CHAPTER ONE

INTRODUCTION AND OBJECTIVES

1.1. Introduction

Food safety is one of many attributes of food together with market value, nutrition, packaging and others (Antle, 1999; Henson and Caswell, 1999). The objective of improving food safety requires consideration of several issues, such as the definition and nature of food safety hazards, the optimal level of food safety and strategies for improvement (Valeeva *et al*., 2004). A food borne hazard can be a chemical, biological or physical agent that is likely to cause food borne illnesses or injury if it is not controlled (Schmidt *et al*., 2003).

Consumer confidence in food safety in Canada has been negatively influenced by several major food safety crises in recent years. For example, a Listeriosis outbreak in September (2008) in deli meats in Canada, a *Salmonella* outbreak in June (2008) and an *Escherichia coli* O157:H7 outbreak in spinach in September (2006) in the United States received significant media attention. Food borne disease relates to a range of microbiological, chemical and physical risks. However, in terms of incidence of disease and economic impact microbiological causes constitute the major risk. These food borne illnesses place a burden on national economies (Veeman, 1999). Consequences of food borne illnesses can include long term health effects, serious disabilities, and even death. However, most cases of illnesses caused by food borne microbiological hazards are not reported since they can be mistaken for other illnesses (Thomas *et al*., 2006). To ensure consumer safety, the implementation of food safety standards based on microbiological criteria for specific food pathogen combinations is currently underway (FSIS, 2008). That requires the active involvement of the other side of the food safety problem the producer side. Interventions at the producer level can effectively reduce the number of pathogens in food. Setting a limit for microbial pathogens for food products is not a new undertaking but has remained controversial with industry and consumers (Unnevehr, 2003). Various interventions are available to producers to reduce pathogens to be able to comply with food safety standards. However, these are costly to producers. Surprisingly, detailed studies on the costs of food safety interventions are rare. (Crutchfield *et al*. 1997). Recently, the assessment of specific interventions, has gained importance in Europe (Mangen *et al*., 2005) and the U.S. (Malcolm

et al., 2004; Nganje *et al*., 2006). According to the National Research Council (NRC 2003), different forms of microbiological criteria exist: microbiological standards, microbiological guidelines and microbiological specifications. Microbiological standards are determined by government agencies and are therefore part of administrative regulations. These standards are mandatory and any products in non-compliance are subject to rejection, reprocessing or destruction as enforced by the regulatory agency. Microbiological guidelines are advisory criteria and used to monitor production processes. One example is the control of critical control points in HACCP. The ultimate assessment and approval of products underlies the judgment of personnel or management as these guidelines can be set and controlled internally. Microbiological specifications are either advisory or mandatory. These are established as purchase requirements for finished products or raw material. Hence, strictly speaking, government intervention concentrates on the setting of microbiological standards. (Unnevehr, 2003) promotes the use of a microbiological standard and finds, for the U.S., that the requirement for meat and poultry processing plants to meet microbial pathogen standards lowers the probability of subsequent contamination and, consequently, food-borne illness.

According to (FAO, 2001) standards for microbiological criteria are designed to determine the acceptability of a food item based on the number of microorganisms or toxins per unit of volume, mass, food lot or area. Standards or criteria are established for reasons of outbreak investigation, routine testing, and verification of HACCP, indicator for shelf life or spoilage of food (Todd, 2004). A wide variety of diseases can be caused by eating food contaminated with pathogenic microorganisms or their products. All of these diseases can be classified as food poisoning (Eely, 1996). *Salmonella* is the most frequently reported cause of food borne illness. In 1996, the Centers for Disease Control and Prevention (CDC) documented 39,027 cases in USA. Any raw food of animal origin, such as meat, poultry, milk and dairy products, eggs, seafood and some fruits and vegetable may carry *Salmonella*. The bacteria can survive to cause illness by cross contamination of foods (El-Begearmi, 1998). *E. coli* is a major facultative inhabitant of the large intestine and ubiquitous in the human environment. It is one of the most frequent causes of the many common bacterial infections, including cholecystitis, bacteremia, cholangitis, urinary tract infection (UTI) and traveler's diarrhea and other clinical infections such as neonatal meningitis. Since 1885, *E. coli* has been recognized as both a harmless commensal and a versatile pathogen (Bower, 1999). While generic *E. coli*

is considered a part of the normal micro flora in the intestinal tract of most warm-blooded animals, including humans, many pathogenic strains can cause diarrheal disease and have been associated with food-borne illness (Doyle, 1990). According to (Antle ,1999), standards can be grouped into performance, design and mixed standards , where a performance standard prescribes a specific level of food safety and the design standard (specification standard) a specific procedure to follow without testing if a level of food safety has been achieved. HACCP is a food safety system that can entail both types of standards. (Antle ,1999), notes that performance standards are more likely to be efficient than design standards when trying to achieve a specific level of food safety since a performance standard allows plant managers to adapt their quality control to the structure of their plant. Hazard Analysis Critical Control Points (HACCP) has become the main safety intervention measure in the food processing sector. HACCP is defined as a logical system to identify hazards and/or critical situations and to produce a structured plan to control these situations. The seven components of a HACCP plan are: 1) conduct a hazard analysis, 2) identify critical control points, 3) establish critical limits for preventative measures, 4) establish monitoring requirements, 5) perform corrective actions, 6) establish a record keeping system and 7) verification procedures (Schmidt *et al.*, 2003). HACCP has been and is being mandated into law in many nations all over the world. In the EU, HACCP principles were adopted through the Directive 93/43 in 1993 (Ziggers, 2000). In the US, HACCP was mandated for seafood in 1995, for meat and poultry in 1998, and for the juice industry in 2001 (FDA, 2001). The Australian Food Standard Code required HACCP-based food safety programs From January 2003 onwards (FSANZ, 2002). In New Zealand, the Animal Products Act 1999 requires all primary animal products processing businesses to have a HACCP-based risk management program in place by November 2002 (MAF, 2001).

Studies on the effectiveness of HACCP show a decline in food borne pathogens with the implementation of the program (Nganje *et al*., 2006). The overall notion is that the benefits of implementing HACCP outweigh the costs of implementation (Crutchfield *et al*., 1997).

1.2. Rational:

Though HACCP method is the best way to minimize hazards in the food production, which prevents hazards before they happen sometimes seems to be difficult and complex because of some hurdles.

Salmonella is one of the leading bacterial food borne pathogens that cause illnesses, deaths and financial losses.

1.3. Objectives

1.3.1. General Objectives

The Objective of this thesis was investigation the status of food control systems currently in force in the Khartoum state and identify the obstacles impeding the application of modern systems in food safety as well as the study are aims to detect the microbial contamination in some food product.

1.3.2. Specific objectives

1. To define the implemented level of food safety system in food businesses and distribution of food products in the Khartoum state.

2. To determine the HACCP knowledge of staff working in food businesses in Khartoum State.

3. To identify problems and obstacles that hinders the implementation of the HACCP system in the state of Khartoum.

4. To detect pathogens in particular *Salmonella spp* in the final product for food intended for human consumption.

5. To determine the microbial contamination of food product, especially Aerobic Plate count and *Escherichia coli*.

CHAPTER TWO

LITERATURE REVIEW

2.1. The hazard analysis and critical control points (HACCP)

2.1.1 History

The Pillsbury Company first developed the concept of HACCP in the early 1960's. This firm worked cooperatively with NASA to develop this new system to ensure safety of the food consumed by the astronauts. At that time, most safety systems were based on end product testing. For this concept to be fully effective, companies must test 100% of their product. Since most testing is destructive, this approach would not be feasible because the entire product would be required (Mortimore and Wallace, 2000). At the 1971 National Conference on Food Protection, the HACCP system was first presented. This new approach to food safety gained interest among food processors and was used as the basis for regulations regarding low-acid and acidified foods. Furthermore, the FDA even began using HACCP for investigation activities. However, after the initial excitement of the new system, interest in HACCP began to fade. According to (Stevenson, 1990), only a few large companies continued to apply HACCP.

During the 1980s, some of the government protection agencies asked NAS/NRC (National Academy of Sciences/National Research Council) to form a committee that would generate some general principles for the application of microbial criteria in foods. This committee proposed the implementation of HACCP in food protection programs. In addition, they suggested that the food industry receive the proper training with regard to the HACCP concept (Stevenson, 1990). Many food industries have implemented HACCP since its inception. Some have done so voluntarily, whereas others have been mandated. Industries currently mandated are Seafood (since 1997) and Juices (effective in 2002). The meat and poultry industry fell under the HACCP mandate in 1998(large plants). Small and very small plants followed in 1999 and 2000, respectively. The smaller plants were given more time to develop their HACCP plans due to fewer resources and personnel compared to larger plants (Bowers, 1998). The canned food industries do not have a mandatory HACCP requirement, but one is highly recommended. The major reason that some

canning companies have implemented HACCP is to control *Clostridium botulinum* (Food Safety and Inspection Service, 2000).

2.1.2. Purpose of HACCP Program

The HACCP program serves several purposes. The main objective of HACCP is to produce a safe product. HACCP is a safety program, not a quality program. Met fragments, microorganisms that cause illness and harmful chemicals are examples of some of the hazards that HACCP will attempt to reduce or eliminate (Swanson and Anderson, 2000). There will never be a process that is absolutely safe, but there must always be a constant effort to achieve zero defects (Snyder, 1991).

Another function of HACCP is to reduce or even eliminate the need for endpoint testing. Before the HACCP concept was developed, many processors depended on endpoint testing to determine if their product was satisfactory. This testing can be very tedious and time consuming. Also, testing can lead to a loss of a portion of the product since some types of testing are destructive (Bauman, 1990). HACCP attempts to reduce endpoint testing by conducting a series of checks throughout the process. At each step in the process, all possible hazards are considered in regards to how to prevent them and what actions will be taken if a significant hazard occurs (Mortimore and Wallace, 2000). By the time the product reaches the end of the process, HACCP attempts to reduce hazards to an acceptable level.

A third purpose of HACCP is to provide documentation to prove that the process is being conducted as written. Without documentation and records, there is no verification that anything has actually taken place.

2.1.3 Advantages HACCP

According to the (FDA, 1999), the advantages of HACCP over other safety systems are that this preventative program:

• Focuses on identifying and preventing hazards from contaminating food

• Is based on sound science

• Permits more effective government oversight because record keeping allows investigators to determine how well a firm is complying with food safety laws over a period of time rather than how well it is doing on any given day.

• Places responsibility for ensuring food safety appropriately on the food manufacturer or distributor.

According to (Mayes, 1994), "Implementation of HACCP is not a quick 'back to the envelope' job done on a quiet afternoon, but it is instead a detailed technical evaluation of a product and process requiring time, commitment, scientific and technical expertise to carry out hazard analyses and establish control and monitoring procedures, and the requisite knowledge, skills and attitude for successful implementation".

2.1.4 HACCP Components

Prerequisite Programs: Before HACCP implementation within the food industry, certain programs were already in place to provide for food safety. For the HACCP system to produce safe products, it must be built on a solid foundation of prerequisite programs.

These programs provide the basic conditions that are necessary for the production of safe food. Some examples of common prerequisite programs are GMPs, SSOPs, letter of guarantee and pest control (NACMCF, 1999). Prerequisite programs ensure that HACCP planes) are functioning effectively (Stier, 1998). Consistent maintenance of these programs is important to the success of the HACCP plan (Bernard *et al*., 1997). Understanding the difference between HACCP and prerequisite programs is accomplished through the recognition of two main points. First, prerequisite programs deal indirectly with food safety, whereas, HACCP focuses solely on food safety. Second, prerequisites tend to be more general and applicable across a processing plant. HACCP plans are only based on hazard analyses that are product or line specific. (Bernard and Parkinson, 1999). Also, there is often the misconception that HACCP replaces the need for prerequisite program. HACCP does not replace any prerequisites. It combines with the prerequisites to form a food safety system (Motarjemi, 1999).

Two of the most common prerequisite programs for HACCP are the Good Manufacturing Practices (GMPs) and the Sanitation Standard Operating Procedures (SSOPs). GMPs emphasize

sanitary effectiveness and hygienic practices during food processing. Many companies require that their supplier conduct regularly scheduled audits to assure that they are adhering to their GMPs (Stier, 1998). SSOPs are a widely used program to maintain proper sanitation within food processing plants even before HACCP was mandated (Gombas, 1998). SSOPs describe all daily procedures that will be conducted to maintain sanitation, specify the frequency of the procedures, and identify those responsible for implementing and monitoring the SSOP (Stier, 1998). Both GMPs and SSOPs are signed and dated by a qualified official and kept with all HACCP related documents (Adams, 1998).

2.1.5 HACCP Team

A HACCP Team has to be developed to champion the operation. However commitment from upper management should be obtained first. Without commitment from the entire plant, HACCP will not function properly. The HACCP team is established of individuals who will execute the duties of implementing and maintaining the HACCP plan. It is important to avoid too much work delegated to one person, but not have too many members so that communication between them becomes difficult. A team consisting of four to six members is ideal, with one of them acting as team leader (Mortimore and Wallace, 2000).

It is recommended that the team consist of at least one expert from Quality Assurance, Operations or Production, and Engineering. The Quality Assurance experience will provide knowledge in what types of hazards can occur and the risks associated with these hazards. The expert from operations or production will have detailed knowledge of the day-to-day operational activity. The engineering representative will be capable of providing expertise on the processing equipment with respect to process capability.

Additional expertise will be needed and can be selected from within the company or from outside consultants. It may be easier to keep the HACCP team internal for communication and availability purposes. These additional experts can be selected based on which will be more beneficial to that particular plant. Someone from research and development can be selected if new products and processes are being developed. Other experts such as purchasing agents, microbiologists and statisticians can be beneficial to the team. Also, a HACCP expert might also

be considered. One who is knowledgeable in setting up HACCP plans will help keep the team focused (Mortimore and Wallace, 2000).

Product Description: Another requirement of a HACCP plan is to develop a product description and intended use of this product. According to (Mortimore and Wallace, 2000), the product description should contain a brief description of the product with regards to storage temperature and shelf life. The description should also describe any hazards associated with the production of the product and how to control these hazards. Furthermore it should give a description of target groups that may consume this product (Ababouch, 2000). The purpose of the product description is to help familiarize the RACCP team with the products and technologies being utilized.

Process Flow Diagram: Prior to conducting the hazard analysis, a process flow diagram must be created. This is a flow chart that represents the process starting with receiving of materials to shipping of the end product. All of those stages on the flow chart that are critical control points must be labeled. The diagram should include time and temperature profiles for each stage of production. The flow diagram does not necessarily have to be an extensive drawing of the facility. A block type flow diagram is used most frequently (FDA, 2000).

Once the flow diagram is completed it should be verified by the RACCP team to ensure completeness and thoroughness. The team should meet and review the diagram to ensure that all stages are included and all other criteria are present. Modifications should be made as necessary (FDA, 2000).

2.1.6 HACCP Principles

After these preliminary steps, the HACCP team should develop the seven RACCP principles. Originally the RACCP protocol consisted of only three principles 1) Hazard analysis and risk assessment, 2) Determine the Critical Control Points (CCPs), and 3) Monitor the CCPs. In 1989 the National Advisory Committee on Microbiological Criteria

for Foods (NACMCF) included four more principles to the HACCP system (Sperber, 1991). According to Snyder (1991), the seven principles that now make up a HACCP plan are:

1. Conduct a Hazard Analysis and Risk Assessment.

2. Determine CCPs.

3. Establish Critical Limits (CL) for each CCP.

4. Establish Monitoring procedures for each CCP/CL.

5. Establish Corrective Actions.

6. Establish Verification Procedures.

7. Establish a Record keeping System.

Principle 1

The first principle involves conducting a hazard analysis, which involves assessing certain risks involved in production of a product. "Hazard Analysis is defined as 'the process of collecting and evaluating information on hazards and conditions leading to their presence to decide which are significant for food safety and therefore should be addressed in the HACCP plan'" (Mayes, 1999). The first part of conducting a hazard analysis involves identifying all possible hazards that could occur within the food product. The HACCP team should hold a brainstorming session to identify every possible hazard. During this session, the team should not consider the significance of a particular hazard. That will be dealt with during the risk assessment. (Mayes, 1999) states that "the Hazard Analysis is probably the key principle in the whole HACCP system and the one people find to be the most difficult." The three types of hazards that must be considered during a hazard analysis are biological, chemical and physical (Tompkin, 1994). Biological hazards are normally those that involve microorganisms. Another type of hazard is a chemical hazard. These hazards involve specific chemicals that may be added to the product or chemicals that contaminate the food during processing. Cleaning compounds and pesticides are two examples of chemicals that could contaminate the product. Other chemical hazards include several added which may be an allergen to the consumer (e.g. Peanuts, eggs or shellfish) (Mortimore and Wallace, 2000). Other hazards are the physical hazards. As the previous two types, these also can occur during any stage in the process. Physical hazards are those that are sharp or hard that could cause injury or choking. Fragments of glass, metal or wood could all be considered physical hazards (Mortimore and Wallace, 2000).

After all potential hazards are identified the RACCP team must now conduct risk assessment. According to (Sohrab, 1999), "Risk assessment is a scientific evaluation of known or potential adverse health effects resulting from human exposure to food borne hazards". An example is where the team determines which identified hazards are significant. A significant hazard is one in which the likelihood of occurrence and severity of illness are high.

When determining the likelihood of a hazard, the RACCP team must research each hazard and identify any trends. If the literature indicates that this hazard does not occur often, the team can indicate that the likelihood of occurrence is low. The team must also research a hazard to understand it's severity if it is not properly controlled. Some hazards may be more severe than others. For example, microorganisms that can lead to chronic illnesses or death are considered very severe. Other microorganisms may only cause small side effects. These are not very severe (Sohrab, 1999).

Principle 2

When the hazard analysis is complete, the RACCP team must go over the flow diagram and decide which steps are critical control points (CCPs). A CCP can be a point in the process where a significant hazard can be eliminated or reduced to an acceptable level. A CCP is also a point where loss of control will lead to a significant hazard. It differs from a control point (CP) in that a loss of control at a CP will not lead to a significant hazard. CCPs require a lot of careful development and extra documentation and that is why they should be limited to only those that are truly critical (Weddig, 1999). When determining which steps are critical control points, some companies use what is called the shotgun approach. This is a method that is not based on any true reasoning; rather CCPs are chosen based on the opinions of the team. This may lead to an excessive number of CCPs resulting in problems for the plant. A more accurate and feasible method that can reduce the number of CCPs is use of the decision tree. This approach asks several questions about each processing step where a hazard is significant (Tompkin, 1994). The questions are in "yes or no" format, and will eventually determine whether that step is a CCP.

Principle 3

Once the CCPs are determined, critical limits are required for each step that is a CCP. A critical limit is a maximum or minimum value to which a specific parameter must be controlled at each

CCP. Common critical limits are temperature, time, moisture, pH and salt concentration. Critical limits are rarely a range of values. Each limit should have some sort of basis whether that is FSIS regulations, FDA action levels, or any other scientific literature (Food Safety and Inspection Service, 1996). An example is the temperature within a freezer. If the critical limit is set at 0° C or below, the temperature must always remain at or below that temperature. The temperature must be watched very closely and monitored to ensure that the limit is not exceeded (King, 1992). Critical limits can be slightly stricter than the regulations set by FSIS. This requirement will ensure that regulatory requirements are still met in the event of a slight deviation from the limit (Food Safety and Inspection Service, 1996).

Principle 4

The next step is to monitor each CCP and critical limit. Monitoring of each critical limit is very important because it helps to ensure that the CCPs are in compliance and the critical limits are not exceeded (Sohrab, 1999). Critical limits can be monitored continuously or non-continuously. If a critical limit were monitored continuously, a temperature monitoring system would be a good investment. A computer system will be devised for measurements at regular increments. Continuous monitoring is ideal when a particular parameter tends to have more variation than normal. This system will also need to be monitored by an individual to ensure the computer system is functioning properly (Tompkin, 1995). If non-continuous monitoring is utilized, a member of the HACCP team must conduct checks at regular increments (i.e. every 30 minutes or every hour). That individual is responsible for keeping an accurate record of each CCP and notifying the proper authority if a critical limit is exceeded. Because non -continuous monitoring is being used, it is important that the frequency of monitoring be adequate to ensure control of the CCP (Sohrab, 1999).

Principle 5

If there is a deviation from the set standards of a critical limit, corrective actions must be taken (Snyder, 1991). Corrective actions are procedures carried out when a loss of control has occurred at a particular CCP. (Sperber, 1991) suggested that all corrective actions as well as responsibilities should be clearly outlined before HACCP is implemented. All records and corrective actions should be documented to prove that corrective actions are being conducted

(Sohrab, 1999). The first step of a corrective action is to stop the processing line and isolate a possibly adulterated product (King, 1992). Once the non-compliant product is segregated, microbial testing will help assess the safety of the product (Kvenberg and Schwalm, 2000). If the product is deemed as unsafe, it will be discarded. However, if testing reveals minimal adulteration, the product can then be reprocessed (Food Safety and Inspection Service, 2000).

Before the processing continues, control must be reached at that CCP. Once the process is stopped, it is up to the individuals responsible to identify why a deviation has occurred and what can be done to bring the process back to conformance. Once this reason is determined, measures will be implemented to prevent the deviation from occurring again (King, 1992). If a deviation occurs too often at one CCP, the HACCP team will have to evaluate whether the HACCP plan is sufficient to control this hazard (Kvenberg and Schwalm, 2000). Corrective actions might even be considered if monitoring indicates a trend towards loss of control at that CCP (Sohrab, 1999).

Principle 6

The next principle that must be addressed is verification. Verification is the application of methods, procedures and tests to determine the company's compliance with the HACCP plan (Mayes, 1999). Verification covers all internal daily activities with regards to HACCP (Lupin, 2000). A few verification procedures include a review of the HACCP system and records, any deviations and product dispositions, and confirmation that the CCPs are kept under control (Mayes, 1999). The only way to be confident that a safe product is being produced is to verify that the personnel have control at each step (Snyder, 1991). Verification can be performed by plant audits with the use of microbial, physical and chemical tests. Government agencies will sometimes review HACCP plans to ensure compliance with standards (Snyder, 1991). The frequency of such audits should be sufficient to verify that the HACCP program is functioning properly (Mayes, 1999). There is often some confusion about how validation differs from verification. Verification determines compliance with the HACCP plan, where validation merely determines that the end results can be achieved (Sperber, 1999).

Principle 7

The seventh principle of HACCP is to establish adequate record keeping procedures. Without records, there is no proof that a plant is doing what their HACCP plan indicates. According to (

Sohrab ,1999), the purpose of recording keeping is to show that the HACCP plan is compliant with the documented system. Records are useful in providing a basis for trends and for systematic improvement of the process over time (Snyder, 1991). All forms pertaining to monitoring results, corrective action logs, or training records must be kept on file for at least 1 year. Any modifications to, or audits of, the HACCP plan must be documented as well (Ababouch, 2000). USDA requires that the HACCP plan and records be filed together and be readily available when requested (King, 1992).

2.1.7 HACCP Assessment

Although HACCP assessment (auditing) can fall under verification, it is not one of the seven principles. Even though both regulators and processors have the same goal of producing safe products, their views differ on how effectiveness should be measured. The goals of a regulatory agency in terms of HACCP are to:

• Make the food supply safer through the prevention of food safety problems

• Enable regulatory agencies to more efficiently utilize their existing resources devoted to ensuring food safety.

• Enhance the ability of the regulatory agency to provide consumers with the assurance that the food supply is safe.

• Underscore the industry's role in continuous problem prevention and problem solving (Kvenberg *et.aI.*, 2000).

The main purpose of HACCP assessment is to establish whether a processor is capable of producing or distributing safe products consistently, i.e. ensuring that the HACCP program is effective in maintaining product safety (Anon, 2000). Assessment should include review of the HACCP manual and an on -site verification to establish whether the HACCP plan is properly implemented (Ababouch, 2000). According to (Mortimore, 2000), the outcome of any assessment should show that the manufacturer has:

1. Implemented a sound HACCP system.

2. The knowledge and experience needed to maintain it.

3. The necessary support (prerequisite) programs in place.

Check sheets can be used to make the assessment more effective. Check sheets have been proven to be an effective tool in assessing HACCP plans. However, check sheets alone will not suffice. It is important for the auditor to have adequate knowledge to identify any deficiencies and address them properly (Ababouch, 2000). It will be up to the discretion of the assessor on how to form their check sheets. Some may use a check sheet as an aide-memoire, but many separate questions must supplement the check sheet, since they are only a broad outline of criteria. There is no set formula for a HACCP plan; therefore check sheets will differ from plant to plant (Mortimore, 2000).

Assessments can be conducted either with an internal assessment team, or with outside consultants. An internal assessment should not be conducted by those individuals involved with the daily activities of the HACCP planes) (Lupin, 2000). One type of HACCP assessment is through the establishment of the effectiveness of in-house HACCP systems. Another assessment would include visiting the suppliers and ensuring their HACCP plan supplies safe incoming ingredients. Occasionally a third type of HACCP assessment may include customers' systems. This assessment will occur when the consumer is partly responsible for distribution of a product (Mortimore, 2000). The frequency at which HACCP assessments are conducted depends on the risk category of the food and the level of commitment from the management. The frequency will also depend on the reputation of the food processor (Ababouch, 2000). An assessment should be conducted any time there are changes to products or processes within a plant. It is a good idea to have audits scheduled throughout the year regardless of other factors that may arise (i.e. recalls, HACCP changes) (Anon, 2000). The current regulation requires at least a yearly audit, but this is a minimum requirement (Lupin, 2000).

2.1.8 Impact of HACCP on Food Safety

During the past decades, the quest for safety has been challenged by important changes in food production, such as innovations in manufacturing processes, reduced intervals between production and consumption, increased product shelf life, and increased prevalence of some microorganisms (Stevenson, 1990 ; Bauman, 1990) .As the food chain became global, FBDs are seen in a new dimension (Motarjemi and Käferstein, 1999) and now represent one of the

greatest health problems worldwide, affecting millions of people a year (Germano, 2003) and leading to significant economic and social consequences (Ruegg, 2003 ; Silva, 1999) .

Data from the World Health Organization show that, in 2005, 1.8 million people died of gastroenteritis caused by contaminated food and water (WHO, 2007). In spite of the technological progress in food production and control, the occurrence of these diseases has recently increased, even in developed countries (Franco and Landgraf, 2003). Food hazards or contamination may come from primary production, still on the farm, from inadequate handling or storage in the food industry, or from errors during preparation at home or in other places where the food is consumed. Although they have not recently become an issue, FBDs have become increasingly important lately, both in terms of magnitude and in terms of health consequences for the general population. Factors related to the supply chain, demographic situation, lifestyle, health system infrastructure, and the environmental conditions of each country influence the prevalence, increased frequency, and consequences of these diseases (Motarjemi and Käferstein, 1999).

When all these facts are taken into account, HACCP is an important tool in modern quality management in the food industry, ensuring the integrity of the product, preventing FBDs, and protecting the health of the consumer (Mortimore and Wallace, 1998). However, HACCP will only become effective when its principles are correctly and broadly applied in all stages of the food production chain. Some of the reasons for the recent increase in FBD frequency all over the world may be failures in implementation or limited application of HACCP, mainly in small companies; lack of knowledge of the final consumer, keeping inadequate food handling practices alive; and low rates of HACCP adoption in developing countries, where most of the FBD outbreaks occur.

2.2. Food safety

Assurance that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use (Codex, 1969).

Food safety is a scientific discipline describing handling, preparation, and storage of food in ways that prevent foodborne illness. This includes a number of routines that should be followed to avoid potentially severe health hazards. In this way Food Safety often overlaps with Food

Defense to prevent harm to consumers. The tracks within this line of thought are safety between industry and the market and then between the market and the consumer (Wikipedia, 2015). In considering industry to market practices, food safety considerations include the origins of food including the practices relating to food labeling, food hygiene, food additives and pesticide residues, as well as policies on biotechnology and food and guidelines for the management of governmental import and export inspection and certification systems for foods. In considering market to consumer practices, the usual thought is that food ought to be safe in the market and the concern is safe delivery and preparation of the food for the consumer. Food can transmit disease from person to person as well as serve as a growth medium for bacteria that can cause food poisoning (Wikipedia, 2015).

2.2.1 Foodborne Illness

The Centers for Disease Control (CDC, 2011) estimates that 47.8 million people get sick annually from foodborne disease, 127,839 are hospitalized and 3,037 die as a result of contracting the illness. While this is a reduction in estimated number of deaths related to foodborne illness in comparison with the estimate of 5000 deaths in 1999 (Scallan *et al.*,2011), the causes of foodborne illness are not always clear. For those cases that can be traced to their origins, many of the cases of advanced foodborne illness and death are traceable to an outbreak either from the consumption of processed food, restaurant food (Hedberg *et al.*,2006) or in home contamination of food, while several others are caused by new or emerging pathogens (Todd, 2004). Additionally, it is estimated that for each reported case of illness, there are 35 cases of foodborne illness that go unreported due to their shorter duration, lower level of severity, medical complications or insurance issues. The majority of these cases arise from contamination with *Salmonella*.spp., *E.coli*.spp., *Listeria monocytogenes, Norovirus, Toxoplasma, Campylobacter* and *Clostridium perfringens*. A recent report on foodborne illness acquired within U.S. borders (Scallan *et al.,* 2011) indicates that of the 36.4 Million cases of illness acquired in the nation (Scallan *et al.*, 2011), 9.4 Million (25%)were foodborne.

2.2.2. Economic Cost of Foodborne Illness

The cost of foodborne illness was estimated at $1,626 per case on average, which equates to an aggregate annual cost of $77.7 billion (Scharff, 2012). The total cost of foodborne illness, in fact,

is composed of health-related costs, loss of productivity that is captured with this enhanced model that accounts for pain, suffering and functional disability in addition to the cost of illness, medical costs and productivity losses. Additionally, societal and business costs may also be considered as lawsuits, insurance costs, outbreak investigations, laboratory and analytical costs and food waste from recalls and regulatory action amount for significant losses that are attributable to foodborne illness, yet not directly borne by the ill individual. The cost to the company responsible for propagating foodborne illness is often too crippling to survive through, particularly for small businesses. Between1988 and 1997, 55 plaintiffs afflicted with a case of foodborne illness were paid $7,330,412,reflecting a 31% success rate from the perspective of the plaintiffs (Buzby *et al.,* 2001).

2.3. Microbiological Criteria to Ensure Safe Food

According to the National Research Council (NRC, 2003), different forms of microbiological criteria exist: microbiological standards, microbiological guidelines and microbiological specifications. Microbiological standards are determined by government agencies and are therefore part of administrative regulations. These standards are mandatory and any products in non-compliance are subject to rejection, reprocessing or destruction as enforced by the regulatory agency. Microbiological guidelines are advisory criteria and used to monitor production processes. One example is the control of critical control points in HACCP. The ultimate assessment and approval of products underlies the judgment of personnel or management as these guidelines can be set and controlled internally. Microbiological specifications are either advisory or mandatory. These are established as purchase requirements for finished products or raw material. Hence, strictly speaking, government intervention concentrates on the setting of microbiological standards. (Unnevehr, 2003) promotes the use of a microbiological standard and finds, for the U.S. that the requirement for meat and poultry processing plants to meet microbial pathogen standards lowers the probability of subsequent contamination and, consequently, food-borne illness.

According to (FAO, 2001), standards for microbiological criteria are designed to determine the acceptability of a food item based on the number of microorganisms or toxins per unit of volume, mass, food lot or area. Standards or criteria are established for reasons of outbreak investigation, routine testing, and verification of HACCP, indicator for shelf life or spoilage of

food (Todd, 2004). Setting thresholds has a long tradition in the economic analysis of environmental problems (i.e. thresholds or standards for the emission of pollutants) (Baumol and Oates, 1988).Standards can be grouped into performance, design and mixed standards, where a performance standard prescribes a specific level of food safety and the design standard (specification standard) a specific procedure to follow without testing if a level of food safety has been achieved. HACCP is a food safety system that can entail both types of standards (Antle, 1999). notes that performance standards are more likely to be efficient than design standards when trying to achieve a specific level of food safety since a performance standard allows plant managers to adapt their quality control to the structure of their plant (Antle, 1999).

2.3.1. *Salmonella*

2.3.1.1. *Salmonella* as a foodborne hazard

Salmonella may be found in all varieties of food production (Koohmaraie *et al*., 2005). The best way to control and eliminate pathogens is to understand their sources and prevalence in the environment. Animal-derived foods have been condemned as the greatest source of human *Salmonella* infections, but more extensive charting, better follow-up and stricter identification are needed to trace the true sources of Salmonellosis (Lindqvist *et al.*, 1999).

2.3.1.2. Characteristics of *Salmonella*.spp.

Since *Salmonella* spp. were first discovered in human tissues in 1880, and then isolated from pigs in 1885 by Salmon (Buxton and Fraser, 1977), their significance as important pathogens has been recognized. To date, more than 2,500 *Salmonella* serovars have been identified (Popoff, 2004), approximately 2, 000 of which are capable of infecting humans. Their host specificity may vary even between variants within a serovar (Wall *et al.*, 1995; Rabsch *et al*. 2002). Epidemiologically, *Salmonella* bacteria can be divided into human-specific serovars, serovars with host adaptation but also able to infect humans, and serovars able to infect both humans and animals (Jay *et al*., 2005). *Salmonella* is a Gram-negative, rod-shaped belonging to the family Enterobacteriaceae, (Marlony *et al*., 2003).

Salmonella are facultative anaerobes, flagellated rod-shaped bacteria with both respiratory and fermentative metabolic pathways. They are oxidase negative, ferment glucose and produce acid

and gas. The organisms grow on citrate as a sole source of energy. They decarboxylate lysine and ornithine, generally produce hydrogen sulfide, and do not hydrolyse urea. One of the characteristics of this genus is that most members do not ferment lactose or sucrose (Yousef and Calstrom, 2003).

2.3.1.3 Nomenclature

Most of the *Salmonella* serotypes cannot be differentiated biochemically (Yousef and Calstrom, 2003). The Kauffmann-White scheme for classifying *Salmonella* assigns a species status to each serotype. Therefore, differentiation between *Salmonella* ser. *typhimurium* and *Salmonella* ser. *enteritidis*, for example, is based on serotyping. Nucleotide sequence relatedness and other molecular methods showed that typical *Salmonella* were 85%-100% related (Yan *et al.,* 2003). Numerical taxonomy supports their similarity (Yousef and Calstrom, 2003). Hence, two species of *Salmonella* are currently recognized which are *Salmonella enterica* and *Salmonella bongori*. According to this scheme, the vast majority of the serotypes are under *Salmonella enterica*, with only 20 serovars belonging to *Salmonella bongori*. The species *Salmonella enterica* is comprised of six subgroups: *Salmonella enterica* subsp. *enterica*, *Salmonella enterica* subsp. *salamae*, *Salmonella enterica* subsp. *arizonae*, *Salmonella enterica* subsp. *diarizonae*, *Salmonella enterica* subsp. *houtenae* and *Salmonella enterica* subsp. *indica*. *Salmonella enterica* subsp. *enterica* is usually isolated from humans and warm-blooded animals and the majority of the serotypes isolated in clinical laboratories belong to this subspecies which includes the pathogens associated with typhoid fever (Kim *et al.,* 2006; Herrera- Leon *et al.,* 2007). The other sub species and *Salmonella bongori* are usually isolated from either the environment or reptiles and therefore are not clinically important (Kim *et al.,* 2006).

2.3.1.4. Serogroups and Serotypes

Salmonella are classified into different serogroups and further into different serotypes according to the Kauffmann-White scheme. Under this serotyping scheme, numbers and letters are assigned to the different O (somatic) and H (flagellar) antigens. Typing the O antigen determines the serogroup and typing the H antigen defines the serotype. This scheme recognizes 46 O serogroups and 114 H antigens that, in various combinations, characterized 2523 serotypes (Popoff *et al.,* 2003). For consistency in the scheme, all serogroups were given a number

designation; however, the most common serogroups (A to E) are commonly designated by letters (Fitzgerald *et al.,* 2007). At least three antibody-antigen reactions are required to identify a particular *Salmonella* serotype, and the less common serovars often requires further tests for correct characterization (Kim *et al,* 2006). Numerous O-grouping antisera along with control antigens are needed to identify the *Salmonella* into serogroups or serotypes. In addition, antiserum to detect the capsular or virulence (Vi) antigen is also required to screen for *Salmonella* serotypes especially *Salmonella* ser. typhi from group D. The O antigen is usually determined by slide agglutination test with group-specific antiserum followed by agglutination with factor antiserum. Growth from non-selective agar or Kliger's iron agar can be used for the determination of O antigen. Strains of *Salmonella* ser. typhi and *Salmonella* ser. paratype C may possess Vi antigen that make the strains non agglutinable in O antisera. These cultures agglutinate in Vi antiserum. They will agglutinate in O antiserum, however, after destruction of the Vi antigen by boiling the culture for 10 minutes. The specific O antigen is confirmed by slide agglutination with factor antiserum. H antigen is usually determined by tube agglutination test. The organisms should be motile and from a liquid culture. The motility of weakly motile organisms can be enhanced by repeated passage in liquid cultures. Determination of the O antigen and the phase 1 H antigen only is usually sufficient for the identification of typhoid fever and paratyphoid fever organisms (WHO, 2003).

In *Salmonella*, the major surface structure is O antigen which is part of the lipopolysaccharide (LPS). The LPS consists of lipid A and core oligosaccharide domain in addition of the O-specific polysaccharide chain (O antigen). Both lipid which serves as the anchor of the entire LPS molecule in the outer membrane and the core oligosaccharides do not vary greatly within a genus and are thought to be invariable within *Salmonella*. On the other hand, O-antigen is extremely polymorphic. It is a polymer with repeating units of three to six sugars in *Salmonella* serogroups A to E. The basis of the variation in O antigen structure is represented by the different types of sugar present (sugar composition) or the arrangement of sugars (linkages between sugar and O units oligosaccharide unit), the addition of branch sugars and modifying side groups and such variation or diversity is used to serotype *Salmonella* isolates (Wyk and Reeves, 1989, Luk*et al*., 1993; Fitzgerald *et al.,* 2003). The Vi antigen was discovered in the 1930s by Felix and Pitt. The virulence capsular polysaccharide (Vi antigen), a homo polymer of Acetyl galactosamineuronic acid that forms a coat on the external surface of the bacterial cell is expressed by *Salmonella* ser.

typhi, *Salmonella* ser. paratyphi C and some of the strains of *Salmonella* ser. dublin, and a few strains of *Citrobacter freundii* (Selander *et al.*, 1992). Flagellar is a necessary organelle for bacterial motility. The flagellar filament is composed of polymerization of approximately 20,000 repeating flagellin proteins. Genes encoding flagellin are highly conserved at the 5' and 3' ends while the middle region is quite variable. The conserved regions encode the core of the flagellar filament and are critical for the assembly of the filament. The central region encodes the surface-exposed antigenically variable portion of the filament (Joys, 1985; Wei and Joys, 1985; Kanto, 1991; Li *et al.*, 1994; Kholodii *et al.*, 2002).

2.3.1.5. *Salmonella* Genome

Salmonella contains over 2600 known lineages, each with distinct biological characteristics, including differences in the niche in which they dwell and the nature of diseases they may cause in their hosts. Genomic sequence analysis is beginning to reveal the genetic basis that determines the phenotypic differences among them. Comparison of eight sequenced genomes of *Salmonella* subgroup I lineages, which infect warm-blooded animals including humans, demonstrates that these pathogens share about 90% of their genes (the "core" genome), with the remaining ca. 10% genes being unique to each of the lineages (the "accessory" genome). Prophages and *Salmonella* Pathogenicity Islands (SPIs) are the main components of the accessory genome. Insertion of large DNA segments, such as SPI7 in *Salmonella* typhi, may disrupt physical balance of the genome between replication origin and terminus and rearrangements of the genome, such as inversions or translocations mediated by homologous sites (rrn operons, prophages, IS200, etc.) may accelerate rebalancing of the genome. Laterally transferred genes are the main driving force in *Salmonella* evolution and speciation; evidence exists indicating that mismatch repair genes may spontaneously regulate bacterial mutability through allele conversion to facilitate or inhibit incorporation of foreign DNA. Further studies may help elucidate the genetic basis of distinct pathogeneses and host ranges among the *Salmonella* pathogens (Steffen, 2011).

2.3.1.6. Human Salmonellosis

Diseases caused most frequently by *Salmonella enterica* are collectively known as salmonellosis (Yousef and Calstrom, 2003).Humans are particularly vulnerable to *S. Typhi* and *S. Paratyphi* A and B, infections, due to the ability of these strains to invade and multiply within host tissues.

Human Salmonellosis comprises several clinical syndromes including enteric (typhoid) fever, local isedenterocolitis and systemic infections by non typhoid microorganisms. Clinical manifestations of enteric fever appear after a period of incubation ranging from 7 to 28 days and may include diarrhoea, prolonged and intermittent fever, abdominal pain and headaches (Mølbak *et al.*, 2002).

Salmonellosis is one of the most common and widely distributed foodborne diseases. It constitutes a major public health burden and represents a significant cost in many countries. Millions of human cases are reported worldwide every year and the disease results in thousands of deaths. Salmonellosis is caused by the bacteria *Salmonella*. Today, there are over 2500 known types, or serotypes, of *Salmonella* (Yousef and Calstrom, 2003). Salmonellosis in humans is generally contracted through the consumption of contaminated food of animal origin (mainly meat, poultry, eggs and milk), although many other foods, including green vegetables contaminated from manure, have been implicated in its transmission. The causative organisms pass through the food chain from primary production to households or food-service establishments and institutions. A total of 2 501 different *Salmonella* serotypes have been identified up to 2004 (Yousef and Calstrom, 2003).While all serotypes can cause disease in humans, they are often classified according to their adaptation to animal hosts. A few serotypes have a limited host-spectrum (affect only one or a few animal species), for example *Salmonella* typhi in primates; *Salmonella dublin* in cattle; and *Salmonella choleraesuis* in pigs. When these strains cause disease in humans, it is often invasive and can be life-threatening. Most serotypes, however, have a broad host-spectrum. Typically, such strains cause gastroenteritis, which is often uncomplicated and does not need treatment, but can be severe in the young, the elderly and patients with weakened immunity (WHO, 2005). This group features *Salmonella enteriditis* and *Salmonella typhimurium*, the two most important serotypes for Salmonellosis transmitted from animals to humans (WHO, 2005). The diseases are enteric fever, gastroenteritis and extra intestinal infection which include bacteremia or septicemia. There are different syndromes of human Salmonellosis. *Salmonella* ser. *typhi* and ser. *paratyphi* cause enteric fever strictly in humans. Typhoid fever accounts for < 5% of cases of Salmonellosis (Yousef and Calstrom, 2003).

2.3.1.7. Foodborne outbreaks caused by *Salmonella*

The majority of pathogens causing foodborne illnesses are considered to be zoonotic (Käferstein and Abdussalam, 1999). The increase in international trade in agricultural, aqua cultural and manufactured food products has facilitated the spread of *Salmonella* (D'Aoust, 1994). *Salmonella* has been the subject of public health concern as an agent causing foodborne diseases for over a century (Hardy, 2004). *Salmonella* has been estimated to be responsible for 30% of the foodborne outbreaks in the United States (Mead *et al.,* 1999), where it was reported to cause approximately one quarter of the hospitalisations and almost half of the deaths among persons with laboratory on firmed infection (CDC, 2004). In Great Britain, *Salmonella* is considered one of the most important pathogens that should be tackled when reducing the number of foodborne cases (Adak *et al.*, 2002). Due to the complexity of its environmental association, *Salmonella* represents a continuing problem for public health (Hardy, 2004) and is still considered one of the most important foodborne pathogens (Humphrey, 2004). Control of foodborne diseases has been emphasized to be in need of a concerted effort on the part of the governments, the food industry and consumers (WHO, 2002).

Salmonella has also been reported as the leading zoonotic disease in humans in the EU (EFSA, 2006). Reporting of foodborne outbreaks has been mandatory for all EU member states since2005. According to EU legislation, an outbreak is an incidence of at least two human cases of the same disease and/or infection, or a situation in which the observed number of human cases exceeds the expected number, and where the cases are (probably) linked to the same food source. The burden of Salmonellosis has been estimated to vary from 4 to 2,741/100,000 regionally in Europe (de Jong, 2006). Most of the human cases in Finland, Sweden, Norway and Austria were reported to have been acquired from abroad (EFSA, 2006). In 2006 there were a total of 160,649 confirmed human *Salmonella* cases, giving an average incidence of 34.6 cases per100, 000 of the population and ranging from zero to 235.9/100,000 in the 24 EU member states, representing a continuous decrease in *Salmonella* cases in the EU (EFSA, 2007). The highest incidences have generally been detected in the age groups 0-4 and 5-14 years. *Salmonella* has also been one of the main reasons for foodborne outbreaks over the years. In 2006 it was the causative agent of 53.9% of all reported outbreaks, involving 22,705 persons, of which 14.0% were hospitalised and 0.1% died. An average European *Salmonella* foodborne outbreak caused 7 human cases in

2006. *S. Enteritidis* accounted for 55.2% of all cases of a specified *Salmonella* serovar, *S.Typhimurium* (4.1%) being the second most frequent. However, the *Salmonella* incidence has decreased, and in 2005 it was about 10% lower, with 24% fewer *Salmonella* -originated outbreaks than in the previous year. Eggs and egg products are regarded as the most frequently implicated sources among the foodborne cases of human Salmonellosis in Europe, followed in order by poultry, pork, beef and mutton (EFSA, 2008).

2.3.1.8. Isolation of *Salmonella*

Isolation and identification of strains involved is an important step in controlling *Salmonella* outbreaks or sporadic clinical cases. Numerous typing schemes have been used to identify *Salmonella* species, including biochemical and serological identification; the latter differentiate *Salmonella* into serovars. Further identification by phenotypic characteristics has also been used both independently and in combination for subdividing serovars. These include phage typing, antibiotic resistance patterns, colic in typing and plasmid characterization. These methods are usually supplemented by genotypic characterization such as plasmid finger print and chromosomal analysis (Threlfall and Forst, 1990; Gonzalez and Mendoza, 1995). Phenotypic and genotypic characterization can provide information on the strain implicated demonstrate an epiderniological link between cases and associate cases with a potential source. Furthermore, these typing schemes can also be used as diagnostic tools and for the assessment of the pathogenic properties of *Salmonella* (Gonzalez and Mendoza 1995; Poppe *et al.*, 1993).Clinical samples are typically cultured directly onto selective agar media, such as Xylose-Lysine-Desoxycholate (XLD) agar, and incubated at 37°C for 18-24 hours. In addition, stool samples are usually inoculated into a selective enrichment broth, such as selenite cystine broth and incubated at 37°C for 18-24 hours, before plating out onto selective agars. There is a current ISO horizontal method, (the International Organization for Standardization, 6579: 2002, for the detection of *Salmonella* spp. in food and animal feed). The method was amended in 2007 to include testing of animal feces and environmental samples from primary production. Similar standard methods have been published elsewhere by other bodies, notably in the United States Food and Drug Administration (USFDA) Bacteriological Analytical Manual (BAM). The first stage in traditional detection methods for most food samples is usually a pre-enrichment culture in a non-selective liquid medium such as buffered peptone water, incubated at 37°C for 18 hours.

Modified pre-enrichment methods may be necessary for samples containing inhibitory compounds. The pre-enrichment culture is then typically sub cultured into two different selective enrichment media, such as Rappaport Vasiliadis Soy broth (RVS) and Muller-Kauffmann Tetrathionate-Novobiocin (MKTTn) broth, and incubated for a further 24 hours at 41.5°C (RVS) or 37°C (MKTTn). The selective enrichment culture is usually inoculated on to at least two selective agar media and incubated at 37°C for 24 hours. The ISO method specifies the XLD agar and one optional selective medium. A variety of alternatives are available, including Bismuth Sulphite agar, Brilliant Green agar and Hektoen Enteric agar. A number of selective chromogenic agar media specifically designed for the differentiation of *Salmonella* colonies are commercially available. Typical *Salmonella* colonies on selective agar are sub cultured onto non-selective media prior to confirmatory testing. (www.rapidmicrobiology.com).

2.3.2 *Escherichia coli*

E. coli is a gram-negative, facultative anaerobic, non-spore-forming rod, which belongs to the Enterbacteriaceae family. The odor *Escherichia* first cultured 'Bacterium coli' in 1885 from the feces of a healthy individual. It was renamed *Escherichia coli* in 1919 in a revision of bacteriological nomenclature (Law, 2000). Many benefits have been found from *E. coli* in human medicine, food industry, and the water industry. Some studies suggest that *E. coli* can serve as a benefit to the human body by synthesizing vitamin K and by using competitive inhibition to out compete other bacteria that might enter the intestinal tract. Differences between strains of *E. coli* lie in the combination of different antigens they possess. There are three types of antigens: the somatic lipopolysaccharideantigen (O), the flagellar antigens (H), and the capsular antigens (K). There areapproximately 174 O antigens, 56 H antigens, and 103 antigens that have beenidentified. There are several stains of *E. coli* that have been isolated. The enteric *E. coli* are divided on the basis of virulence properties into enterotoxigenic (ETEC),enteropathogenic (EPEC), enteroinvasive (EIEC), verotoxigenic (VTEC), enterohemorrhagic (EHEC), and enteroaggregative (EaggEC). ETEC can be found in humans, pigs, sheep, goats, cattle, dogs, and horses; EPEC is found in humans,rabbits, dogs, cats, and horses; EIEC and EAgg EC are only found in humans; VTEC is found in pigs, cattle, dogs, and cats; while EHEC is found in humans, cattle, and goats and attack porcine strains that colonize the gut in a manner similar to human EPEC strains (Fratamico et *al*., 2002).*E. coli* grows optimally at 37°C with a generation time of

approximately 20 min in rich media (Meng and Schroeder 2007). It is catalase positive and oxidase negative (Flatamico and Smith2006; Meng and Schroeder 2007). *E. coli* strains do not grow under refrigeration conditions but can survive in temperatures of 4 or -20 °C for weeks, water activity (aw) of at least 0.95, and in NaCl concentration of 8.5% (Flatamico and Smith, 2006).

2.3.2.1 Pathogenic *Escherichia coli*

Pathogenic *Escherichia* coli, specifically *E. coli* O157: H 7 has emerged as a foodborne pathogen of great concern in beef products. While generic *E. coli* is considered a part of the normal microflora in the intestinal tract of most warm-blooded animals, including humans, many pathogenic strains can cause diarrheal disease and have been associated with food-borne illness (Doyle, 1990). These pathogenic strains have been classified into four subgroups.

Enteropathogenic *E .coli* (EPEC) historically have been linked to outbreaks of infantile diarrhea (Doyle, 1990; Reed, 1994). These outbreaks typically occur in hospital nurseries, especially those in developing countries, where the importance of sanitation and hygiene is not clearly understood. Symptoms of EPEC infections include fever, malaise, vomiting and diarrhea (Levine, 1987). The pathogenic mechanisms for this subgroup of *E. coli* are thought to be attachment and effacing adherence to the intestinal tract, rather than toxin production (Doyle, 1990; Levine, 1987).

Enteroinvasive *E. coli* (EIEC) produces symptoms similar to *Shigella* dysenteriae, including fever, abdominal cramps, malaise, watery diarrhea, and toxemia (Doyle, 1990; Levine, 1987; Reed, 1994). These organisms also resemble *Shigella*, in that they are non-motile and unable to ferment lactose. Serologically, EIEC and *Shigella* often produce cross reactions (Levine, 1987). Pathogenicity of EIEC is caused primarily by invasion of the epithelial tissue of the colon, which leads to inflammation and ulceration of the mucosa as the bacteria replicate (Doyle, 1990; Kornacki and Marth, 1982). Most outbreaks are thought to be caused by person-to-person transmission and are more common in underdeveloped countries (Doyle, 1990; Levine, 1987).

Enterotoxigenic *E. coli* (ETEC) are commonly associated with a disease known as traveler's diarrhea, which frequently strikes individuals traveling from areas of good hygiene to areas of poor hygiene (Doyle and Padhye, 1989; Kornacki and Marth, 1982; Reed, 1994). In tropical

areas and developing countries, ETEC may be a cause of diarrhea in all age groups, particularly infants and children (Doyle, 1990; Levine, 1987; Reed, 1994). While man is believed to be the primary reservoir for ETEC, many outbreaks have been foodborne, presumably due to poor sanitation practices. Enterotoxigenic *E. coli* cause disease by penetrating the mucosal layer of the small intestine, after surviving the harsh stomach environment. After adherence, the bacteria produce heat-labile (LT) enterotoxins and/or heat-stable (ST) enterotoxins (Doyle, 1990; Kornacki and Marth, 1982; Levine, 1987; Reed, 1994). Symptoms of ETEC infection include watery diarrhea, nausea, abdominal cramping, and fever (Levine, 1987).

Enterohemorrhagic *E .coli* (EHEC) contain the most virulent strains of pathogenic *E. coli*, including *E. coli O157:H7*. These strains are associated with hemorrhagic colitis (HC), a clinical syndrome characterized by abdominal cramps and watery diarrhea, followed by a hemorrhagic discharge resembling gastrointestinal bleeding (Dorn, 1993). This discharge eventually leads to edema, erosion, and hemorrhage of the mucous layer of the intestinal tract (Riley, 1987). Complications associated with HC may result in a condition known as hemolytic uremic syndrome (HUS), in which kidney tissue is severely damaged, often resulting in microangiophatic hemolytic anemia, thrombocytopenia, and acute renal failure, sometimes requiring dialysis (Dorn, 1993). A further complication stemming from EHEC infections is thrombotic thrombocytopenic purpura (TTP), wherein the central nervous system becomes involved, resulting in fever, neurological abnormalities, seizures and prolonged coma (Doyle and Padhye, 1989).

Although over 100 *E. coli* serotypes have been classified as EHEC, serotype O157:H7 has received the most widespread attention, since it appears to be the most virulent and most common cause of major outbreaks. This particular organism can be differentiated from other *E. coli* in that it is unable to ferment sorbitol rapidly (March and Ratnam, 1986) and does not produce ß-glucuronidase (Thompson *et al.*, 1990). While approximately 95% of E. *coli* has an IMViC pattern of ++--, *E. coli* O157:H7 belongs to the 5 % that produces a -+-- pattern (Doyle and Padhye, 1989). Most strains of O157:H7 are susceptible to a variety of antibiotics, including ampicillin, trimethoprim-sulfamethoxazole, tetracycline, and quinolones. It has however, been found to be resistant to erythromycin, metronidazole, and vancomycin (Ratnam *et al.*, 1988; Pai *et al.*, 1984).

2.3.2.2 General Method of Isolation and enumeration for *E. coli*

Standardized methods (e.g., ISO methods) are usually considered the reference analytical methods for official controls. In most cases, they are traditional culture methods that use selective liquid or solid culture media, to grow, isolate, and enumerate the target microorganism and simultaneously prevent the growth of other microorganisms present in the food (Jasson *et al.*, 2010). Enumeration of the microorganisms present in a sample is normally performed by plate count method or the most probable number (MPN) method. The plate count method is based on culturing dilutions of sample suspensions in the interior or on the surface of an agar layer in a Petri dish. Individual microorganisms or small groups of microorganisms will grow to form individual colonies that can be counted visually. The MPN method calculates the number of viable micro organisms in a sample by preparing decimal dilutions of the sample, and transferring subsamples of 3 serial dilutions to 9 or 15 tubes containing liquid culture medium, to carry out the method on 3 or 5 tubes, respectively. The tubes are incubated, and those that show growth (turbidity) are counted. Taking into account the dilution factor, the final result is compared to a standard MPN table, which will indicate the MPN of bacteria in the product (Blodgett, 2010).

2.3.3. Aerobic plate count

The aerobic plate count is designed to provide an estimate of the total number of aerobic organisms in a particular food. A series of dilutions of the food homogenate is mixed with an agar medium and incubated at 35°C for 48 hr. It is assumed that each visible colony is the result of multiplications of a single cell on the surface of the agar (Andrews, 1992).The total aerobic plate count is useful for indicating the overall microbiological quality of a product and, thus, is useful for indicating potential spoilage in perishable products. The aerobic plate count is also useful for indicating the sanitary conditions under which the food was produced and/or processed (Andrews, 1992).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study design

3.1.1. Sampling plan and data collection

To conduct this research questionnaires method were used to collect data about food safety and implementation of HACCP system in Khartoum state. Furthermore, random samples were collected the finished product of food supply to the consumer for the detection of microbial content.

3.1.2. Study area

This study was carried out in Khartoum State. The laboratory work was done in the Microbiology Laboratory, Sudanese Standards and Metrology Organization.

3.1.3. Questionnaire

This survey was conducted from May 2015 to January 2018 involving fifty employees from food businesses in Khartoum City - Khartoum state- Sudan.

3.1. 3.1. Questionnaire Design

The design of survey questionnaire was inspired by the existing literature studying the process of HACCP implementation worldwide (Martin and Anderson, 2000; Colatore and Caswell, 2000; Mortlock *et al.*, 2000; Buchweitz and Salay, 2000; Henson and Holt, 2000).

The questionnaire consisted of a first set of 4 demographic questions (age, sex, education level and training received), followed by, 12 items related to general HACCP knowledge (Appendix 1). The questionnaire also contained food safety practices implemented in food businesses by 11 items (Appendix 2). Furthermore, the questionnaire also contained knowledge obstacles that hamper the implementation of the HACCP system according to the opinion of employee's by 13 items (Appendix 3).

3.1. 4. Target samples

Different samples of food products were collected from factories of foods in Khartoum State.

3.1.4.1. Inclusion criteria

Meat and meat products samples were included in this study.

3.1.4.2. Exclusion criteria

Freezed meat and food products were excluded from this study.

3. 2. Study duration

This study was conducted during the period from May 2015 to May 2018

3.3. Methods

3.3.1. Sample size and sampling technique

One hundred samples of food were collected from factories in Khartoum State. The samples were taken in accordance with the International Organization for Standardization as follows; food products were collected randomly by hands with sterile gloves with the aid of sterile scalpel and forceps. Each sample was put into a labeled sterile plastic bag. They were placed into sterile plastic containers in an ice box. All samples were transported to the laboratory within 2 hours of collection or were stored them at 2 ºC ± 2 °C for a maximum of 24 h. (ISO17604:2003/Amd.1: 2009 (E)).

3.3.2. Preparation of sample:

An amount of Twenty five gram from each sample was transferred to a sterile Stomacher bag. Then 225 ml buffer peptone water (BPW) (Appendix 4) was added to the bag initial dilution (10^{-1}). The contents were homogenized in the Stomacher apparatus for 30-60 seconds then make further decimal dilutions.

3.4. *Salmonella* isolation

Isolation and identification of *Salmonella* species were done according to the International Organization for Standardization (ISO 6579:2002/Amd.1:2007(E)).

3.4.1. Bacteriological culture media

All culture media and reagents were obtained from Oxoid and Mast, UK and prepared according to manufacturers' instructions (Appendices 4-17).

3.4.2. Preparation of pre-enrichment sampling cultures

An amount of Twenty five gram from each sample was transferred to a sterile Stomacher bag. Then 225 ml buffer peptone water (BPW) (Appendix 4) was added to the bag. The contents were homogenized in the Stomacher apparatus for 30-60 seconds, then sealed and incubated at 37 °C for 18-24 hours. Portions of 1.0 and 0.1 ml of BPW pre-enrichment culture were transferred to 10 ml of Muller-Kauffmann Tetrathionate-novobiocin (MKTTn) broth (Appendix 5), and 10 ml of Rappaport-Vassiliadis medium with soya (RVS broth) (Appendix 6), respectively. The MKTTn broth enrichment cultures were incubated at 37 °C for 24 h while the RVS broth enrichment cultures were incubated at 42 °C for 24h.

3.4.3. Selective plating

After incubation period, enrichment broth tubes were mixed by vortex mixer. A loopful from each tube was streaked on plates of Xylose Lysine Deoxycholate (XLD) Agar (Appendix 7) and bismuth sulfite (BS) agar (Appendix 8). Then the plates were incubated for 24 hours ± 2 hours at 37 °C. After that the plates were examined for *Salmonella*. Five colonies suspected were taken for confirmatory test. Selected colonies were streaked on the surface of pre-dried nutrient agar plates (Appendix 9) and incubated at 37°C ± 1°C for 24h ± 3h.

3.4.4. Confirmation Isolate

Pure cultures were used for biochemical and serological confirmation.

3.4.4.1. Biochemical tests

Isolated colonies were examined biochemically on:

a. Glucose lactose fermentation, gas and H_2S production tests

TSI agar (Appendix 10) slant surface was inoculated by streaking and the butt was stabbed with the suspected colony, and was incubated for 24 h at 37 °C. The changes in the medium were interpreted as follows: Typical *Salmonella* cultures showed alkaline (red) slants and acid (yellow) butts with gas formation (bubbles) and formation of hydrogen sulfide (blackening of the agar).

b. Urease test

Urea agar (Appendix 11) was inoculated with the suspected colony and was incubated for 24 h at 37 °C. If the reaction is positive, splitting of urea liberates ammonia changed the color of phenol red to rose-pink and later to deep cerise. The reaction was often apparent after 2h to 4 h.

C. Voges-Proskauer (VP) test

Three ml of the VP medium (Appendix 12) in test tube was inoculated with a loopful of the suspected colony and incubated at 37ºC ± 1ºC for 24 h ± 3h.

After incubation, two drops of the creatine solution, three drops of the ethanolic solution of 1-naphthol and two drops of the potassium hydroxide solution were added. The content of the tube were shaken well. The formation of a pink to bright red color within 15 min indicates a positive test.

d. Indole test

A tube containing 5 ml of the tryptone/tryptophan medium (Appendix 13) was inoculated with the suspected colony and incubated at 37 °C ± 1 °C for 24 h ± 3h. After incubation, 1 ml of the Kovacs reagent (Appendix 14) was added. Formation of a red ring indicates positive reaction; a yellow-brown ring indicates negative reaction.

e. L-lysine decarboxylase test

L-lysine decarboxylation medium was inoculated with the suspected colony and incubated at 37 °C ± 1 °C for 24 h ± 3h.Turbidity and purple color after incubation indicates positive test; a yellow color indicates negative test (Appendix 15).

3.4.4.2. Serological tests

The isolates were further confirmed by slide agglutination test using polyclonal O-, Vi- and H-antiserum specific for genus *Salmonella* ((procured from MAST, England).

I. Examination for O-antigens.

One drop of saline solution was placed onto a clean glass slide. Portion of the colony under test was picked by loop and dispersed in the drop to obtain a homogeneous and turbid suspension. One drop of the anti-O serum was added and the slide was gently rocked for 30s to 60s. A positive reaction was shown by formation of agglutination.

II. Examination for H-antigens

One drop of saline solution was placed onto a clean glass slide. Portion of the colony under test was picked by loop and dispersed in the drop to obtain a homogeneous and turbid suspension. One drop of the anti-H serum was added and the slide was gently rocked for 30s to 60s. A positive test was shown by formation of agglutination.

III. Examination for Vi-antigens

One drop of saline solution was placed onto a clean glass slide. Portion of the colony under test was picked by loop and dispersed in the drop to obtain a homogeneous and turbid suspension. One drop of the anti-Vi serum was added and the slide was gently rocked for 30s to 60s. A positive test was shown by formation of agglutination.

3.5. *E. coli* isolation and calculation

Isolation and identification of *E. coli* were done according to the International Organization for Standardization (ISO 16649-2:2001(E)).

By using of a sterile pipette, 1 ml of the tested sample was transferred to a sterile Petri dish. initial dilution (10^{-1}) inoculated in to two plates ,then repeated this procedure with the further decimal dilutions, after that poured into each Petri dish approximately 15 ml of the (TBX) medium(Appendix16), previously cooled at 44°C to 47°C in the water bath . Carefully the inoculums mixed with the medium and allowed the mixture to solidify. Inverted the inoculated dishes so that the bottom is uppermost and placed them in an incubator set at 44 °C for 18 h to 24 h.

3.5.1. Counting the colony-forming units

After the specified period of incubation counting the typical CFU of glucuronidase-positive *Escherichia coli.*

3.6. Calculation of Aerobic Plate Count (APC)

Calculation of APC was done according to the International Organization for Standardization (ISO 4833:2003(E)).

Two sterile Petri dishes transferred to each one by means of a sterile pipette 1 ml of the tested sample from initial suspension (10^{-1} dilutions). The same procedure was used for the another dilution 10^{2} then Poured 15 ml of the plate count agar(Appendix17) into each Petri dish and allowed to solidify and incubated at 30 °C ± 1 °C for 72 h ± 3 h.

3.6.1. Counting of colonies

Using the colony counting bacteria isolate were counted between 15 and 300 colonies per plate.

3.7 Data analysis

Data were analyzed using SPSS computer program to calculate, frequency, mean, chi square and correlation values were calculated.

CHAPTER FOUR

RESULTS

4.1. HACCP knowledge scores

The questionnaire was designed as the highest point of 60 for each participant. The lowest score were obtained from this survey was 1 and the highest was 60. According to this score design of the questionnaire were grouped the scores as "low knowledge", "middle knowledge", and "high knowledge" with the score ranges of 1–40, 41–50, and 51–60, respectively. Accordingly, (42%) of the respondents were seen to have low knowledge, 20% of the respondents had satisfactory high knowledge (table 4.1).

4.2. Demographic characteristics

A total of 50 employees were questionnaired these were male and female participants with the ratio of (58%) and (42%), respectively. Most participants were aged 21–40 and over 40 (88% and 12%, respectively).

Considering the participant's terms of their education level, high school, Graduate, Postgraduate studies, with the ratio of (2%), (86%), and 12%, respectively. The participants 48%were received training on food safety and (52%) did not. Participants' level of HACCP knowledge was compared with gender, age, level of education and the training they received on food safety. As a result of low HACCP knowledge scores, male employees were found to have (37.93%) lower than female workers (47.61%).

Low HACCP knowledge scores for the age group 41 and over (9.5%) was found to be lower than the scores of age groups 21–40 (90.5%).The findings of Low HACCP knowledge indicated that the employees who received food safety training had lower (47.6%) compared to untrained employees (52.4%)(table 4.2).

4.3 Food safety practices implemented in food businesses

Food safety practices implemented in food businesses, for taking and recording end-point temperature of all foods that only (76%) of employees always implemented. Sanitizer concentration usually was not checked in food businesses (14%). Employees of food businesses reported that did not developing procedures for storing food (30%), In addition they were taking and recording refrigerator/freezer units temperature (24%), personal hygiene (10%) and cleaning

and disinfection (12%). Some of the employees of food businesses (20%) did not send food samples or swabs (28%) to the laboratory for testing bacterial contamination (table 4.3).

4.4 Barriers identified employees to implementing food safety systems

Lacking of prerequisite (24%) or knowledge on HACCP (82%), or cost (72%) and lacking of time (46%). Also there was no support from authorities (80%). The employees (56%) had volume paper work, but they were needed simple guidelines (82%). While most of interviewers locking personnel training (92%) were the most common barriers in food businesses were recorded by the employers(table 4.4).

Table (4.1): HACCP knowledge scores (n=50)

Score groups	Score range	frequency	presents
Low knowledge	1 to 40	21	42%
Middle knowledge	41 to 50	19	38%
High knowledge	51 to 60	10	20%

Table (4.2): Demographic characteristics of the food business employees (n=50)

Characteristics	**frequency(n=50)**	**Presents (%)**
Male	29	28%
Female	21	42%
Age < 40	44	88%
Age > 40	6	12%
high school level	1	2%
Graduate	43	86%
Postgraduate studies	6	12%
received food safety training	24	47.6%
Untrained in food safety system	26	52.4%

Table (4.3): Food safety practices implemented in food businesses (n=50).

Food safety practices	**yes**		**No**	
	n	**%**	**n**	**%**
Take and record end-point temperatures of all cooked foods	38	76%	12	24%
Take and record temperature of food on the serving line	36	72%	14	28%
Check concentration of sanitizing solutions	43	86%	7	14%
Take and record food temperature upon receiving	38	76%	12	24%
All equipment and cutting boards are sanitized between uses	40	80%	10	20%
Take and record refrigerator/freezer units temperature	34	68%	16	32%
Developed food storage procedures	35	70%	15	30%
Developed personnel hygiene procedures	45	90%	5	10%
Developed cleaning and disinfestations procedures	44	88%	6	12%
Send food product samples to a laboratory for bacterial testing	40	80%	10	20%
Take swabs of food production equipment and counters to determine bacterial count	36	72%	14	28%

Table (4.4): Barriers identified to implementing food safety management systems (n=50).

Barriers	Yes		No	
	n	%	n	%
Lack of prerequisite programs	12	24%	38	76%
Lack of knowledge about HACCP	41	82%	9	18%
Cost	36	72%	14	18%
Time	23	46%	27	54%
Staff turn-over	30	60%	20	40%
Lack of management	25	50%	25	50%
Lack of physical conditions	31	62%	19	38%
Lack of employee motivation	33	66%	17	34%
Complicated terminology	12	24%	38	76%
Need for simple guidelines	41	82%	9	18%
Volume of paperwork	28	56%	22	44%
Lack of personnel training	46	92%	4	8%
Not enough support from the authorities	40	80%	10	20%

4.5 Microbiological Criteria

In the present study, a total of 100 samples of meat and meat products were collected from factories and analyzed. The samples included 20(20%) burger, 30 (30%), sausage, 25 (25%) kofta, 12 (12%) minced meat, and 13 (13%), different type of meat products (Figure 4.1).

Culture and different biochemical and serological tests were used to detect *Salmonella* spp, *Escherichia coli* and aerobic Plate count. The results showed that 4 (4%) of samples were positive for *Salmonella.* spp (Figure 4. 2). Also the results showed that 44 (44%) of samples were positive for *Escherichia coli* (Figure4. 3).The results obtained confirmed the existence of *Salmonella* spp. in all positive samples (Appendices 18 and 19). In addition to 71 (71%) of samples exceeded the limit 10^5 cfu/g of the Sudanese Standard for Aerobic Plate count test (Figure4. 4).

Different biochemical test were used for identification of *Salmonella* spp. These including glucose fermentation test, urease test, voges-proskauer test, indole test and 1-lysine decarboxylase test (Table 4.3).

From pure colonies 4 isolated organisms were confirmed by slide agglutination method against *Salmonella* O-, Vi- and H-antisera (Table 4.4).

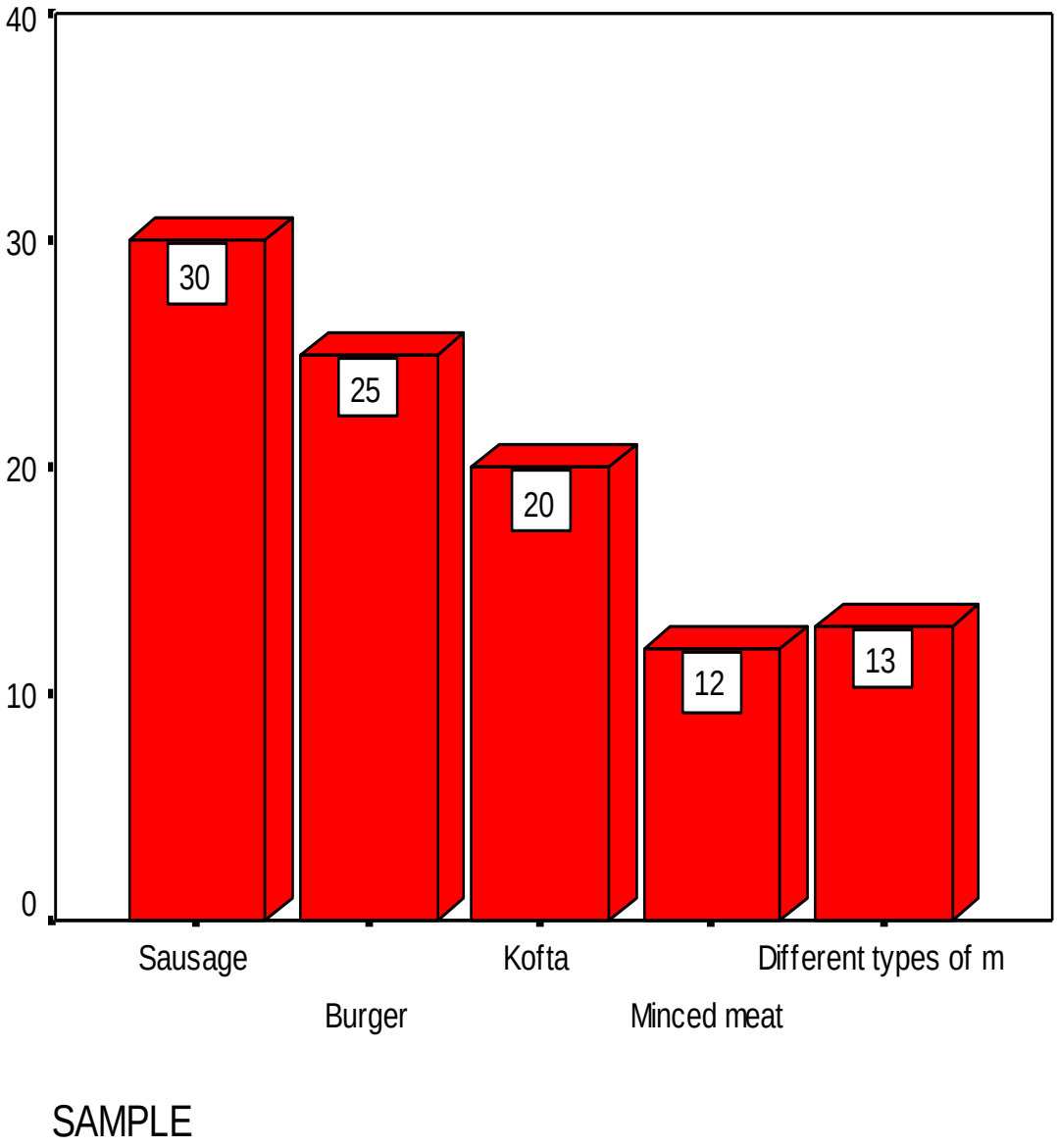


Figure 4.1 Type and number of meat product samples

Table4. 5 Biochemical reactions of *Salmonella* (n= 4)

Test	Result
Glucose fermentation	+
Urease test	–
Voges-Proskauer test	–
Indole test	–
1-Lysine decarboxylase test	+

Table 4.6 Serological reaction of *Salmonella* spp. (n= 4)

Test	Result
Polyvalent O Antisera	Agglutination (+)
Polyvalent H Antisera	Agglutination (+)
Polyvalent Vi Antisera	Agglutination (+)

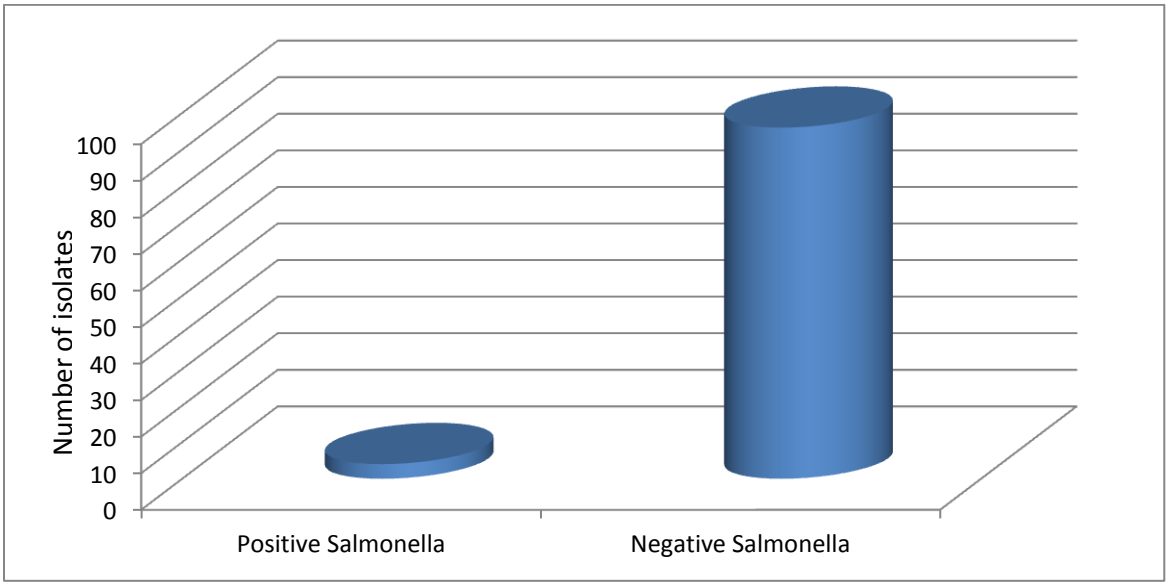


Figure 4.2. *Salmonella* isolated from meat samples

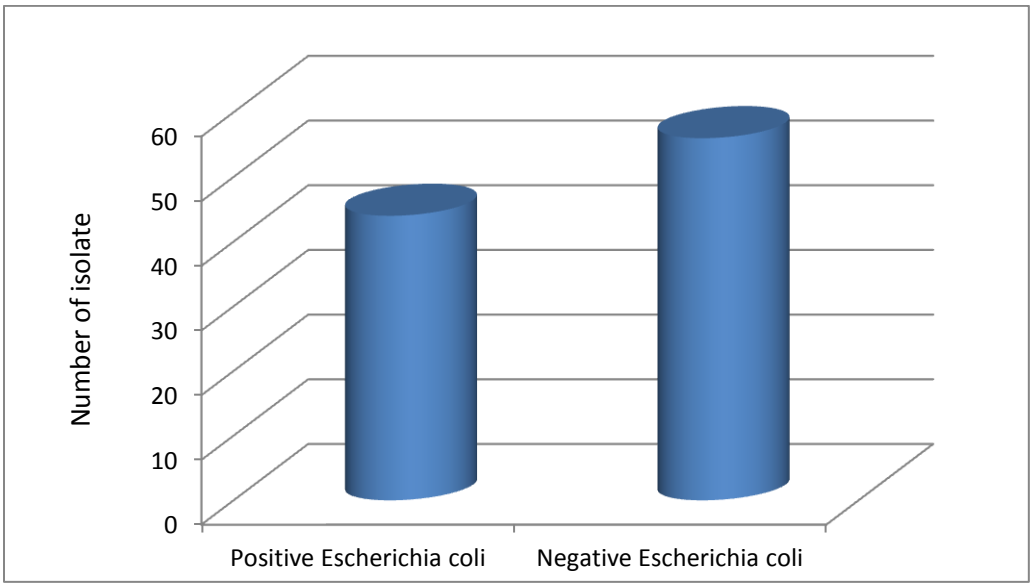
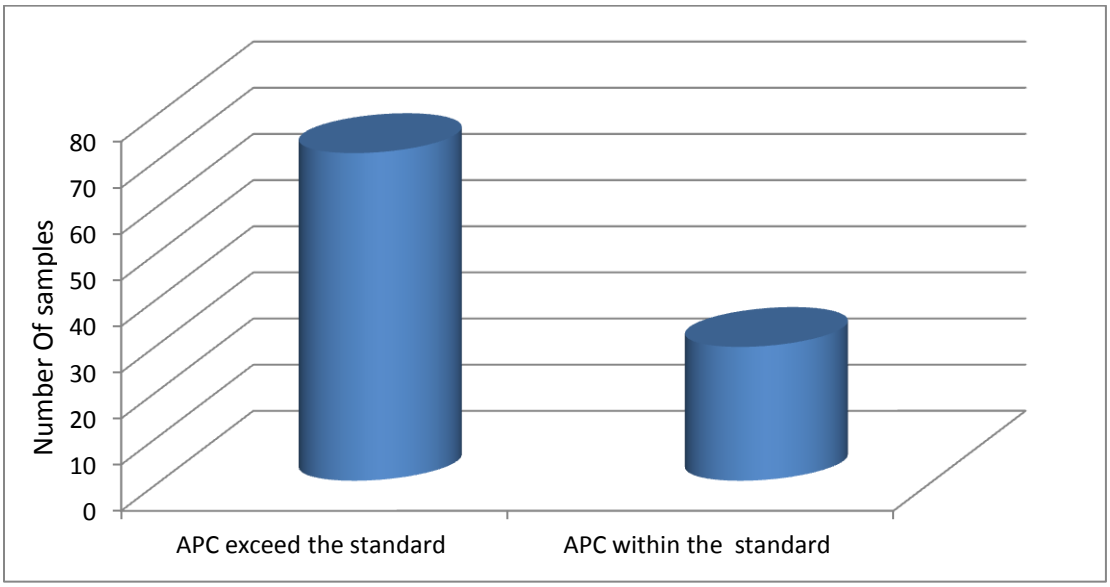


Figure4.3. the number of *Escherichia coli* isolated from meat samples



Fugger4.4. Aerobic plate count result from meat samples according to standard (10^5 cfu/g)

CHAPTER FIVE

DISCUSSION

5.1 Discussion

HACCP has become an international standard in food safety assurance and recommended or mandatory use of HACCP is found in the regulations of several countries, and governments, industries, and consumers are showing growing acceptance of the system (Fermam, 2007). The Richmond Report in 1990 recommended that food safety control in all UK food premises be based on the principles of HACCP.

The system can be considered as an efficient tool for both industry and health authorities to prevent foodborne diseases if it is based on understanding and proper implementation, because it is not HACCP system itself which makes food safe, but its correct application (Motarjemi and Kferstein, 1999).

In the present study, most participants were male 58% ,their age range was between 21-40 years, 86% of them Graduate, 48% Of the participants received training on food safety this results is in agreement with (Ulusoya and Çolakoğlu, 2013) who reported that most participants their age under 40years , graduated and received training on food safety system.

In this study the most of the interviewed employees did not implemented food safety practices in food businesses. Twenty-four percent of respondents not recorded end-point temperature of all foods. Sanitizer concentration usually was not checked in food businesses (14%). Employees of food businesses reported that did not developing procedures for storing food (30%). Some of the employees (20%) did not send food samples or swabs (28%) to the laboratory for testing bacterial contamination. The findings obtained from the study of Bas and co-workers (2006) indicated the food safety practices implemented in food businesses. Taking and recording end-point temperature of all foods was the practice that only 16.5% of food business Employees always implemented. In addition, only 29.6% of food business managers reported sending food product samples to a laboratory for bacterial testing. In a study, 55% of food employees received formal food hygiene training, and 63% of managers had under taken formal food hygiene training in UK food businesses (Walker *et al*., 2003).

The findings of our study indicated difficulties and barriers for the implementing of HACCP and food safety systems in food businesses in the Khartoum state. The main barrier was lack of

personnel training also Lack of knowledge on HACCP, lack of cost, lack of employee motivation, staff turnover ,lack of time ,lack of prerequisite , lack of management, complicated terminology, Lack of physical conditions . Lack support from the authorities, Volume of paperwork, need for simple guidelines.

Bas and co-workers (2007) conducted a survey on difficulties and barriers for the implementing of HACCP and food safety systems in food businesses in Turkey. Lack of knowledge on HACCP, lack of employee motivation, complicated terminology and lack of personnel training were the most common barriers in food businesses. In addition, Panisello and Quantick (2001) identified constant turnover of employees as a barrier to the proper implementation of the HACCP system, as employees need time and training in order to fully comprehend and use the system. Time and money were identified as the greatest barriers to improve food safety (Bas, 2007).

Several studies have examined barriers to HACCP implementation in food businesses. Hwang, Almanza, and Nelson (2001) found that Indiana school food service managers identified time to establish a HACCP program, time to run the program, and labor costs as being the three biggest obstacles. In addition, "lack of training funds, time to get used to running the HACCP program, and union problems" were other identified obstacles. (Giampaoli *et al*., 2002) conducted a national study and found three types of barriers: resource management, employee motivation, and employee confidence.

The purpose of microbial testing was to confirm that all possible avenues contamination have been identified and that these avenues are being controlled (Kvenberg and Schwalm, 2000). Meat samples were selected for this study because they are reported to frequently harbor various enteric organisms. The present study showed that the prevalence of *Salmonella* species in meat and meat products constituted 4%. These results were in line with the results of others who obtained a relatively contamination of meat and meat products with different serotypes of *Salmonella* (Campbell and Gilbert, 1995). *Salmonella.* spp. was found in minced meat, kofta and burger (Mohamed, K. 2013). The lowest percentage of *Salmonella* spp. In this study agree with finding of 4% by (Fatin *et al*., 2004) and disagreed with obtained by (Essa *et al*., 2009) which are 23.3%.

Escherichia coli and fecal coli forms are considered to be the most important and compulsory measure of microbiological quality of food and food related products in terms of hygiene. Their

presence is used as indicators of fecal pollution. Among these, *E. coli* is often preferred as a more specific indicator of fecal contamination because it is specific and most reliably reflects fecal origin (Feng and Hartman, 1982) and (Doyle and Erickson, 2006).Testing for generic *E. coli* is one method that is required in meat and poultry processing plants (Eisel *et al*., 1997).

In this study the over-all prevalence of *E. coli*. In all meat products was 44% which similar with some other studies. Ayla, E and Seza, A (2012) reported the results of microbiological analysis of retail meat samples relative to the contamination levels of *Escherichia coli* are 53.6%.

In Australia, *E. coli* was detected on 15.4% of meat and meat products samples.

In present study 71 (71%) of samples contaminated with aerobic plate count organisms exceed the limit allowed by the Authority of Sudanese standards and Metrology Organization 10^5 cfu/g. The higher aerobic count in meat indicated that sanitary measures during handling, manufacturing process, and packaging were neglected and also low quality of meat was used. The variations in total aerobic count in meat samples might be due to the contamination from equipment or the environment.

The total aerobic plate count is useful for indicating the overall microbiological quality of a product and, thus, is useful for indicating potential spoilage in perishable products. The aerobic plate count is also useful for indicating the sanitary conditions under which the food was produced and/or processed (Andrews, 1992).

Testing against microbiological criteria provides a way of measuring how well the operator has controlled the production processes to avoid and control contamination. The results of testing can be used to validate whether the operator's HACCP-based procedures are controlling food safety and food quality and verify they are being correctly applied.

5.2 Conclusion

The study concluded HACCP and food safety system Knowledge is low in Khartoum state and there is a high microbial contamination in meat products. We believe that there is a significant relationship between the knowledge of HACCP and the microbial content of the product while difficulties and barriers for the implementing of HACCP and food safety systems in food businesses in the Khartoum state Lack of knowledge on HACCP, needed for more checks by the authorities, need for simple guidelines and lack of personnel training were the most common barriers in food businesses were recorded by the employers.

5.3. Recommendations

1- Periodical training for HACCP applications must be provided by the government.
2- In order to increase the knowledge and awareness of HACCP, training has been found to be important.
3- To reduce microbiological risk, HACCP knowledge should be increased in food business.
4- Activate the role of government control and oblige food companies to implement an effective food safety system.
5- More researches should be done regarding the attitude and behavior situation in Khartoum state food business.

CHAPTER FIVE

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APPENDICES

Appendix (1)

Items regarding general HACCP knowledge	**Yes**	**No**
The principle of HACCP system is preventing the hazards in the stages before the endpoint of the production.		
According to prerequisite programs it is enough to wash the hands only before starting the work		
HACCP is an obligatory system that all food related plants should apply.		
HACCP is not a very effective system to provide food safety.		
HACCP is a mandatory system in Turkey's food law.		
Each hazard that may reflect to end product should be identified and recorded according to HACCP principles.		
HACCP is a food safety law specific to our country.		
Prerequisite programs that include all hygiene rules must be fulfilled prior to the implementation of the HACCP system.		
The HACCP system requires staff training in Hygiene.		
Prerequisite programs are accepted as infrastructure of any food business.		
Microbiological hazards cannot be included in HACCP.		
It is essential to keep track of and to record every step of food production in HACCP system		

Appendix (2)

Food safety practices	**Yes**	**No**
Take and record end-point temperatures of all cooked foods		
Take and record temperature of food on the serving line		
Check concentration of sanitizing solutions		
Take and record food temperature upon receiving		
All equipment and cutting boards are sanitized between uses		
Take and record refrigerator/freezer units temperature		
Developed food storage procedures		
Developed personnel hygiene procedures		
Developed cleaning and disinfectation procedures		
Send food product samples to a laboratory for bacterial testing		
Take swabs of food production equipment and counters to determine bacterial count		

Appendix (3)

Barriers identified by employee to implementing food safety management systems.	**Yes**	**No**
Lack of prerequisite programs		
Lack of knowledge about HACCP		
Cost		
Time		
Staff turn-over		
Lack of management		
Lack of physical conditions		
Lack of employee motivation		
Complicated terminology		
Need for simple guidelines		
Volume of paperwork		
Lack of personnel training		
Not enough support from the authorities		

Appendix (4)

Buffered Peptone water

Composition

Enzymatic digest of casein	10.0 g
Sodium chloride	5.0 g
Disodium hydrogen phosphate dodecahydrate	
($Na_2HPO_4.12H_2O$)	9.0 g
Potassium dihydrogen phosphate (KH_2PO_4)	1.5 g
Water	1000 ml

Preparation

Dissolve the components in the water, by heating if necessary.

Adjust the PH, if necessary, so that after sterilization it is 7.0 ± 0.2 at 25 °C.

Dispense the medium into flasks of suitable capacity to obtain the portions necessary for the test.Sterilize for 15 min in the autoclave set at 121°C.

Appendix (6)

Muller-Kauffmann tetrathionate-novobiocin broth (MKTTn)

Base medium

Composition

Meat extract …….……4,3 g

Enzymatic digest of casein …….. 8,6 g

Sodium chloride (NaCl) ……..……...2,6 g

Calcium carbonate (CaCO3) …….....…..38,7 g

Sodium thiosulfate pentahydrate (Na2S2O3·5H2O) 47, 8 g

Ox bile for bacteriological use 4, 78 g

Brilliant green …….9,6 mg

Water …….…..……..1000 ml

Preparation

Dissolve the dehydrated basic components or the dehydrated complete medium in the water by boiling for 5 min. Adjust the pH, if necessary, so that it is 8, 2 ± 0, 2 at 25 °C. Thoroughly mix the medium. The base medium may be stored for 4 weeks at 3 °C ± 2 °C.

Iodine-iodide solution

Composition

Iodine ……..……...…….20,0 g

Potassium iodide (KI) …… …….……..…….25, 0 g

Water …….……100 ml

Preparation

Completely dissolve the potassium iodide in 10 ml of water, then add the iodine and dilute to 100 ml with sterile water. Do not heat.

Store the prepared solution in the dark at ambient temperature in a tightly closed container.

Novobiocin solution

Composition

Novobiocin sodium salt …….……0,04 g

Water …….....…….……5 ml

Preparation

Dissolve the novobiocin sodium salt in the water and sterilize by filtration. Store for up to 4 weeks at 3 °C ± 2 °C.

Preparation

Aseptically add 5 ml of the novobiocin solution to 1 000 ml of base medium Mix, then add 20 ml of the iodine-iodide solution .Mix well. Dispense the medium aseptically into sterile flasks of suitable capacity to obtain the portions necessary for the test. The complete medium shall be used the day of its preparation.

Appendix (6)

Rappaport-Vassiliadis medium with Soya (RVS) broth

Solution A

Composition

Enzymatic digest of soya	5.0 g
Sodium chloride	8.0 g
Potassium dihydrogen phosphate (KH_2PO_4)	1.4 g
Dipotassium hydrogen phosphate (K_2HPO_4)	0.2 g
Water	1000 ml

Preparation

Dissolve the components in the water by heating to about 70 °C if necessary.

The solution shall be prepared on the day of preparation of the complete RVS medium.

Appendix (7)

Xylose lysine deoxycholate agar (XLD) agar

Base medium

Composition

Yeast extract powder	3.0 g
Sodium chloride (NaCl)	5.0 g
Xylose	3.75 g
Lactose	7.5 g
Sucrose	7.5 g
L-Lysine hydrochloride	5.0 g
Sodium thiosulfate	6.8 g
Iron (ш) ammonium citrate	0.8 g
Phenol red	0.08 g
Sodium deoxycholate	1.0 g
Agar	9 g to 18 g
Water	1000 ml

Preparation

Dissolve the dehydrated base components or the dehydrated complete base in the water by heating, with frequent agitation, until the medium starts to boil. Avoid overheating. Adjust the PH, if necessary, so that after sterilization it is 7.4 ± 0.2 at 25°C**.** Pour the base to tubes or flask of appropriate capacity. Heat with frequent agitation until the medium boils and the agar dissolves. Do not overheat.

Preparation of the agar plates

Transfer immediately to a water bath at 44°C to 47°C, agitate and pour into plates. Allow to solidify. Immediately before use, dry the agar plates carefully (preferably with the lids off and the agar surface downwards) in the oven set between 37°C and 55°C until the surface of the agar is dry. Store the poured plates for up to 5 days at 3°C ± 2°C.

Appendix (8)

Bismuth Sulfite (BS) agar

Composition

Tryptone	5.00 g
Peptic digest of meat	5.00 g
Meat extract	5.00 g
Glucose	5.00 g
Disodium phosphate	4.00 g
Ferrous sulfate	0.30 g
Ammoniacal bismuth citrate	1.85 g
Sodium sulfite	6.15 g
Brilliant green	25.0 mg
Bacteriological agar	14.70 g

Preparation

Suspend 47.0 g of dehydrated medium in 1 liter of distilled or deionized water. pH of the ready-to-use medium at 25 °C : 7.6 ± 0.2. Slowly bring to boiling, stirring with constant agitation until complete dissolution. Do not autoclave.

Preparation of the agar plates

Cool and maintain the medium at 44-47 °C. Homogenize the medium in order to disperse the precipitate. Pour into sterile Petri dishes at 20 mL per dish. Let solidify on a cold surface. Dry in an incubator with the covers partially removed.

Appendix (9)

Nutrient agar

Composition

Meat extract	3.0 g
Peptone	5.0 g
Agar	9 g to 18 g
Water	1000 ml

Preparation

Dissolve the components or the dehydrated complete medium in the water, by heating if necessary. Adjust the PH, if necessary, so that after sterilization it is 7.0 ± 0.2 at 25 °C. Transfer the culture medium into tubes or bottles of appropriate capacity. Sterilize for 15 min in the autoclave set at 121 °C.

Preparation of nutrient agar plates

Transfer about 15 ml of the melted medium to sterile small Petri dishes. Dry the agar plates carefully in the oven set between 37 °C and 55 °C until the surface of the agar is dry.

Appendix (10)

Triple sugar iron agar (TSI agar)

Composition

Meat extract	3.0 g
Yeast extract	3.0 g
Peptone	20.0 g
Sodium chloride (NaCl)	5.0 g
Lactose	10.0 g
Sucrose	10.0 g
Glucose	1.0 g
Iron (ш) citrate	0.3 g
Sodium thiosulfate	0.3 g
Phenol red	0.024 g
Agar	9 g to 18 g
Water	1000 ml

Preparation

Dissolve the components or the dehydrated complete medium in the water, by heating if necessary. Adjust the PH, if necessary, so that after sterilization it is 7.4 ± 0.2 at 25 °C. Dispense the medium into test tubes or dishes in quantities of 10 ml. Sterilize for 15 min in the autoclave set at 121 °C. Allow to set in a sloping position to give a butt of depth 2.5 cm to about 5 cm.

Appendix (11)

Urea agar (Christensen)

Base medium

Composition

Peptone	1.0 g
Glucose	1.0 g
Sodium chloride (NaCl)	5.0 g
Potassium dihydrogen phosphate (KH_2PO_4)	2.0 g
Phenol red	0.012 g
Agar	9 g to 18 g
Water	1000 ml

Preparation

Dissolve the components or the dehydrated complete base the water, by heating if necessary. Adjust the PH, if necessary, so that after sterilization it is 6.8 ± 0.2 at 25 °C. Sterilize for 15 min in the autoclave set at 121 °C.

Urea solution

Composition

Urea	400 g
Water, to final volume of	1000 ml

Preparation

Dissolve the urea in water. Sterilize by filtration and check the sterility.

Complete medium

Composition

Base	950 ml
Urea solution	50 ml

Preparation

Add, under aseptic condition, the urea solution to the base, previously melted and then cooled to 44 °C to 47 °C. Dispense the complete medium into sterile tubes in quantities of 10 ml. Allow to set in a sloping position.

Appendix (12)

Reagent for Voges-Proskauer (VP) reaction

VP medium

Composition

Peptone	7.0 g
Glucose	5.0 g
Dipotassium hydrogen phosphate (K_2HPO_4)	5.0 g
Water	1000 ml

Preparation

Dissolve the components in the water, by heating if necessary. Adjust the PH, if necessary, so that after sterilization it is 6.9 ± 0.2 at 25 °C. Transfer the medium to tubes in quantities of 3 ml. Sterilize for 15 min in the autoclave (6.1) set at 121 °c.

Creatine solution (N-amidinosarcosine)

Composition

Creatine monohydrate	0.5 g
Water	100 ml

Preparation

Dissolve the creatine monohydrate in the water.

1-Naphthol, ethanolic solution

Composition

1-Naphthol	6 g
Ethanol, 96 % (volume fraction)	100 ml

Preparation

Dissolve the 1-Naphthol in the ethanol.

Potassium hydroxide solution

Composition

Potassium hydroxide	40 g
Water	100 ml

Preparation

Dissolve the potassium hydroxide in the water.

Appendix (13)

Reagents for indole reaction

Tryptone/tryptophan medium

Composition

Tryptone	10 g
Sodium chloride (NaCl)	5 g
DL- Tryptophan	1 g
Water	1000 ml

Preparation

Dissolve the components in the boiling water. Adjust the PH, if necessary, so that after sterilization it is 7.5 ± 0.2 at 25 °C. Dispense 5 ml of the medium into each of several tubes. Sterilize for 15 min in the autoclave set at 121 °C.

Appendix (14)

Kovacs reagent

Composition

4-Dimethylaminobenzaldehyde	5 g
Hydrochloric acid, $\boldsymbol{\rho}$ = 1.18 g/ml to 1.19 g/ml	25 ml
2-Methylbutan-2-ol	75 ml

Appendix (15)

L-Lysine decarboxylation medium

Composition

L-Lysine monohydrochloride	5, 0 g
Yeast extract	3, 0 g
Glucose	1, 0 g
Bromocresol purple	0,015 g

Water	1 000 ml

Preparation

Dissolve the components in the water, by heating if necessary. Adjust the pH, if necessary, so that after sterilization it is 6, 8 ± 0, 2 at 25 °C. Transfer the medium in quantities of 2 ml to 5 ml to narrow culture tubes with screw caps. Sterilize for 15 min in the autoclave set at 121 °C.

Appendix (16)

TRYPTONE BILE X-GLUCURONIDE MEDIUM (TBX)

Composition	**gm/liter**
Tryptone	20.0
Bile Salts No. 3	1.5
Agar	15.0
X-glucuronide	0.075

pH 7.2 ± 0.2 @ 25°C

Preparation

Suspend 36.6g of TBX Medium in 1 litre of distilled water. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and pour the medium into sterile Petri dishes.

Appendix (17)

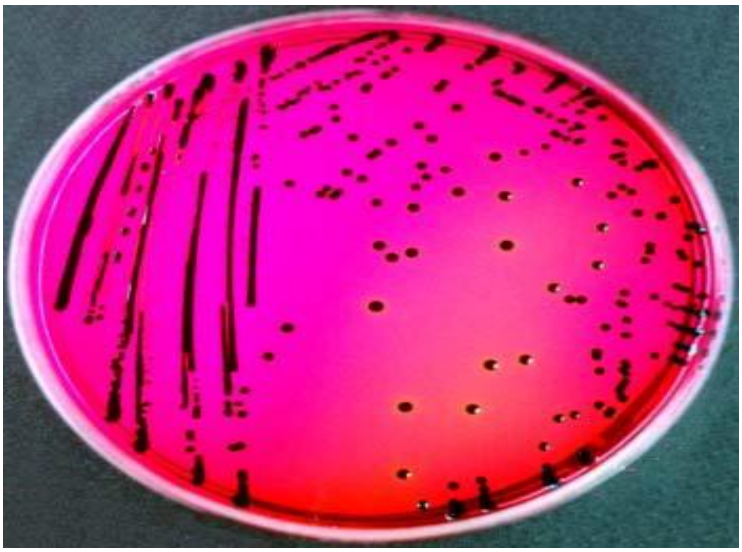
PLATE COUNT AGAR

Composition	**gm/liter**
Tryptone	5.0
Yeast extract	2.5
Glucose	1.0
Agar	9.0

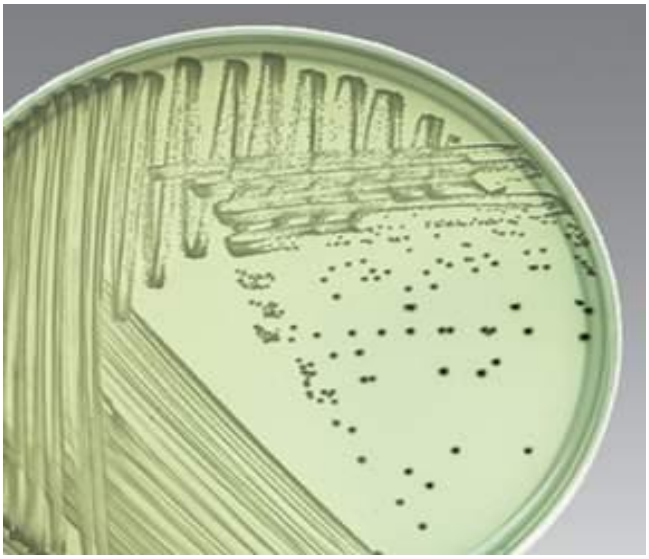
pH 7.0 ± 0.2 @ 25°C

Preparation

Add 17.5g to 1 litre of distilled water. Dissolve by bringing to the boil with frequent stirring, mix and distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes.



Appendix 18. *Salmonella* spp. on Xylose Lysine Deoxycholate Agar (XLD) medium showed black colonies



Appendix 19. 24 hours old culture of *Salmonella* spp. in Bismuth Sulfite (BS) agar showing black colonies