or *Staphylococcus* have been shown to be so

ubiquitous that their presence in some commercial food is unavoidable (Elliot and Michener, 1961).

A most important part of any of the proposed standard is a 'total count' of viable aerobic count. The relation between bacterial level and quality is open to less controversy. Bacterial levels is said to be a measure of sanitation, adequacy of refrigeration, or speed of handling.

2.2.4 Advantages of bacteriological standards

i. Bacteriological standards are convenient and necessary; bacteriological standards have the advantage of putting questions of safety and quality in foods on a numerical basis. Bacteriological standard is a convenient measure for decision as to the acceptability of a food (Elliot and Michener, 1961). According to Thatcher (1955), in the absence of definite standards, food control agencies have often been greatly handicapped in efforts to improve quality.

ii. Bacteriological standards enhance sanitation: setting a bacteriological standard results in a cleaner food product by bringing bacteriological aspects of production to the fore in the minds of food producers.

iii. Low bacterial counts are associated with safe foods: low bacterial counts tend to parallel safety from food poisoning pathogens. In general, those foods suspected of causing food poisoning have counts greater than 1 million per gram. In cases where counts are very high in a food, the process of production would permit the entry into the food of both spoilage types and food poisoning types. Foods with large numbers of spoilage organisms are not wholesome, whether or not pathogenic organisms are present or not.

2.3 Hazard Analysis And Critical Control Points

This is a systematic means of controlling any microbiological hazard that may arise in a food processing or handling operation and aims to identify problems before they occur. The first step is to establish the hazardous organisms associated with a particular food product. It is based on a list of all those bacteria that are known to cause food borne disease in man, following an evaluation of raw materials, the production process and possibilities for contamination. More evaluation of the hazards is usually made during the identification of CCPs and the setting of control criteria at each CCP. The HACCP system is internationally accepted as a system of choice for food safety management. It is a preventative approach to food safety based on the following seven principles:

1. Identify any hazards that must be prevented, eliminated or reduced.
2. Identify the critical control points at the steps at which control is essential.
3. Establish critical limits at critical control points.
4. Establish procedures to monitor the critical control points.
5. Establish corrective actions to be taken if a critical control point is not under control.
6. Establish procedures to verify whether the above procedures are working effectively.
7. Establish documents and records to demonstrate the effective application of the above measures.

   The HACCP approach provides a systematic way of identifying food safety hazards and making sure that they are being controlled day-in, day-out. This involves four steps; plan, do check and act.

   Plan- plan what needs to be done to maintain food safety and write it down. It is particularly important to;

1. Minimize the likelihood of food poisoning bacteria containing meat and associated products.
2. Avoid physical and chemical contamination of meat.
3. Reduce the potential for growth of food poisoning bacteria on meat and meat products.
4. Minimize the potential for cross contamination of ready-to-eat foods by food poisoning bacteria on meat during further processing or in the kitchen.

HACCP essentially controls the process of food production. However, there are many environmental issues which affect food safety. These often apply across the board, regardless of what food is being produced or how it is processed. These issues are referred to as "prerequisites". This means that, in order to ensure food safety, these matters must all be addressed alongside the processing hazards.

2.3.1 Hazards

A food safety hazard is anything present in food with the potential to harm the consumer, either by causing illness or injury. There are three major hazards introduced into the food supply anytime during harvesting, processing, transporting, preparation, storage and serving food. These hazards may be microbiological, chemical or physical. Good food safety practice must be science-based and a thorough understanding of hazards is the first essential step in their control.

2.3.2 Microbiological hazards

These occur when food becomes contaminated by microorganisms found in the air, food, soil, animals and the human body. Microorganisms are helpful and necessary for life itself e.g *Penicillum notatum*. However, given the right conditions some microorganisms may cause a food borne illness. Microbiological hazards pose the greatest immediate food safety threat to the consumer. For example, the capacity of food poisoning bacteria to cause large outbreaks of acute

illness is an ever present threat in the food supply chain. It is microorganisms and food borne parasites that are of most concern as biological food safety hazards.

Most food poisoning occur due to the continuous growth to dangerous levels of microorganisms particularly bacteria in food. Food handlers should know about food poisoning bacteria and the conditions they require for growth to ensure food borne illness is avoided. A number of bacterial species are food safety hazards. Some, such as *Salmonella* and *Listeria monocytogenes*, are familiar, whereas others are much less well understood. *Campylobacter* is an example of a less well known cause of food borne illness. Few people have heard of this organism, yet it is now the cause of bacterial food poisoning in the developed world than any other agent, including *Salmonella*.

The seven principles of HACCP aim to focus attention on the identification and control of microbiological, as well as chemical and physical food safety hazards during production. The hazard assessment and the regular monitoring of critical control measures must be documented to provide the basis for audit checks and possible evidence of due diligence in the event of legal action. In meat plants HACCP plans will focus on control measures that can reduce the likelihood of contamination of meat from microbiological hazards such as *Salmonella, E.coli* 0157 and *Campylobacter* during production. These meat-borne pathogens can be carried by healthy animals and cannot be detected by sight or smell. Although thorough cooking kills most bacteria.

2.3.3 Food borne illnesses

The preparation, processing, handling of ready-to-eat foods may portend a great danger to consumers in particular and the public in general. Various reports (Adesiyun *et al.,* 1994) have highlighted the potential for serious health consequences associated with vended foods. Dawson

1992 reported cases of disease outbreaks, morbidity and even cases of mortalities attributed to the consumption of ready-to-eat foods.

Food security is a complex issue, where animal proteins such as meats, meat products, fish and fishery products are generally regarded as high risk commodity in respect of pathogen contents, natural toxins and other possible contaminants and adulterants (Yousuf *et al.,* 2008). Food borne infections and illnesses is a major international health problem with consequent economic reduction.

It is a major cause of illness and death worldwide (Adak *et al.,* 2005). Recognizing this, the World Health Organization (WHO) developed its Global Strategy for Food Safety (Adak *et al.,* 2005). In the developing world, food-borne infection leads to the death of many children and the resulting diarrheal disease can have long-term effects on children's growth as well as on their physical and cognitive development (Adak *et al.,* 2005). In the industrialized world, food-borne infection causes illnesses, heavily affecting healthcare systems (Adak *et al.,* 2005). According to Clarence *et al.*(2009), food borne diseases are diseases resulting from ingestion of bacteria, toxins and cells produced by microorganisms present in food. The intensity of the signs and symptoms may vary with the amount of contaminated food ingested and susceptibility of the individuals to the toxin (Clarence *et al.,* 2009).

Meat is the most perishable of all important foods since it contains sufficient nutrient needed, to support the growth of microorganisms (Magnus, 1981). The chief constituents of meat are water, protein and fat, phosphorus, iron and vitamins are also contained in meat. The major primary unit of meat is called carcass. The edible parts of a carcass include lean flesh, fat flesh and edible

glands or organs such as heart, liver, kidney tongue and brain. Meat is considered as the most nutritive source of protein consumed by humans.

Age and sex of the animal has a major influence on the quality of meat that is produced from animals (Rao *et al.,* 2009). Most meat have high water content corresponding to the water activity approximately 0.99 which is suitable for microbial growth (Rao *et al.,* 2009).

Meat is considered to be spoiled when it is unfit for human consumption. Meat is subjected to changes by its own enzyme, by microbial action and its fat may be oxidized chemically. Microorganisms grow on meat causing visual, textural and organoleptic change when they release metabolites (Jackson *et al.,* 2001). In fact, tissue from healthy animals are sterile however, it has been pointed that during slaughter, dressing and cutting, microorganisms came chiefly from the exterior of the animal and its intestinal tract but that more added from knives, cloths, air, carts and equipment in general. External contamination of meat is a constant possibility from the moment of bleeding until consumption (Lawrie, 1984). Among the factors that affect microbial growth in meat are intrinsic properties (physical and chemical properties of meat) and extrinsic (environmental factors) (Rombout and Wout, 1994), however the factors having the greatest influence on the growth of microorganisms in meat and meat products are the storage temperatures, moisture and oxygen availability (Forest *et al.,* 1985).

Food-borne microbiologic hazards may be responsible for as many cases of illness as possible each year and are thus an important food safety challenge. To lower the incidence of food borne diseases, many experts and stakeholders urge the development of a science- and risk-based food safety system, in which decision makers prioritize hazards and interventions using the best available data on the distribution and reduction of risks (Batz *et al.,* 2005). Such a system

requires an understanding of the many risk factors between the point of production and the point of consumption and the ability to systematically target intervention efforts along this "farm-to-fork" continuum (Batz *et al.,* 2005). The preservation of meat as a perishable food usually is accomplished by a combination of preservation methods which greatly lengthen the keeping quality of the meat. So, to increase meat quality assurance in accordance with microbial load assessment is deemed necessary (Yousuf *et al.,* 2008).

It has been reported that gram negative bacteria account for approximately 69% of the cases of bacterial food borne disease (Clarence *et al.,* 2009). Turtura (1991) reported that the most frequently coliform identified on meat were *C. freundii, E coli, En. agglomeram* and less frequently strains are of the genera *Klebsiella, Shigella sonnie, Salmonella, Proteus. E. coli* and *S. aureus* are normal flora in human and animals, their presence in foods are indications of excessive human handling. Members of the gram negative bacteria e.g. *E. coli* are widely distributed in the environment. Contaminated food and water are the major sources by which the bacteria are spread (Clarence *et al.,* 2009). Selected strains can cause a wide variety of infections in hospitals and community setting (Donnenberg, 2005). *Escherichia coli* is commonly used as surrogate indicator, its presence in food generally indicate direct and indirect fecal contamination (Clarence *et al.*, 2009).

2.3.4 Causes of food-borne illnesses

Microbial contamination of food is indicative of the presence of both pathogenic and non-pathogenic organisms which may be due to inadequate sanitary practices during preparation and storage of such food (Brandly *et al.,* 1966; Anderson, 1990). Pathogenic organisms are introduced into food by raw materials, unclean cooking utensils, or by persons involved in the

handling of food during processing, distribution and sales (Raji, 2001). In addition, lack of adequate supply of pipe borne water and personal hygiene, improper wastes and garbage disposal have all been incriminated in microbial contaminated of food (Dawson, 1992; Umoh and Odoba, 1998).

2.3.5 Management of food-borne illnesses

Although most foods-borne diseases or infections are self-limiting, the general approach to their management includes: restoration of acid base balance, fluid electrolyte replacement in severe cases, the use of antimicrobial drugs and the use of antidiarrheal and intestinal protectants (Majkowski, 1997).

2.3.5.1 *Restoration of acid-base balance and fluid electrolyte replacement*

Oral and parenteral fluid electrolyte therapy can be administered to correct cases of dehydration and electrolyte imbalance. Several oral electrolyte preparations are available such as mixtures of sodium chloride, glucose and sodium bicarbonate. Intravenous fluid therapy are indicated in severely dehydrated and weak individuals (Jawetz *et al.*, 1989; Wakawa. 1999).

2.3.5.2 *The use of antimicrobial agents*

Broad spectrum antimicrobial and antibiotic drugs are commonly used in the treatment of diarrhea. These drugs includes aminoglycosides, gentamycin, neomycin, tetracycline, tylosine, spiramycin and sulphonamides. These drugs are indicated and have therapeutic effects against enteric bacteria (Aliu , 2007).

2.3.5.3 *The use of antidiarrhoeal agents*

Different formulations of antidiarrheal drugs are readily available an used extensively in the control of diarrhea. These drugs are intended to absorb and eliminate toxins and prevent them from coming in contact with the intestinal mucosa of infected individuals (Jawatz el al., 1989; Wakawa, 1990; Aliu, 2007). Absorbents such as kaolin, a natural aluminium silicate has been used. Likewise, opiate derivatives such as loperamide, codein phosphate and diphenocyclates have been used in the management of diarrhoea.

2.3.6 Sources of food contamination

The sources of food contamination can be classified into two namely; primary and secondary sources of food contamination.

2.3.7 Primary sources of food contamination

The primary sources of food-borne illnesses come directly from infected raw materials, foods or spoilt materials during its transportation and storage.

2.3.8 Secondary sources of food contamination

Secondary sources of food-borne diseases comes from infected humans, such as persons involved in the handling of food during its preparation, distribution and sale, or those who fail to effectively wash their hands after defecation (Raji, 2001). It could also be from unsanitary environment whereby waste materials and garbages on the streets, encouraging food susceptibility to contamination by flies and dust laden microorganisms. Formites e.g water, road, air and plant may serve as vehicles for food-borne disease agents (Hubbert, 1966; FAO, 1988a; Raji, 2001).

2.3.9 Common microbial contaminants of foods

A contaminant as defined by Codex Alimentarius (1993) is any substance not intentionally added to food, which is present in such food as a result of the production (including operations carried out in crop husbandry, animal husbandry and veterinary medicine), manufacture, processing, preparation, treatment, packing, packaging, transportation and distribution. Microorganisms such as *Staphylococcus aureus, Bacillus cereus, Salmonella spp, Clostridium spp* and *Escherichia coli* have all been linked with food-borne diseases (Lucas and Gilles 1990).

2.3.9.1 *Prevention of food contamination*

Prevention of microbial contamination is an integral aspect of safe and wholesome food consumption. The need for a safe and wholesome food/meat pie cannot be overemphasized. It should be the responsibility of both producers and consumers alike. The need to prevent microbial food contamination is a global phenomenon, as it cuts across boundaries, culture, ethnicity, divides and occupations, race and religious inclinations.

Furthermore, since food is an essential carrier of disease organisms, healthy, safe and wholesome considerations will always form basic part in food-borne illness prevention. The following considerations below are essential in achieving food contamination prevention;

i. Ensure general good sanitary protocols in the preparation, processing, handling and serving of food.
ii. Discourage infected individuals or carriers from handling food, because they may serve as vehicles through which food may be contaminated.
iii. Ensure all cooking utensils, equipments, tables, cutting boards, vessels and containers are properly cleaned or disinfected immediately when they are suspected to be contaminated.

iv. Adequate preservation and storage of perishable foods using refrigerator, equipments should be done regularly.

v. Pathogen-bearing vermins should be prevented always from gaining entrance to the environment where foods are prepared.

2.4 *Salmonella species*

Salmonella, a member of the family *Enterobacteriaceae* are Gram negative short rods, non-spore forming, motile, facultative and non-encapsulated. The new classification of *Salmonella* indicated that the genus *Salmonella* consists of only two species, *Salmonella bongori* and *Salmonella enterica*, with the latter being divided into six subspecies (I–VI); *S. enteric* subsp. *Enteric* (I), *S. enteric* subsp. *Salamae* (II), *S. enteric* subsp. *Arizonae*(IIIa), *S. enteric* subsp. *Diarizonae* (IIIb), *S. enteric* subsp. *Houtenae* (IV), and *S.enterica* subsp. *indica*(VI) (Popoff *et al.*, 2003).

They grow at optimum temperature on MacConkey (appearing as colourless colonies) and *Salmonella-Shigella* agar after enrichment in tetrathionate broth. *Salmonella* are principally of intestinal origin (Tabukum, 1990). The major source of *Salmonella* infection is carcass contamination during slaughter through fecal materials or contaminated soils in direct contact with the carcass (Tabukum,1990). Other sources of food contamination by *Salmonella* includes infected persons handling food with unwashed hands after defaecation, faecal contaminated water used during food preparation (Bryan *et al.*, 1992; Wei *et al.*, 1995). In Nigeria several reports exist on *Salmonella* contamination of foods (Nkanga and Uriah 1981; Adesiyun 1984a; 1984b; Adesiyun *et al.*, 1987*). Salmonella* in food produce enterotoxins (Adesiyun *et al.*, 1987).*Salmonella typhi* is a bacterium that causes typhoid fever (enteric fever), an acute, life-

threatening febrile illness (CDC, 2008). The disease is a cause for concern and a major public health problem in developing countries (Asia, Africa); especially in Nigeria due to poor sanitary conditions and lack of or inadequate potable water (Ibekwe *et al.*, 2008). It is mainly transmitted through food or drink or water, contaminated with urine or faeces of infected people or a chronic carrier (CDC, 2008; Ibekwe *et al.*, 2008). Since 1987, *Salmonella enteritidis* has been one of the most frequently isolated *Salmonella e*associated with foodborne outbreaks, which have been linked with consumption of chickens, eggs, and foods that contain eggs and it presents an interesting challenge from an epidemiologic perspective (Zheng *et al.*, 2007).

Infections with non-typhoidal *Salmonella* have increased during the last 3–4 decades, and although a decrease has been reported over the last decade, *Salmonella* infections continues to be a major public health concern in many countries. These *Salmonellae* are zoonotic, and the infections are generally food-borne (Helms *et al.*, 2005). The main reservoir of zoonotic *Salmonella* is food animals, and the main sources of infections in industrialized countries are animal-derived products, notably fresh meat products and eggs. Rapid spread of a limited number of successful *Salmonella* clones in different sectors of food animal production (swine, broiler chickens, and particularly layer hens) has been suggested as the most important cause of this increase.

Bacterial gastrointestinal infections continue to cause illness and death and contribute to economic loss in most parts of the world, including high-income countries that have developed surveillance and control programs (Ternhag *et al.*, 2008).

The possible sources of these bacteria are likely to come from the skin of the animal from which the meat was obtained. Other potential sources of microbial contaminations are the equipment

used for each operation that is performed until the final product is eaten, the clothing and hands of personnel and the physical facilities themselves (Rombouts and Nouts, 1994).

Retail cut could also result in greater microbial load because of the large amount of exposed surface area, more readily available water, nutrient and greater oxygen penetration available (Forest, *et al.,* 1985) hence retail cuts displayed are conducive for microbial growth and proliferation which leads to spoilage of the meat (Ayres, 1995).However in Nigeria, a number of foods (meat inclusive) have been reported to have high incidence of bacteria (Nkanga and Uraih, 1981; Okonko*et al.,* 2008a, b, c, 2009a, b; Clarence *et al.,* 2009). There is limited information on the health challenges from food borne diseases from fresh meat retailed within a highly populous community.

2.4.1 Diseases caused by *Salmonella*

A report by Indiana State Department of Health (2009) mentioned that salmonellosis is a disease caused by *Salmonella*, the most common which are *Salmonella typhimurium* and *Salmonella enteritidis*. Symptoms include diarrhea, fever, and abdominal cramps 12 to 72 hours after infection. Canadian food inspection agency indicated that most people recover without treatment. However, in some people, symptoms can be severe and can cause dehydration, which may lead to hospitalization. In the case of severe illness, complications such as abscesses and pneumonia can occur. Complications can sometimes cause death unless treated with the appropriate antibiotics. Some people may develop Reiter's syndrome or even colitis. Reiter's syndrome is a condition that develops in response to an infection in another part of the body. It can last for months or years and may lead to chronic arthritis (Indiana State Department of Health, 2009).

2.4.2 Economic cost due to Salmonellosis

Salmonella infection is the most frequent food-borne gastrointestinal disease transmitted from animals to humans. Typhoid fever still remains endemic in many developing countries. Non-typhoidal salmonellosis also is a major food-borne disease worldwide and is estimated to be responsible for the death of more than 500people each year, with costs of $1.1–$1.5 billion annually (Ul-Hassan *et al.,* 2004).

2.4.3 Salmonellosis

Is usually considered as an asymptomatic or self-limiting illness, but it can also become invasive and fatal, especially for patients who are young or immune compromised (Wilson *et al.,* 2003).

The incidence of non-typhoidal *Salmonella* is usually highest in infants and young children. The incidence rate of some serotypes increases slightly in young adults in their twenties. There is also a slight increase among elderly person as shown in figure1 Molbak *et al.*,2006).

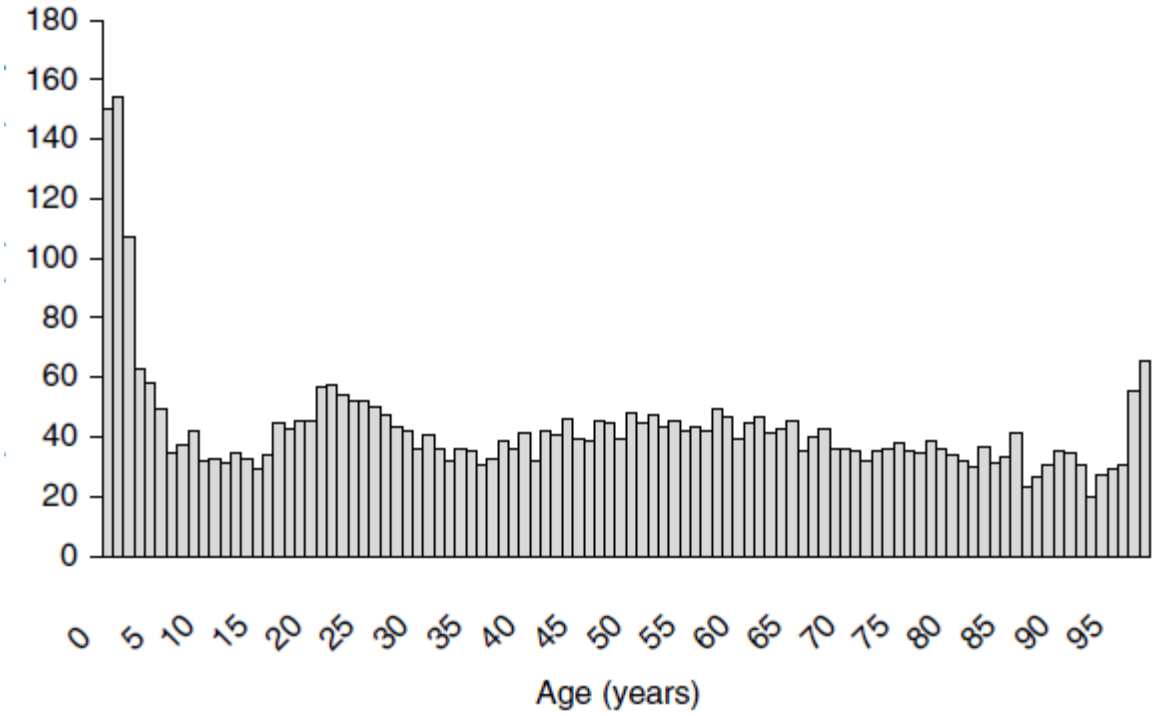

Figure 2.1: The age specific incidence of human *Salmonella* infections in Denmark, 1999–2003 (Molbak *et al.*, 2006).

In the first peak, among children below 2 years of age, the incidence was highest in boys (relative rate 1.15; 95 % CI 1.01–1.30). Around the second peak, among individuals between 20 and 25 years of age, the incidence was higher in females than in males (relative rate 1.17; 95 % CI 1.02–1.33) (n = 11 900 episodes) (Molbak *et al.*, 2006).

Non-typhoidal *Salmonella* strains are important causes of infections in both humans and animals. This disease is caused by *Salmonella* serotypes other than *S. typhi* and *S. paratyphi*. It is a major food-borne infection with worldwide distribution. The majority of cases are self-limiting gastroenteritis (Kariuki *et al.*, 2002).

2.5 Pathology of *Salmonella* Infection

All *Salmonella* infections begin with the ingestion of organisms in contaminated food or water. Conditions that increase gastric pH reduce the *Salmonella* infectious dose, which suggests that

gastric acidity represents a significant initial barrier to infection. *Salmonella* exhibits an adaptive acid-tolerance response on exposure to low pH, possibly promoting survival in acidic host environments (Ohl and Miller, 2001).

Certain species of *Salmonella* are virulent enough to be able to penetrate the intestinal walls, and can then enter the lymphatic system and cause widespread infections. In order to do this, the bacteria have to pass through the lacteals of the small intestine i.e. the capillaries through which fatty acids are transported into the lymphatic system and be taken to the lymph nodes from which they can gain entrance into the blood stream. In this way, septicemia can occur (Tam, 2008).

Salmonella are thought first to colonize the intestine and penetrate the intestinal barrier. Invasion of the intestinal mucosa results in an extrusion of the infected epithelial cells into the intestinal lumen and destruction of microvilli, which leads to loss of absorptive surface. The invasion of epithelial cells elicits the production of the proinflammatory cytokines, which stimulate the influx of polymorphonuclear leucocytes into the infected mucosa (Zhao *et al.,* 2002).

2.6 Detection Techniques of *Salmonella*

2.6.1 Bacterial identification by classic methods

Traditionally, bacterial species have been identified by classic tools depending on culturing processes coupled with morphological, physiological, and biochemical characterization. Phenotypic identification is based on direct comparison of phenotypic characteristics of unknown bacteria with those of type cultures. The reliability of this kind of identification is in direct proportion to the number of similar phenotypic characteristics. When classifying microorganisms, all known characteristics are taken into account. However, certain characteristics are selected and used for the purpose of identification. Primary identification

usually involves a few simple assays such as colony morphology, Gram staining, growth conditions, catalase and oxidase tests **(**Duerden *et al.,* 1998).

Cultural methods for *Salmonella* detection involve a nonselective pre-enrichment followed by selective enrichment and plating on selective and diagnostic agars. Suspect colonies are confirmed biochemically and serologically. The complete test requires three to four days to obtain a negative result and up to seven days to get a confirmed positive result. Number of rapid methods for the detection of *Salmonella* in foods has been developed, including electrical techniques, immunoassays and nucleic acid probe analyses. However, there are still problems with their sensitivity and specificity (Jenikova *et al.,* 2000).

In the last decades, the progress of microbiological identification turned to more rapid and sensitive methods, including antibody-based assays and DNA-based methods, together with important advances in bioinformatics tools. Thus, some methodologies such as ELISA or PCR already became classic. PCR coupled to sequencing tools has provided a big amount of information that has been deposited in public databases and is freely available. Recently, the development of rapid and high sensitive techniques, such as real-time PCR(RT-PCR), DNA microarrays and biosensors, provoked the replacement of traditional culturing methods in the field of bacterial identification in clinical diagnostics, as well as in the food sector (Feng, 2007; Mohania *et al.,* 2008).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was carried out in Zaria, Kaduna state, which is located in the Northern part of Nigeria, within latitudes 11° 07, 11° 12′N and longitudes 07° 41′E (figure3), with an altitude of 500-700 meters above sea level and a total area of 300km^2. The town is characterized by a tropical climate, a monthly mean temperature ranging from 13.8°C to 36.7°C and annual rainfall of 1092.8mm. It has an estimated population of 408,198 and a density of 1,360.7 km^2 (NPC, 2006). The main occupation of the people in Zaria is primarily Agriculture. In Zaria, cattle population was reported to be 1,144,000 and about 99% of these cattle were being managed under semi-intensive, extensive or pastoral system (Aliyu *et al.*, 2014). Zaria also is home to major tertiary institutions with a large student population. Commercial activity is low, however, there is the presence of large commercial food retailers including Mr Biggs, Chicken Republic and other small scale vendors.

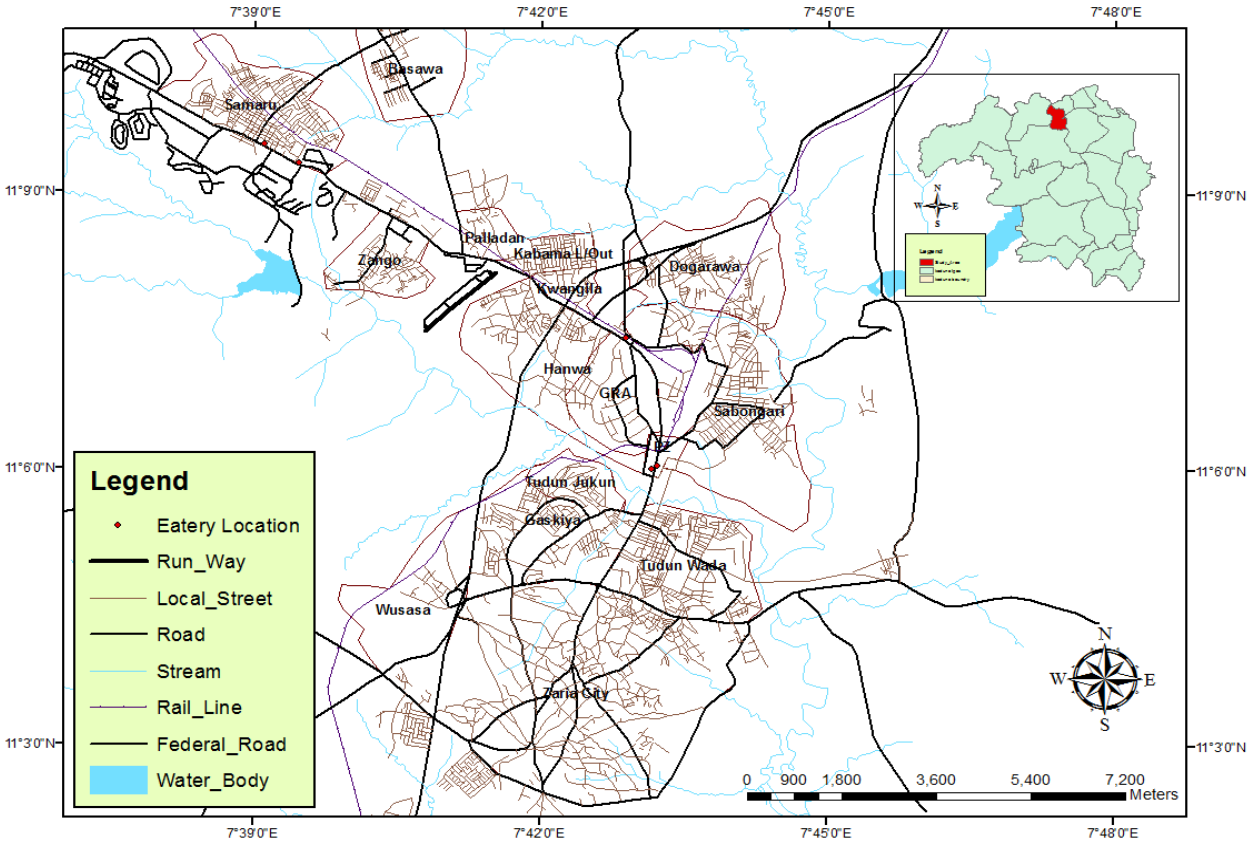

Figure 3.1: Map of Zaria showing the main areas (Source: Zaria Master plan, 2000)

3.2 Study Design

A cross sectional study was conducted. Meat pie samples and swabs were collected fromfour retail points in Zaria, viz; Samaru, Kwangila, Palladan, PZ area.

3.3 Sample Collection and Transportation

Samples 200g of meat pie samples were collected aseptically with sterile metal spoon. Samples of ingredients used in the preparation were also collected. Samples were put into sterile polythene bags, which were usually fastened at the top with bag fasteners. Samples of water (20ml) were aseptically poured into sterile sample bottles during sampling. Swab samples from tables, cutting boards, knives and various utensils used including hands of persons involved at different stages of

preparation were taken by rubbing the surfaces with sterile swabs previously soaked in physiological saline. A total of 480 samples were collected consisting of 30 samples each of flour, egg, water, green beans, carrot, meat, potato, spices, dough, fillings, palm swab, knife swab, container swab, pan swab, rolling pin swab and meat pie.

All samples and specimens were appropriately labeled and transported to the Bacteriology Laboratory, Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria on the day of collection. Observations were made for the likely sources of contamination and opportunities for cross contamination. Data were evaluated using charts and tables. Potential sources of contamination (raw meat, eggs, potatoes, spices, flour, equipment and utensils and persons preparing the products) are noted on the diagram. Critical control points of the operations that require monitoring are indicated in Figure 4:1. The persons who were involved in the operations did so in their usual manner and were not given any special instructions. Operations were observed to identify sources and routes of contamination by the persons preparing the products, utensils and equipment surfaces or by any other circumstances, which could have led to contamination.

3.4 Description of the Meat Pie Production Process

The production units selected for this study are located in Zaria and they were selected based on convenience. These units are patronized by street vendors, retailers and even students. The production units starts their operations as early as 06:00 (6am) and end at about 19:00 (7 pm) daily, except on Sundays. The pastries prepared for the day would all have been sold and the assemblage and preparation of raw materials for the following day's operation would start thereafter until the unit closes for the day. For convenience of operation the production section

was divided into four : the baking unit, a kneading unit (the mixing unit, washing unit and cooking unit), the store and the customer unit.

The kneading unit which comprises the mixing, washing and cooking units is where most of the activities are carried out. In the mixing unit flour is mixed with egg, butter, water and baking powder which give rise to the dough. At the washing unit all ingredients and utensils are washed before use and then at the cooking unit meat is boiled along side other ingredients like carrot, green beans, potatoes and other spices which give rise the sauce or filling that is then filled into the dough at the kneading unit and finally baked at the baking unit and finally taken to the customer unit for sales.

3.5 Hazard Analyses

Hazard analyses consisted of:

a) Observing meat pie preparation, handling and storage practices to identify sources and modes of contamination.
b) Collecting samples of meat pie before and after sequential stages of preparation and testing them microbiologically.

3.6 Materials and Procedures in Meat Pie Production

1. Fresh meat (CCP)

Fresh meat is a sensitive raw material and requires high level of control, fresh meat should not be stored for longer than 24h to avoid pathogenic bacteria growth. It should be tested for:

a)antibiotics b)pesticides and c)microbiological agents

2. Mixing-mixing the ingredients (flour, eggs, salt, baking powder) in an electric mixer or manually until getting compact dough.

3. Coverage of dough on a work desk- After mixing in a container, the compact dough was transferred on work desk for additional processing..

4. Frying meat with other ingredients- Cooking (frying) of meat is the most important step to the manufacturing process regarding safety temperature and time control is very important.

5. Filling with meat

6. Transferring to baking dish- Physical hazard the presence of foreign materials from personel, chemical hazard, possible cross-contamination from the cleaning chemicals. Wash baking dishes thoroughly. Good manufacturing practices must be applied, mainly cleaning and sterilization.

7. Baking at 200^{0}C for more than 20mins (CCP)- Baking of meat pie is the most important step of the manufacturing process regarding microbiological safety.

8. Serving- Good manufacturing practices must be applied, mainly cleaning and sterilization.

9. Packaging- Physical hazard, the presence of foreign materials from personnel.

3.7 Laboratory Procedures

Ten grams of each meat pie samples was aseptically weighed out using electrical weighing balance, sterile forceps and scissors. Samples for analyses were homogenized according to International Standards Organization (ISO,1980). For most of the meat pie, water samples and swabs, 10g and 10ml respectively were homogenized in 90ml of 0.1% peptone water. The suspensions were then poured into stomacher bags, fastened and blended using the stomacher

machine (Stomacher Laboratory Blender 400), and by means of vertical and horizontal agitation for a few seconds. In addition to *Salmonella* isolation, total aerobic plate counts and coliform counts were made by surface plating method on nutrient agar and MacConkey agar. Appropriate serial dilutions of all samples were carried out and 0.1ml portions of the serially diluted homogenates were spread on nutrient agar and MacConkey plates respectively with the aid of sterile glass spreaders (hockey sticks) and the incubated at37^0C for 24h. These techniques were used for the enumeration of total aerobic and coliform viable counts.Media used were prepared according to the Manufacturer's instructions. *Salmonella* was confirmed by biochemical tests namely: Triple Sugar Iron (TSI), Simmons citrate, indole, motility, hydrogen sulphide, urease and methyl-red voges-proskauer (MR-VP) tests.

3.7.1 Total aerobic counts

Nutrient agar was used to achieve this and 10-fold serial dilutions was carried out. The serial dilutions were done using a sterile micropipette to aspirate 0.1mls of the homogenized samples and then dispensed into 9.9mls (double fold dilution) physiological saline. Serial dilutions were made up to 10^{-4}.

The nutrient agar plates were then inoculated with 0.1ml of 10^{-4} serial dilution. The inoculum was spread uniformly using sterile glass rod, the inoculated plates were then incubated at 37^0C for 24h, after which the colonies were then counted and expressed as colony forming unit per gram (CFU/g).

3.7.2 Coliform counts

MacConkey agar was used to obtain coliform plate counts. Ten fold serial dilutions were carried out and then used to inoculate the prepared MacConkey plates. A sterile glass rod was used to

spread the 0.1ml inocula. The inoculum was incubated for 24h at 37^0C and then counted. The counts for each plates were expressed as colony forming unit of the suspension (CFU/g)

3.7.3 Cultural isolation of *Salmonella* species

Each sample was analyzed in line with recommended techniques by the International Organization for Standardization of *Salmonella* species (Shabnam *et al.,* 2010) the stages of analysis are highlighted as follows;

3.7.4 Selective enrichment

One ml of the inoculated dilutions was transferred using a 10ml sterile syringe into 9mls of Rappaport-Vassiliadis Soy (RVS) broth and then incubated at 37^0C for 24h.

3.7.5 Selective isolation

A loop full of each enriched broth was streaked unto Bismuth Sulfite agar plate using sterile wire loop and then incubated at 37^0C for 24h. Colonies identifiable as discrete on the Bismuth Sulfite agar were carefully examined macroscopically for cultural characteristics such as shape colour, size and consistency. Bacterial isolates were characterized based on microscopic appearance, colonial morphology and gram staining reactions, which was then subjected to biochemical tests.

3.8 Biochemical Characterization

Presumptive *Salmonella* colonies sub-cultured on nutrient agar slants were confirmed biochemically in line with guidelines of the ISO 6579 (2002). These tests include glucose (TSI), motility test (SIM), oxidase, indole, methyl red-voges proskauer (MRVP), simon citrate and urea production (Shabnam *et al.,* 2010).

Presumptive colonies that were blackish in colour were transferred into tubes of Triple Sugar Iron(TSI) agar, simmon's citrate, urea, methyl Red, voges-proskauer and incubated at 37^0C for 18-24hrs. Further confirmation of the presumptive *Salmonella* isolates were carried out using a commercial bacterial identification kit (Microbact GNB 24E).

3.9 Biochemical Testing

3.9.1Triple sugar iron agar test

In this test, the triple sugar iron agar prepared according to the Manufacturer's instruction was inoculated with the isolates both on the butt and the slant by stabbing and streaking, respectively. This was followed by incubation at 37^0C for 24-48 h. It was then observed for hydrogen sulfide production indicated by a black precipitate at the butt of the tube) and carbohydrate fermentation, indicated by gas production and colour change. (Quinn *et al.*,1994).

3.9.2 Sulphur, indole and motility tests

This is a differential medium. It tests the ability of an organism to reduce sulphur, produce indole and migrate through the agar. Sulphur, Indole motility (SIM) is commonly used to differentiate members of *Enterobacteriaceae.* Sulphide, Indole motility, (SIM medium) was prepared according to the Manufacturer's instructions. The pure isolates were inoculated into the medium by stabbing and incubating at 37^0C for 18-24h. They were then observed for hydrogen sulphide (H_2S) production, (indicated as a black coloration in the tube) and motility (indicated by migratory movement through the medium and away from the line of stab). Three drops of kovac's reagent were then added and shaken gently.

3.9.3 Citrate utilization test

In this test a needle was used to pick a single isolated colony which was lightly streaked on the surface of the Simmon's citrate agar slant (prepared according to manufacturer's instruction), which contains pH indicator (Bromothymol blue) in a test tube (whose screw cap was placed loosely) and incubated at 37^0C for 18-24hours (Cowan and Steel, 1993).

3.9.4 Methyl red-voges proskauer test

Samples were inoculated into 5mls of MR-VP broth and incubated at 37^0C for 18-24h. After incubation, about 1ml of the broth was transferred to a small serological tube followed by the addition of 2-3 drops of methyl red and the colour on the top of the medium was read immediately. A red colouration on addition of the indicator signified a positive methyl red test. (Cheesbrough,1985).

3.9.5 Urease test

In this test, pure culture was used to treat the entire surface of the urea slant prepared in a test tube under sterile conditions. The inoculated test tubes were then incubated for 18-24 hours at 37^0C. Urease production was indicated by a bright fuchsia pink colour on the slant which identifies those organisms that are capable of hydrolyzing urea to produce ammonia and carbondioxide, for which *Salmonella* is negative (Cheesebrough, 1985).

3.9.6 Additional biochemical tests

Sugars and some amino acids tests were carried out and various reactions were observed and recorded; nitrate positive, lysine decarboxylase positive, oxidase negative, ferments glucose, mannitol, ducitol, and maltose but fail to ferment lactose. Sucrose, adonitol and raffinose (Tafida *et al.*,2013).

3.9.7 Sugars

Mannitol, maltose, rhamnose, and xylose were tested. 1g of each sugar was weighed and 1.5g of Andrade peptone water was also weighed and mixed in a conical flask and dissolved in 100mls of distilled water and then 5mls dispensed into sterile test-tubes and then steamed at 115^0C for 5-10 minutes and then allowed to cool, after which isolates were subcultured on to a selective media and then 2 to 3 colonies picked and suspended into the test tubes, after which they were kept in the incubator at 37^0C for 24-48h and then read. Red colour indicates positive, while light pink colour indicates negative.

3.9.8 Amino acid decarboxylase test (Arginine,Ornithine and Lysine).

1g of each amino acid was weighed along with 1.5g of Andrade peptone water and then dissolved in 100mls of distilled water, then dispensed into sterile test-tubes and then heated for 5-10 minutes and then allowed to cool. There-after the stored isolates were inoculated into the tubes and then 2 to 3 drops of mineral oil was then added to them and then the tubes were covered and kept in the incubator for 18-24 h and then read (Møller, 1954).

3.10 Microbact 24E kit

Commercially available test called Microbact24E was used according to the manufacturer's instructions to confirm isolates as*Salmonella*spp based on the results of the biochemical tests.

3.10.1 Procedure for testing an organism with Microbact 24E

The microbact kit is a plate with 24 well. The wells (1-24) contain the following substrates/tests: lysine, ornithine, H_2S, glucose, mannitol, xylose, o-nitrophenyl-β-d-galactopyranoside (ONPG), indole, urease, vogesproskauer (VP), citrate, tryptophandeaminase (TDA), gelatin, malonate, inositol, sorbitol, rhamnose, sucrose, lactose, Arabinose, Adonitol, Raffinose, Salicin and Arginine respectively.

The isolates were grown on a selective media (BSA). Using a sterile straight wire, 2 to 3 colonies were picked and emulsified in 5ml of peptone water and incubated at 37^0C for 4h . A sterile pipette was then used to dispense one drop of the peptone water culture into 5ml of sterile normal saline solution (0.85%)

The wells of the individual substrate sets were exposed by cutting the end tag of the sealing strip and slowly peeling it back (using a sterile micropipette, 100µl of the bacterial suspension was added to each well in the set). Mineral oil was then used to overlay the substrates in wells 1,2 and 3 using micropippete. The inoculated rows were then resealed and labeled at the end of the tag with the specimen identification number followed by incubation at 37^0C for 24h.

On 24-h incubation period, the sealing tape on the test strips were peeled back and evaluated. Results were recorded in a report form as positive or negative by comparing them with a colour chart and making reference to the table of reactions provided. However, to well 8 (indole

production), two (2)drops of indole (kovacs) reagent was added and the result evaluated within 2 minutes of addition of the reagent. Well 10 (Voges-Proskauer reaction), 1 drop each of VP1 and VP2 reagents were added and the result evaluated within 15 to 30 minutes after the addition. To well 12 (tryptophan Deaminase), 1 drop of TDA reagent was added and the result evaluated immediately.

The 24 wells were then inoculated with Four (4) drops of test organism adjusted to 0.5 mineral oil was put into well 1, 2 and 3 and then incubated at $35^0C \pm 2^0C$ for 24hrs.

The reaction colours for negative reactions were; Yellow, Yellow-green, Straw colour, Blue-green, Blue-green, Blue-green, Colourless, Colourless, Straw colour, Green, Straw-colour, Colourless, Green, Blue-green, Blue-green, Blue-green, Blue-green, Blue-green, Blue-green, Blue-green, Blue-green, Blue-green, Yellow, Yellow-green colour to the 24 corresponding wells.

The reaction colours for positive reactions were; Blue-green, Blue, Black, Yellow, Yellow, Yellow, Yellow, Pink-red, Pink-red, Pink-red, Blue, Cherry-red, Black, Blue, Yellow, Yellow, Yellow, Yellow, Yellow, Yellow, Yellow, Yellow, Yellow, Yellow, Green-blue, and Blue.

Furthermore, reactions in 3 consecutive wells were summed up to give an Eight (8) digit number, which was fed into the computer software to provide information on the identity of organisms, including percentage (%) probability. Values $\geq$ 75% for each organism was accepted.

3.10.2 Interpretation of results

In Microbact 24E, an octal coding system has been adopted in which each group of three reactions produce a single digit of the code. Using the results recorded on the report forms, the indices of the positive reactions are circled and the sum of these indices in each group of three

reactions formed a code of eight numbers. The code obtained was then entered into the computer aided identification package and the resulting identity of the organism and its percentage probability recorded.

3.11 Serological Identification

All biochemically positive isolates were confirmed serologically using *Salmonella* polyvalent 'O' group A-S antiserum according to the manufacturer's instructions (OXOID). Using the slide agglutination test.

3.12 PCR Detection of *Salmonella* Species

3.12.1 Extraction of *Salmonella* dna

DNA was extracted from biochemically confirmed *Salmonella* isolates sub-cultured on nutrient agar, 18-24 h pure culture of each isolates was transferred to microtubes containing 400µl of lysis buffer and then 25µl of proteinase K was added to the suspensions which were then votexed and incubated at 65^0C for one hour. The suspensions were votexed every 20 minutes during the incubation. Following incubation, 400µl of phenol chloroform was added to each suspension, votexed and centrifuged at 13,000 revolutions per minute (rpm) for 10minutes. The supernatants were collected into fresh, sterile tubes and 400µl of chloroform was added. These were again votexed and centrifuged at 13,000rpm for 5minutes. The supernatants were afterwards collected in fresh, sterile tubes and 1000µl each of absolute ethanol was added followed by 40µl each of 3 molar sodium acetate. These were incubated overnight at -20^0C. The incubated suspensions were centrifuged at 13,00rpm for 10 minutes and the supernatants discarded, 400µl of 70% ethanol was then added to each residue and centrifuged at 13,000rpm for 5minutes. Again, the

supernatants were discarded and each residue was allowed to air-dry after which 50µl of ultra pure water was added.

3.12.2 Primers reconstitution

PCR primers (5'- GTG AAA TTA TCG CCA CGT TCG GGC AA - 3' Forward and 5' – TCA TCG GAC CGT CAA AGG AAC C – 3' Reverse) with amplicon size of 284 bp (Rahn *et al*., 1992) were used to target the *inv*A gene. The primers were reconstituted by adding 118µl and 142µl of deionized water to the forward and reverse primers, respectively according to manufacturers (Bioneer Inc., Korea) instructions.

3.12.3 PCR Amplification Procedure

The procedure described by Oludairo *et al*. (2013) was employed in which twenty five microlitre of $MgCl_2$, Taq (*Thermus aquaticus*) polymerase and dNTPs were mixed with 10µl template DNA, 2.5 µl forward 2.5µl reverse primer and 10µl nuclease free water in a PCR microtube. The cycling parameter involved initial denaturation at 95^0C for 2 minutes and 30 amplification cycles consisting of denaturation at 94^0C for 30 seconds, annealing at 53^0C for 30 seconds, extension at 72^0C for 60 seconds and final extension at 72^0C for 7 minutes. The PCR was then performed in a DNA thermal cycler according to manufacturer's instructions.

3.12.4 Electrophoresis of PCR product

Nine µl of the PCR product was separated by electrophoresis on 1.5% agarose gel with ethidium bromide dye and a 100bp DNA ladder. Amplicons from electrophoresis were viewed under ultra violet illumination (Bio-Rad, USA) for *inv*A gene (284bp band size).

CHAPTER FOUR

4.0 RESULTS

Flow processes, hazards and critical control points of the meat pie production line are presented in Figure 3.2. It was observed that temperature was a critical control point (CCP) as most samples which were exposed to either baking (meatpie) or thorough cooking (did not contain *Salmonella* from Plate 4.1. Most samples (meat, sauce, dough, egg) that had direct contacts with personel were contaminated with *Salmonella* and this could be due to poor hygiene, improper washing or cross contamination from Plate IV. All the samples investigated had bacterial growths on nutrient agar the counts ranged from the counts ranged from 5.18 $\log_{10}$CFU/cm^2 to 8.00 $\log_{10}$CFU/cm^2 for swab samples and ranged from 5.39 $\log_{10}$ CFU/g or ml to 8.12 $\log_{10}$ CFU/g or ml for meat pie samples and other liquid samples and MacConkey agar the counts ranged from 7.4 $\log_{10}$CFU/cm^2/g/ml to 10.14 $\log_{10}$CFU/cm^2/g/ml for swab samples, meat pie and other liquid samples.

4.1 Total aerobic plate counts: Total aerobic counts as shown in Appendix1 was conducted on a 10^{-7} dilution by surface plating on nutrient agar. The counts ranged from 5.18 $\log_{10}$CFU/cm^2 to 8.00 $\log_{10}$CFU/cm^2 for swab samples and ranged from 5.39 $\log_{10}$ CFU/g or ml to 8.12 $\log_{10}$ CFU/g or ml for meat pie samples and other liquid samples.

4.2 Total coliform count: It was done on MacConkey agar using dilution of 10^{-5}. The number of small to medium size pinkish smooth colonies were seen and counted as shown in Appendix 2. The counts ranged from 7.4 $\log_{10}$CFU/cm^2/g/ml to 10.14 $\log_{10}$CFU/cm^2/g/mlfor swab samples, meat pie and other liquid samples.

The mean microbial counts and the standard deviation ranged from 6.68log $_{10}$ ±1.88 to 7.17 log $_{10}$ ± 0.77 from processor 1to 10 which were very high, indicating that the poor hygiene level in the

production units was a general situation, because the maximum level allowed for total aerobic counts is 5×10^3CFU/g.

From Table 4.1 Kwangila had the highest total aerobic plate count with a mean log of 7.00×10^8 CFU/ml and from Table 4 PZ area had the highest total count with a mean log of 9.21×10^6 CFU/ml.

4.3 Demonstration and the occurrence of *Salmonella:* From Table 4.3 out of four hundred and eighty samples collected from production line of meat pie, 5 (1.04%) yielded *Salmonella*, 1 (3.33%) out of 30 'eggs' samples was positive for the organism, 2 (6.66%) out of 30 'meat' samples was positive for the organism, 1 (3.33%) out of 30 sauce (fillings) samples was positive for the organism, and 1 (3.33) out of 30 'dough' samples was positive for the organism. None of the meat pie, spice, water, carrot, green beans, potato, swabs yielded any isolates. Therefore, the overall isolation frequency of *Salmonella* was 5 (1.04%) out of 480 samples tested from Table 4.5.

4.4 Identification and Characterization of Isolates From Table 4, twenty nine (29) isolates were identified as suspects based on conventional biochemical tests. The 29 suspects were then subjected to confirmation using the Microbact rapid kit for Enteriobacteriaceae. The Microbact system identified 5 out 29 that were tested as *Salmonella,* only isolates that met the test criteria (75%) for inclusion were considered.

4.5 Serological Identification of Isolates: using *Salmonella* polyvalent 'O' group A-S antiserum, the 5 suspected isolates from Microbact rapid kit were further subjected to agglutination using the *Salmonella* polyvalent 'O' group A-S antiserum and strong agglutination was observed for the 5 suspect.

4.6 Virulence Potentials of Isolates by PCR

(Figure4): Of the 5 *Salmonella* isolates that were screened for *inv*A gene, all of them gave a 284bp DNA fragment which suggested the presence of *inv*A gene and further confirmed that the5 isolates were *Salmonella* (Plate 4.1).

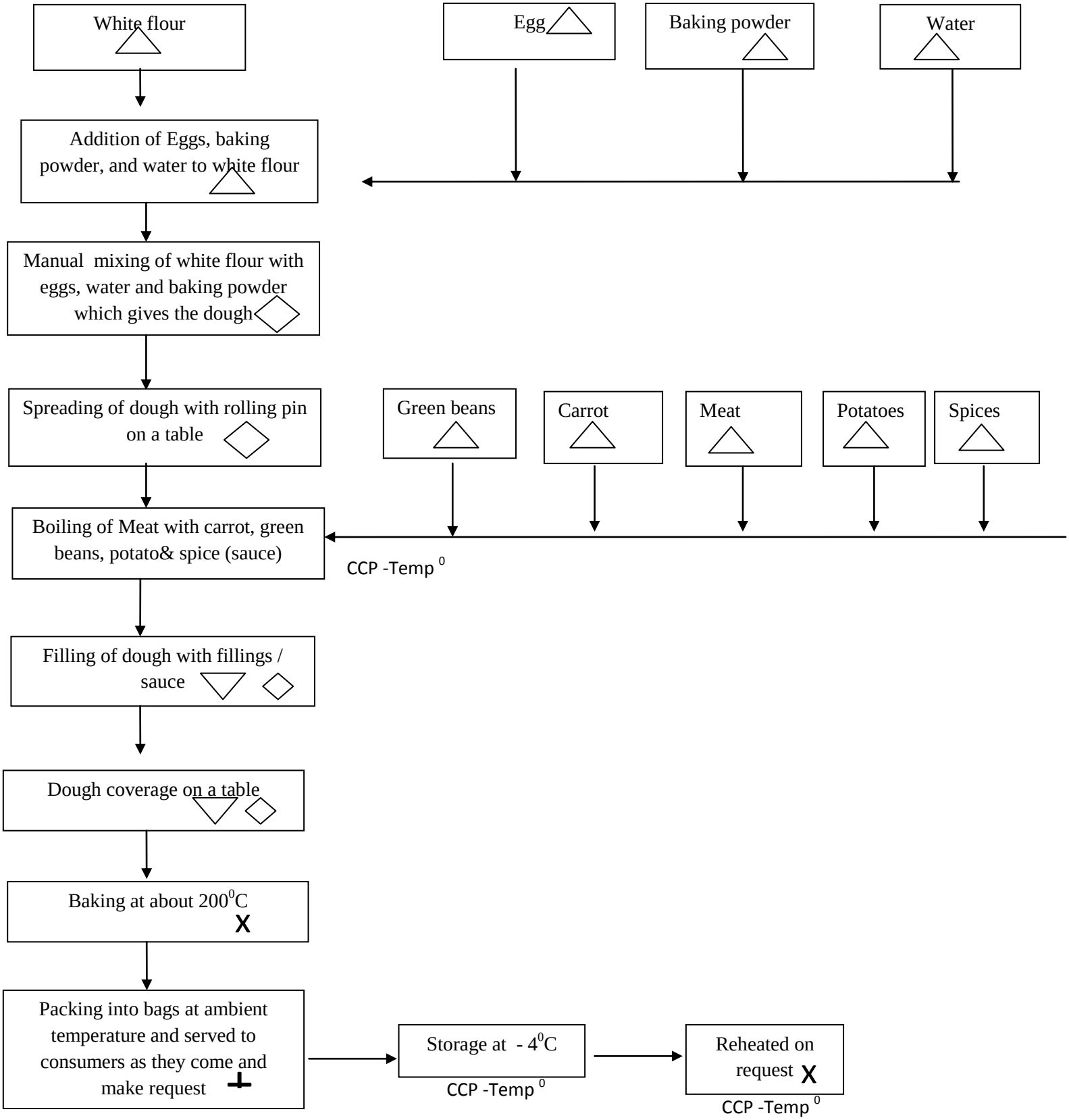

Figure 4:1 Contamination, survival and growth of microorganisms associated with the preparation of meat pie and critical control point s of operations in some retail outlet in Zaria. Nigeria (Okolocha *et al.*, 2006)

LEGENDS

 Initial contamination likely

 Contamination with equipments, utensils and surfaces likely

 Contamination by persons handling products likely

 Propagation of bacteria likely

X - Inactivation of vegetative forms of bacteria

CCP - critical control points

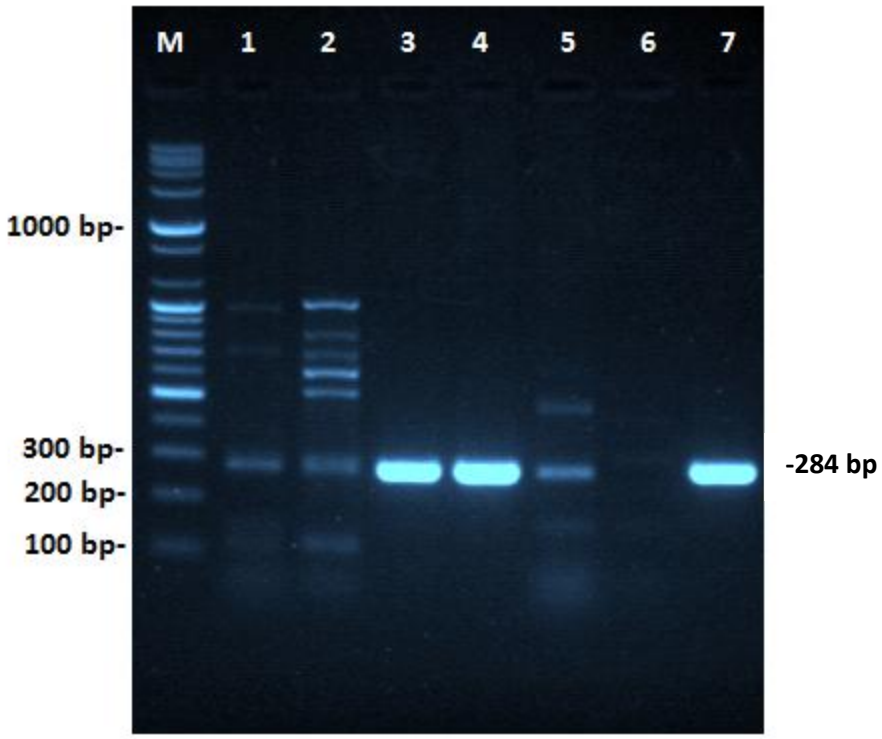

Plate IV: PCR Amplification of *inv*A gene of 5 *Salmonella* isolates.

Lane1: Dough; 2: Sauce (fillings); 3, 4: Meat; 5: Egg; 6: Negative control; 7: Positive control and M represent molecular weight markers.

Table 4.1 Mean log CFU/ml total aerobic plate counts of samples from meat pie production in Zaria.

Location	No. of sample	Mean log (CFU/ml) ±SD
Samaru	144	6.97± 2.50
PZ area	144	7.00 ± 2.48
Kwangila	96	7.14 ± 3.07
Palladan	96	7.11 ± 3.01
Total	480	

Table 4.2 mean CFU/ml total plate count on samples collected from meat pie production in Zaria.

Location	No. of samples	Means log (CFU/ml) ±SD
Samaru	144	9.13 ± 2.50
PZ area	144	9.21 ± 2.47
Kwangila	96	9.19 ± 3.06
Palladan	96	9.08 ± 3.04
Total	480	

Table 4.3: Frequency of *Salmonella* isolation from samples of meat pie production in Zaria.

S/No	Sample type	No of sample examined	No (%) of positive for *Salmonella*
1.	Meat pie	30	0(0)
2.	Fillings /sance	30	0(0)
3.	Carrot	30	0(0)
4.	Water	30	0(0)
5.	Dough	30	1 (3.33)
6.	Green beans	30	0 (0)
7.	Potato	30	0 (0)
8.	Eggs	30	1 (3.33)
9.	Meats	30	2 (6.66)
10.	Spice	30	1(3.33)
11.	Palm swab	30	0 (0)
12.	Knife swab	30	0 (0)
13.	Pan swab	30	0 (0)
14.	Rolling pin swab	30	0 (0)
15.	Container swab	30	0(0)
16.	Flour	30	0(0)
	Total	480	5(1.04)

Table 4.4: Frequency of *Salmonella* spp isolated from samples of meat pie production n=5

S/No	Sample ID	*Salmonella*	No. of isolales	%
1.	Water	*S. arizonae*	1	84.99
2.	Meat	*S. arizonae*	1	76.44
3.	Egg	*S. arizonae*	1	50.99
4.	Meat	*S. arizonae*	1	58.55
5.	Spice	*S. arizonae*	1	98.29

CHAPTER FIVE

5.0 DISCUSSION

Salmonella infections still occur at high frequencies in industrialized nations and developing countries. In addition *Salmonella* serotypes continue to be a major global health problem.

This study was aimed at determining the bacteriological quality of samples from meat pies sold in retail outlets in Zaria, Nigeria. On comparing the bacterial contamination between the various locations sampled (Samaru 9.13, PZ 9.21, Kwangila 9.19, and Palladan 9.08 log $_{10}$ CFU/ml) exceeded the maximum limits permitted in foods. The maximum limit allowed for total coliform count is 5×10^3CFU/g and all meat samples should be negative for *Salmonella.* The bacteriological investigation of 480 samples from meat pie production revealed that 475 samples were negative for *Salmonella* and only 5(1.05%) were positive. *Salmonella* species such *Salmonella typhi* is a bacterium that causes acute typhoid fever, which is a life threatening febrile illness (CDC, 2008). The disease is a cause for concern and a major public health problem in developing countries especially in Nigeria due to poor sanitary conditions and lack of or inadequate portable water (Ibekwe *et al.,* 2008). It is mainly transmitted through food, drink or even water, contaminated with urine or faeces of infected people or a chronic carrier (CDC, 2008, Ibekwe *et al.,* 2008).

The results of this work show the importance of conducting hazard analysis and critical points to detect food-borne disease hazards and to focus attention on solutions where control action is needed. This approach is very important in a country like Nigeria where there is little or no food borne disease surveillance activities (Okolocha *et al*., 2006). The hazards observed during the preparation of meat pie were the white flour, eggs, meat, potatoes, dough, spice, carrot, green

beans, water, swabs from equipments, rolling pins, baking pins, tables, knives, tables, the bare hands used in handling the meat pie. These may be sources of pathogens or spoilage microorganisms (Frazier, Westhoff, 1991). The primary control points for the preparation of meat pie are

I. Cooking thoroughly (boiling and baking) and freezing
II. Cleaning of utensils and equipment
III. Handling of the meat pie and other hygienic practices

The total aerobic plates counts which ranged from 5.18log to 8.12log CFU/ml or cm^2as the case may be is a clear pointer to the poor sanitary practice in meat pie production unit. Generally ready to eat foods with microbial counts exceeding $5\log_{10}$ CFU/ml or cm^2 is considered unfit for human consumption(Appendix 1 and 2) are usually considered unfit for human consumption (Adesiyun *et al.,* 1994). The highest total plates counts were observed in the meat samples, this is attributed to cross contamination from carcass to the processed beef. Thus the high microbial counts could be attributed to improper sanitary practices and very poor hygiene.

Samples obtained from the raw ingredients which includes; carrot, water, green beans, egg, potato and also from swabs of equipments, utensils and palm swabs i.e hands involved in the preparation also had high microbial load, this could be explained by the fact that the persons involved in the preparation, the implements, the utensils and water used may have served as a source of contamination (Okolocha*et al*., 2006).

The isolation rate of*Salmonella*was 1.05%, which was seen in the raw ingredients, this prevalence was found to be lower than the 6% isolation rate reported by Esona *et al.,* (2004) from beef in Zaria. Adesiyun and Oni (1989) obtained a prevalence of 5% from slaughter cattle.

Furthermore Kwaga *et al.*, (1985) obtained a prevalence of 8.4% in raw beef in Zaria, in Egypt, Abd El-Atty and Meshref (2007) detected *Salmonella* with a prevalence of 4% in sausages and 2% in spiced minced meat. Likewise in Ethiopia, Molla, Daniel and Woubit (2003) isolated Salmonella from slaughtered cattle with a prevalence of 4.2% and 12.1% in minced meat. The high percentage in processed beef was attributed to food handling and cross contamination from carcass to the processed meat.

Howevermeat pie did not yield any *Salmonella* possibly due to the high temperature employed during baking. This shows that temperature is an important critical control point, though other ingredients had high counts and *Salmonella* was isolated before the final cooking. It is obvious that temperature, whether high or low, played a more positive preventive role in meat pie preparation (Okolocha *et al*., 2006). The major factor that contributes to outbreaks of food borne diseases in developing countries is holding foods at ambient (room) temperature for several hours (Bryan, 1988.Dave, 1985, Roberts 1982). This situation contributes to food borne outbreaks in developing countries. If the duration of holding is short, pathogenic bacteria will have little time to multiply to large numbers. Avoiding hand contact of cooked foods is an important measure to minimize chance of contamination, if either hand contact of foods cannot or will not be avoided, effective time- temperature control during holding must follow (Okolocha *et al*., 2006).

Hand washing by staff of the operation units, which was either not done properly or done with water that had been used several times for the same purpose must have attributed to high microbial counts. Utensils and vessels rinsed in water and damp multipurpose cloth, non usage of aprons, absence of hair covering must have also contributed in a general way to the contamination. In addition the presence of stagnant water and vehicular movements of the road,

may increase the air-borne particles with concomitant increase in microbial contamination (Oranusi and Olorunfemi, 2011).

Cooking is a critical control point for foods that contain poultry eggs, meat, water, which are often contaminated by enteric pathogens. For most foods, a temperature of 73.9^{o}C will inactivate, large numbers of viruses, parasites and vegetative bacteria in less than a minute (Bryan *et al.,* 1992). Cooking is also necessary to remove microorganisms of potential public health significance. This explains the no isolation of *Salmonella* from meat pie itself.

Freezing is another critical control point. This may not be effective because of the unsanitary practices observed within the environment and also instable supply of light leading to post freezing contamination. Nevertheless freezing is an important control measure to prevent growth of bacteria in foods. (Bryan *et al*.,1992)

The presence of this organism in ready-to-eat food is of serious public health significance as similarly observed by Abdullahi *et al.,*(2006). They reported the possibility of micro organisms to multiply and thrive, based on the fact that the ambient temperature at which this meat product is stored coupled with the moisture content could favour its multiplication. Meat pie did not yield any *Salmonella* possibly due to the processing techniques of this ready-to-eat food, which is usually exposed to prolonged high ambient temperature (baking), as a result the low moisture nature of the products leads to reduced water activity which does not promote the growth and survival of micro organisms, which may have contributed to the 0% isolation rate from this meat product. The reduced water activity of these ready-to-eat products also makes them shelf stable at room temperature (25-26^{0}C) and differentiates them from perishable fresh products. *Salmonella* species have a water activity growth limit value of 0.95 (Gibbs and Gekas, 2010).

However, further studies need to be carried out to determine the water activity in this meat products and their relationship with the presence of food borne pathogens.

The microbact 24E kit confirmed 5 out of 29 *Salmonella* suspects identified by conventional biochemical test carried out. The use of the microbact kit for the identification of *Enteriobacteriaceae* was observed to be more sensitive, less labourious, rapid and convenient method for the identification. It was also observed that out of the five *Salmonella* isolates identified by microbact only two were further confirmed based on PCR conducted , the presence of this organism in raw ingredients is of serious public health concern as similarly observed by Abdullahi et al. (2006). Abdullahi further reported the possibility of microorganisms to multiply and thrive; based on the fact that the ambient temperature at which this meat product is stored coupled with the moisture content could favour the multiplication. Hence, the need for rapid identification of organisms cannot be over-emphasized. The convenience and efficiency demonstrated by the microbact kit suggests a possibility for it to be used for rapid identification of food-borne organisms and also can be used to improve diagnosis in laboratory settings.

The hazards are identified by hazard analyses or epidemiological and other scientific studies, preventive measures which are practical under prevailing customs and circumstances must be chosen from known measures or they must be devised. The high microbial counts recorded in this study can be attributed to mainly lack of infrastructure, poor and unhygienic handling. It is obvious from the foregoing that the main quality control points to consider concerns hygiene. (Okolocha *et al*., 2005).

Food safety activities must therefore concentrate on informing personnel involved in the preparation of foods they usually prepare and practical prevention measures needs to be focused

on. There is need to educate the producers on the HACCP of meat pie preparation. Such control and monitoring procedures like cooking thoroughly, washing hands at intervals with soap, washing equipments and utensils before and after use, washing ingredients properly, preparing the meat pie in quantities that can be easily sold-off, especially where there are no means of refrigeration, are necessary for the preparation for a safe product (Oranusi *et al.,* 2003).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The following conclusions were drawn from the results of the study.

1. *Salmonella* is present in sample collected from meat pie production line in Zaria and was found in the following samples: dough, sauce, meat and egg. Salmonellosis has most often been associated with consumption of contaminated foods of animal origin such as poultry, eggs, milk and dairy products. Several epidemiological studies conducted globally indicate the association of wide variety of foods related to outbreaks of salmonellosis.
2. Meat pies sold in Zaria metropolis has high load of microbial agents
3. PZ area was observed to present the highest microbial load as compared to other sample locations
4. Eggs, meat, sauce (fillings) and dough were contaminated with *Salmonella* at 3.33%, 6.66%, 3.33%, and 3.33%, respectively.
5. Out of 480 samples collected, only 5 (1.03%) were positive for *Salmonella.*
6. As a result of the increased consumption of ready-to-eat foods such as meat pie in our environment and the possible contamination of such foods by pathogenic organism such as *Salmonella* with regards to the consequences of such contamination on public health, this study was able to:

i. Highlight the public health implications of consuming meat pie contaminated with *Salmonella spp*

ii. Educate meat pie producers, vendors and consumers on the need to adopt and adhere strictly to hygienic practices during preparation, sale and consumption of meat pies

iii. Make appropriate recommendations to relevant authorities on the need to take necessary actions and steps to prevent, and where necessary control food-borne illnesses associated with the consumption of meat pies contaminated with *Salmonella sp.*

6.2 Recommendations

The following recommendations are made on measures to reduce *Salmonella* and other microorganisms in samples from the production line of meat pie:

The fact that *Salmonella* was detected in meat pie sample, requires

1. An appropriate cleaning and disinfection program should be implemented to decrease the infection with *Salmonella* and other food borne diseases.

2. There is a need to establish permanent programmes for surveillance of *Salmonella* and all other food-borne pathogens.

3. *Salmonella* testing services should be reviewed periodically and linked to external quality control to improve services.

4. More rapid and sensitive tests should be employed for *Salmonella* detection, such as nested PCR.

5. Intensive monitoring and surveillance program should be conducted by the concerned authorities.

6. Food handlers and consumers should be educated on the need for personal hygiene to minimize contamination

7. Individuals engaged in the food industry be taught and be encouraged to imbibe the HACCP principles in food production to improve consumer safety.

8. Food-borne disease surveillance programme should be instituted by the government and implemented by appropriate government agencies. Data generated as a matter of policy should be used to give direction to food safety measures.

9. Strict sanitary measures should be observed during meat pie preparation, distribution and sales to ensure wholesomeness.

10. Further studies should be carried out on the presence of other possible pathogens in meat pies in Zaria, Nigeria.

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APPENDIX

Appendix 1: Mean log total aerobic plate count from sample of meat-pie production (cfu/ml/g/cm^2)

S/No	Sample ID	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
1	Flour	7.78	5.99	5.54	6.37	6.39	5.39	5.82	6.76	5.57	6.64
2	Egg	7.66	7.72	7.58	7.56	7.95	7.96	7.58	7.54	7.92	7.84
3	Water	7.55	7.81	7.58	7.75	6.77	6.86	7.97	7.90	7.70	7.79
4	G/Beans	7.75	7.89	7.87	7.72	7.32	7.77	7.61	8.00	7.34	7.57
5	Carrot	6.90	7.79	7.59	7.45	7.53	7.64	7.63	7.71	7.96	7.84
6	Meat	7.85	7.39	7.27	7.32	7.12	7.92	7.00	7.79	7.86	7.54
7	Potato	6.90	7.55	7.64	7.56	7.64	7.56	7.38	7.95	7.90	8.11
8	Spices	7.85	7.70	7.97	7.53	7.53	7.95	8.00	7.55	7.44	7.80
9	Dough	6.47	7.85	7.77	7.47	6.60	7.97	7.75	7.06	7.61	7.78
10	Fillings	7.06	7.76	7.67	6.97	6.90	7.75	7.79	7.84	6.77	7.06
11	Palm swab	8.00	6.40	5.91	6.3	5.18	5.24	5.51	6.75	5.18	6.05
12	Knife swab	6.42	6.59	6.03	6.3	6.42	6.49	6.27	6.58	6.19	6.29
13	Container swab	6.37	6.23	6.23	6.41	6.22	6.37	6.36	6.02	6.25	6.51
14	Pan swab	6.42	6.40	6.62	6.41	6.45	6.16	6.45	6.33	6.68	6.25
15	Rolling pin swab	6.23	6.05	5.94	6.25	6.49	6.27	6.50	6.29	6.27	5.52
16	Meat pie	7.54	7.81	6.62	7.53	7.68	6.9	7.63	8.05	7.87	7.61

KEY: P: Processors

P1-P3: Samaru P7-P8:kwangila

P4-P6: PZ area P9-P10: Palladan

APPENDIX 2: Mean log total coliform count from samples of meat pie production (CFU/ml, CFU/g and CFU/cm^2)

S/No	Sample ID	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
1	Flour	7.89	5.99	5.54	5.57	6.37	6.39	5.39	5.82	6.76	6.64
2	Egg	7.66	7.72	7.58	7.56	7.95	7.96	7.58	7.54	7.92	7.84
3	Water	7.55	7.81	7.58	7.75	6.77	6.86	7.97	7.90	7.70	7.79
4	G/Beans	7.75	7.89	7.87	7.72	7.32	7.77	7.61	8.00	7.34	7.57
5	Carrot	6.90	7.79	7.59	7.45	7.53	7.64	7.63	7.71	7.96	7.84
6	Meat	7.85	7.39	7.27	7.32	8.12	7.92	7.00	7.79	7.86	7.54
7	Potato	6.90	7.55	7.64	7.56	7.64	7.38	7.95	7.90	8.11	7.62
8	Spices	7.85	7.70	7.97	7.53	7.53	7.95	8.00	7.55	7.44	7.80
9	Dough	6.47	7.85	7.77	7.47	6.60	7.97	7.75	7.06	7.61	7.78
10	Fillings	7.06	7.76	7.67	6.97	6.90	7.75	7.79	7.84	6.77	7.06
11	Palm swab	8.00	6.40	5.91	6.30	5.18	5.24	5.51	6.75	5.18	6.05
12	Knife swab	6.42	6.59	6.03	6.30	6.42	6.49	6.27	6.58	6.19	6.26
13	Container swab	6.37	6.23	6.23	6.41	6.22	6.37	6.36	6.02	6.25	6.51
14	Pan swab	6.42	6.40	6.62	6.41	6.45	6.16	6.45	6.33	6.68	6.25
15	Rolling pin swab	6.23	6.15	5.94	6.25	6.49	6.27	6.50	6.29	6.27	5.52
16	Meat pie	7.54	7.81	6.62	7.53	7.68	6.9	7.63	8.05	7.87	7.61

Appendix 3: Frequency of organisms isolated from samples of meat pie production n=29

S/no	organisms	No. of isolates	percentage range%
1	*S.liquifaciens*	6	44.75-54.60
2	*C.freundii*	3	45.62-83.2
3	*E.gergoviae*	2	39
4	*C.youngae*	3	53.6-95.88
5	*S.marcescens*	3	76.92-96.8
6	*C.amalonaticus*	2	70.01-85.98
7	*E.agglomerans*	1	6.34
8	*S.arizonae*	5	50.99-98.29
9	*Citrobacter*	2	58.22-74.91
10	*K.cryocrescens*	1	50.15