Sudan University of Science and Technology
College of Graduate Studies

Investigation of Food Safety Status in the Restaurants of Salalah State Municipality in Sultanate of Oman

التحقيق في سلامة الأغذية في مطاعم بلدية صلالة في سلطنة عمان

By

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Dedication

To my mother, sisters , brothers and sincerely to my wife and son. I would also like to dedicate this work to the soul of my father

Acknowledgments

Above all, praise is to my almighty Allah for giving me a good health, wisdom, ability, and strength to carry out this work and for all other graces.

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Abstract

A cross-sectional study was conducted from June to December 2012, in Salalah Municipality, Sultanate of Oman. A total number of 142 samples were collected from 21 restaurants from 7 different areas (3 restaurants in each), namely; Al-Haafah, Al-Sinaat Al-Jadeedah, Al-Goof, Awgaad, Al-Saadah North and South and Salalal Al-Wustaa. Samples were randomly collected as follow: 41 ready to eat food, 38 water, and 63 swab samples. The food samples included cooked meats (chicken, fish and beef) and beans (lentil) and vegetables (potatoes and others). The swab samples were collected from hands worker ؛ the surfaces؛

kitchenware (knives, and cutting boards) used for food preparation. Parallel to that questionnaire-guided interviews with 21 worker were conducted. Isolation and identification of bacteria revealed two prevailing species of bacteria; *E. coli* was prevalent in 5.0% (95% CI, 0.71 - 9.29) and *Staphylococcus aureus* was in 3.0% (95% CI, -1.08 - 7.08) of the investigated samples. However, no any *Salmonella* species, *Bacillus* species, *Listeria* species and Yeast and molds were detected in any of the studied sites/restaurants; prevalence of 0.0% (95% CI, 0.0 - 0.0). The study revealed no statistical significant difference, at *p-value* ($p \leq 0.05$), observed in the Total Bacterial Count (TBC) and the *Enterobacteriace* Enumeration estimated between the samples; the highest contamination levels in meat (3.3×10^5 cfu/cm^{-2}) in Al-Sinaat area, in vegetables (1.3×10^4 cfu/cm^{-2}) in Al-Saadah South area, in utensils (1.0×10^5 cfu/ml) in Al-Haafah and Al-Saadah North areas, in surfaces (2.0×10^5 cfu/ml) in Al-Haafah area and on hands of workers (1.6×10^5 cfu/ml) in Al-Saadah South area. While the EE revealed the highest contamination levels in meat (0.037×10^3) in Al-haafah and Al-Sinaat area, 1in vegetables (1.000×10^3) in Salalaha Al-Wustaa area, in utensils (3.400×10^3) in Al-Saadah North area, in surfaces (1.500×10^3) in Salalaha Al-Wustaa area and on hands of workers (0.267×10^3) in Al-Saadah North area. The respondents were asked if they wear gloves when working, wash hands before putting on the gloves, wear an apron and a mask and put on a cap when working, wash hands before and after touching raw meat, wash hands after the rest time when coming back to work, eat and/or drink and smoke at work place. They were also asked how often do they use the products of their working plants and how often do they recommend the products of your working plants to others, they were answered 10, 20,20,2,16,20,21,21,0,0,20,21 from all respondents 21 respectively.

ملخص الدراســــــــــة

وقد أجريت دراسة مستعرضة من يونيو إلى ديسمبر 2012م، في بلدية صلالة، سلطنة عمان. حيث تم جمع عدد 142 عينة من 21 مطعم من 7 مناطق مختلفة (عدد 3 مطاعم في كل منطقة)، وهي؛ آلحافة، آلصناعية الجديده، القوف، عوقد، السعادة الشمالية ؛ السعادة الجنوبية وصلالة الوسطي . تم جمع عينات بشكل عشوائي على النحو التالي: 41 عينة طعام جاهز ومعد للاكل ، 38عينة مياه ، و 63 من العينات مسحة. وشملت عينات المواد الغذائية واللحوم المطبوخة (الدجاج والسمك ولحم البقر) والفاصوليا (العدس) والخضار (البطاطا وغيرها). تم جمع عينات مسحة ايدي العمال ؛

الأسطح وأدوات المطبخ (السكاكين، وألواح التقطيع) المستخدمة في إعداد الطعام. كما تم عمل الاستبيان ل 21 عامل من معدي الاطعمة بالمطاعم . كشفت العزل والتعرف على البكتيريا نوعين من البكتيريا السائدة. ك ايكولاي السائدة في 5.0٪ (95٪ ،CI 0،71-9،29) و المكورات العنقودية الذهبية في 3.0٪ (95٪ 7.08 - 1.08- ،CI) من العينات التي تم التحقق منها . ومع ذلك، لم يكن هناك أي نوع من السالمونيلا ،الأنواع العصوية، الأنواع الليستيريا والخميرة والعفن تم الكشف عنها في أي من المواقع / المطاعم. انتشار 0.0٪ (95٪ 0،0-0،0 ،CI). وكشفت الدراسة عدم وجود فروقات ذات دلالة إحصائية، في القيمة ص ($p \leq 0.05$)، لوحظ في العدد الكلي للبكتيريا (الدرن) و Enterobacteriace تعداد يقدر بين العينات. أعلى مستويات التلوث في اللحوم (3.3 × 105 وت م / سم 2) في منطقة الصناعية ، في الخضراوات (1.3 × 104 وت م / سم 2) في منطقة آل سعادة الجنوبية، في الأواني (1.0 × 105 وت م / مل) في مناطق الحافة و آل-سعادة الشمالية، في السطوح (2.0 × 105 وت م / مل) في منطقة آلحافة وعلى أيدي العمال (1.6 × 105 وت م / مل) في منطقة السعادة الجنوبية . في حين كشفت EE أعلى مستويات التلوث في اللحوم (0.037 × 103) في منطقة الحافة ومنطقة الصناعيات والخضروات((1،000 × 103 ي منطقة صلالة الوسطي، وفي الأواني (3.400 × 103) في منطقة السعادة الشمالية ، في السطوح (1،500 × 103) في منطقة صلالة الوسطي وعلى أيدي العمال (0،267 × 103) في منطقة السعادة الشمالية.. كما تم سؤال المستطلعين إذا كانوا يرتدوا القفازات عند العمل، وغسل اليدين قبل ارتداء القفازات، وارتداء مئزر وقناع ووضع قبعة علي الراس عند العمل، وغسل اليدين قبل وبعد لمس اللحوم النيئة، وغسل اليدين بعد وقت الراحة عندما العودة إلى العمل، وتناول الطعام و / أو الشراب والدخان في مكان العمل. كما سؤئلوا أيضا عدد المرات التي يستخدموا فيها منتجات عملهم وهل يوصوا الاخرين باستخدام منتجاتهم , حيث اجابوا 10‘20‘20‘2‘16‘20‘21‘21‘0‘0‘20‘21 من جميع المستطلعين 21 بالتوالي .

Introduction

Food safety is one of the most important indicators of the economic and health development of Nations (WHO, 2000). Despite the importance of food safety, there seem to be few quality control systems to guard against food-related illnesses, in developing countries, some of which may be fatal while others can lead to expensive medical care (Snyder, 1992). Illnesses from food related diseases is estimated to be more than illnesses from all other environmental factors combined. Over 66% of food-borne illnesses are caused by bacterial pathogens (Byran, 1992). Globally, the number of diseases that causes diarrhoea alone has been estimated to be 400 million cases annually, this is pointing out to a serious food safety problem (Byran, 1992). The direct cost of food-borne illness outbreak can approximate $75,000 per food service establishment and these can include investigation clean- up, restaffing, restocking, product loss, settlements and increased regulatory sanctions (Hannington, 1992).

Food service workers and personnel have a major responsibility concerning the safety of the food since their actions can affect the health of many people. Food-borne diseases are major public health problem estimated to affect up to 10% or more of the population in the industrialized countries (WHO, 2005). Food and water-borne diseases are prevalent in many in countries, especially the developing ones, and epidemiological studies have shown that a great proportion of food-borne diseases occur as result of poor food sanitation and unhygienic handling of foods in restaurants and other eating outlets (Antoria, 2002).

Hazard Analysis Critical Control Point (HACCP) has been endorsed by the National Academy of Sciences, the Codex Alimentarius Commission which is an international food standard setting organization, and the National Advisory Committee on microbiological criteria for foods (ICMSF, 1980). HACCP is considered the best system available for designing programmes to assist food firms in producing foods that are safe for consumption (Food Codex, 1995). The biggest advantage of HACCP over the other systems is that it pre-empts all the activities in the food process thus reducing risks in food-borne diseases. According to Taber (1993), the hazard of any material is determined by chemical, physical and biological properties. Processing and preparing can be a risky business and precautions must be implemented to prevent problems and to correct them if they do occur. HACCP, a system for ensuring food safety, was developed in 1971 in a cooperative effort by the United States Army Natick

Laboratories, the National Aeronautics and Space Administration and the Pillsbury Company (Pierson and Corlett, 1992). The system is endorsed as an effective and rational means of assuring food safety from harvest to consumption. Preventing problems from occurring is the basis of the HACCP system. It is termed superior to all the conventional food microbiological quality control procedures in the market because it only addresses significant food safety hazards.

Byran (1992) emphasizes that food safety concerns are magnified when an outlet prepares foods from raw materials and points out that foods mostly involved in the outbreaks of diseases include milk and milk products, vegetables, salads and puddings, meat and meat products among others. Perhaps the introduction of a HACCP system could improve and reduce the incidence of food poisoning in urban restaurants. It can also aid inspection by regulatory authorities and promote international trade by increasing confidence in food safety. It provides a more specific and critical approach to the control of microbiological hazards in foods than that provided by traditional inspection and quality control approaches (Amref, 1982).

In the countries of the third world lack of the knowledge and skills of Good Manufacturing Practices (GMPs) have significantly contributed to poor hygienic practices in food service establishments. However, due to the inadequacy of the studies on food safety and the shortage in the availability of data in public restaurants the health administrative departments have taken the evaluation of food safety and hygienic practices during food processing (WHO, 1999). These reports show that about 80% of all diseases and more than 1/3 of all deaths in the developing countries are caused by contaminated food and water (WHO, 2004). This study attempted to establish whether the scenario is the same in the restaurants of Salalah Municipality in Sultanate of Oman with a general goal of increasing knowledge on aspects related to food safety issues.

Objectives

1. To determine the microbial load of foods and water consumed in the restaurants of Salalah Municipality in Sultanate of Oman
2. To isolate and identify of different types of bacteria that colonize the restaurants.
3. To establish the viability of implementing a HACCP system as a strategy for quality control in the restaurants .

Chapter One

Literature Review

1.1. Food Safety

Food safety can be expressed as food risk for example it could be the possibility of not getting infected by an illness that was a result of ingesting a certain type of food. In the general way, food safety can be defined as the wide spectrum of food‘s nutritional values, chemical composition and the concerns that evolve in regards with newly introduced foods that have an unfamiliar composition, as in the uneasiness regarding genetically modified foods (Seward *et al.*, 2003).

Two types of food safety include objective measures and subjective perception. Objective food safety is a scientist‘s measure regarding the evaluation of the risks that come with a certain food. Subjective is a consumer‘s perception in regards with the safety of a certain food. It is generally recognized that objective and subjective food safety diverge in many situations (Grunert, 2005). In developed countries it is more obvious that the public are concerned about health risks related to food safety and proper sanitary standards (Pinstrup-Andersen, 2000).

Food safety is very important for restaurants. Once a restaurant is implicated in a food-borne illness; it can result in damaging publicity, consumer interest and trust loss, as well as community health regulation and legal charges. Considering the significance of food safety, it is astonishing how there are few studies that examine the consumers‘awareness of food safety at restaurants. Even though food safety complications can arise during any part of food production, restaurants are a crucial final step in this series from the farm to fork (Seward *et al.*, 2003).

1.2. Restaurant Cleanliness

The purpose of routine restaurant inspections is to prevent food-borne illness by promoting safe food handling and preparation. Although different jurisdictions enforce different standards of sanitation and cleanliness, inspections are required by food sanitation codes in the Sultanate of Oman. Food safety is the basis of these restaurants inspections and abiding by the food safety rules is a necessity to obtain a good inspection grade (Meng and Doyle, 2002). Olsen *et al*. (2000) found out that restaurants inspections scores with poorer results on inspections were more likely to have food-borne disease outbreaks. These outbreaks were a result of violating critical laws, and family to use food protection practices, and this reflected on the inspection time period (how long it took to inspect) and the overall grading of the restaurant. HACCP guidelines

are incorporated into the Food and Drug Administration‘s Food Code and are followed by the food industry, but restaurant inspections are done by past measures and have not been updated. Moreover some restaurants operate under their states food codes, which may be different from the federal food code. Although HACCP systems do control hazardous food processes and monitor continually dangerous conditions, inspection criteria and serious violations that were applicable in the past decade may possibly inadequately reflect the reasons of restaurant food-borne outbreaks these days (Meng and Doyle, 2002).

From a publicity viewpoint, it may be helpful for restaurants to advertise their food safety standards and policies (Cruz *et al.*, 2001). Snyder (2005) speaks about a HACCP program for marketing manufacturing processes. Jin and Leslie (2005), endorse the acceptance of hygiene grading methods at restaurants. They consider that hygiene grading cards may provide an economic incentive for restaurants to improve hygienic standards and public health outcomes (Meng and Doyle, 2002; Lee *et al.*, 2009). It is vital to comprehend in what way customers observe all the settings in the food chain to conceptualize awareness of restaurants, as food safety occurs within a food system. Additionally, food safety concerns may possibly influence where customers buy their meals. For instance, if restaurants are seen as being less safe than supermarkets, customers might choose to buy ready to eat meals at the supermarket instead of eating their meals at a restaurant. In spite of the greater emphasis on food safety by the restaurant business, an important proportion of restaurants still conduct insufficient food safety practices. The U.S. Food and Drug Administration‘s (FDA) Retail Program Steering Committee (2000) report stated that only 60 % of full-service restaurants and 74% of fast-food restaurants were in compliance with the FDA Food Code in regards to the five risk factors that are associated with foodborne illness (Knight *et al.*, 2007).

Henson *et al*. (2006) found that hygiene was the most often mentioned characteristic used by customers to define food safety at restaurants. Other characteristics used by consumers to assess food safety at restaurants included: general excellence of the restaurant, density of customers, and outside data, such as restaurant reviews, different views of visitors such as friends and family, and inspection grading cards. Even though restaurants in the US undergo inspections by their local health departments, studies have constantly shown that a large proportion (60% restaurants) regularly has insufficient food hygiene practices (Knight *et al*., 2007). Even though health departments inspect restaurants on a routine basis to see if the

restaurant in abiding by the established hygienic standards, little data is accessible in regards with the effectiveness of the hygiene standards in preventing foodborne illness (Knight et al, 2007).

The impact of a restaurant hygiene grading system on foodborne- illness hospitalizations in Los Angeles County was described by Buchholz et al (2005). This restaurant hygiene grading system utilized publicly posted grade cards on the doors of restaurants reflecting the hygienic levels of that restaurant. The grading system was introduced in January 1998, (Buchholz et al., 2002; Simon et al, 2005) and patient hospital discharge files on foodborne illness cases during the period of 1993–2000 were examined in the Los Angeles County area and, as a control, for the rest of California (Simon et al, 2005). In 1999 the restaurant hygiene grading program was associated with

1.3. Global Perspective on Food Safety

The food safety development (FSD) strives to reduce the serious negative impact of food-borne diseases world-wide (Gessner and Beller, 1994). Food and water-borne diarrhoeal diseases are leading causes of illness and death in less developed countries, responsible for affecting 1.8 million people annually. Recent trends in global food production, processing, distribution and preparation are creating an increasing demand for food safety research in order to ensure a safer global food supply. WHO works closely with FAO (2002) to address food safety issues along the entire food production chain by the use of HACCP system. These methods provide efficient, science-based tools to improve food safety, thereby benefiting both public health and economic development (Gessner and Beller, 1994).

To improve food safety and strength consumer confidence, concerns over safety and quality for governments, food producers, industrial traders and consumer are increasing. The burden of food-borne diseases is significant in all parts of the world. In the European region, some food safety and quality problems have endangered consumer health. Food can be contaminated by water used as an ingredient (Ilboudo and Traoré, 2005).

1.4. Consumer Information and Demand

The implementation of food safety principles should be confined not only to developed countries but also to developing countries because this is a clear indication of factors of development allowing the destructive eventualities of potential health incidents, which can be avoided (WHO, 2005). Consumers who are well-informed will be able to fight for their rights and ensure that they are provided with safe and good quality products and services.

Countries without effective food control systems cannot ensure safe foods, although the range of foods eaten may affect our individual health in the long term, food safety discussions usually focus on the more immediate effects that arise from consuming foods contaminated with some undesirable biological or chemical agents. Food quality control is the science, which deals with the basic standards of food safety maintenance to be accepted by the human race (FAO / WHO, 2002).

The importance of food technologies in the prevention of diseases and health remains unrecognized in public health establishments and they are thought to be causes of food-borne diseases (WHO, 2005). The role of food technologies in the life and health of people is wide and very important in improving the nutritional quality of food ensuring safety and preventing food-borne diseases. They reduce losses due to spoilage and contamination and therefore prevent malnutrition and starvation. There are socio-economic implications which facilitate and promote trade in food, provide employment, women facilitation in family‘s food preparation thus fully participating in social life. They also increase the customers‘ pleasure and provide a greater choice of products.

1.5. Public Health Aspects

Food safety is a priority for consumers and customers as they want safe health food, which keeps them strong and healthy (Hayer, 1994). Major case for food contamination with pathogens is unsanitary practices during product handling, processing and distribution. Food poisoning agents (infection and intoxication), that are associated with foods include *Escherichia coli, Salmonella spp, Vibrio cholera, Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes and Clostridium* perfringens (Sockett, 1991).

Staphylococcus aureus is a human associated bacterium isolated from the human skin and nasal membrane and its presence in food indicates lapse in the maintenance of personal hygiene (Adesiyun, 1984). *Salmonellosis* is one of the major food-borne health hazards and is associated with animal food such as poultry, meat, milk, eggs and fish (Garner and Nunn, 1995). They produce enzymes that degrade carbohydrates, fats and proteins thus resulting in softening and flavor deterioration of foods (Maff, 1995). Under favorable conditions during harvesting, processing and storage of food commodities, moulds produce toxic metabolites called mycotoxins which are a concern to global food safety because of their effects on human health. Most mycotoxins are heat stable and capable of producing diseases of acute or chronic nature when ingested with food. They can affect organs like the liver, the kidney and nervous systems, endocrine and immune systems. Uses of an integrated management system of risks that reflects the HACCP concepts and emphasizes on good manufacturing practices have been recommended (Kapperud, 1995).

1.6. Food Safety versus Food Spoilage

Spoilage is any change in the food that causes the development of undesirable flavors, textures and appearances. Examples of spoilage include soft rot in potatoes which is a biological change, rancidity in oils a sign of chemical change and crushing of food during shipping which is a physical change (Thorner, 1983). There are two types of food borne illnesses, intoxication and infection. An illness caused by consuming harmful (toxic) chemical is intoxication while that caused by microorganisms invading the body is an infection (Desenclos, 1996).

Bacillus cereus food poisoning was associated with rice at a day care centre in Virginia (Khdor, 1993) while in Quatemala transmission of a newly introduced epidemic strain by street vendors caused infection (Koo, 1996). This can also happen in Kenya and therefore necessary measures have to be taken to avoid this happening to safe guard the public from food poisoning.

Disadvantages of HACCP badly done could include lower quality products, less save products, low customer satisfaction, bad customer relationships, legal/civil/prosecution, loss of reputation and profit. Advantages of the system well done could be higher quality products, safer products, high customer satisfaction and relationship, focus of resources, premium prices and better margin (WHO, 2005). In small business sector the barriers and challenges of

implementing HACCP system is attributed to inadequate infrastructure and facilities, lack of expertise and information, psychological constraints, basic hygiene and human resource (ICMSF, 1980). Others could be perception and finance, legal and government commitment, business, customer and consumer awareness. Also lack of formal education, expertise, technical support and inadequate communication and training programs (Tartakow, 1981)

The toxin produced by one strain of *Clostridium botulinum* (type B) was so powerful that when a bite-size piece of beef containing it was diluted about 108 times, all the mice into which it was injected died. This indicates that if a human being had eaten a small amount of that meat, he would have died (Nickelson, 1990). Bacteria are killed by heat at a rate that is referred to as a logarithmic order of death. The *D*-value is defined as the time needed at a given temperature to destroy 90% of a microbial population. Each 90% reduction of bacteria at *D*-value of 2.5 minutes at 250ºF (121ºC) is described as a reduction of one —log cycle‖ (Vieira, 1999).

1.7. HACCP and Food Safety

This was developed to ensure the safety of food for United States astronauts nearly 30 years ago (Pierson and Corlet, 1992). It is now being used in the restaurants because these guidelines make good sense. When customers go into a restaurant, most of them are looking for a clean, safe place to eat. By applying the basic principles of HACCP to the restaurant business, you are making sure that you serve safe food to the customers (Ndungu, 2002).

1.7.1. Application of HACCP to Food Service and the Underlying Benefits

HACCP is a management system in which food safety is addressed through the analysis and control of biological, chemical and physical hazards from raw material production, procurement and handling, to processing, preparation, distribution and consumption of the finished product. A firm commitment to HACCP by top management provides company employees with a sense of the importance of producing safe food (WHO, 2002).

The system is designed for use in all segments of the food industry from growing harvesting, processing, manufacturing, distributing and merchandising to preparing food for consumption. Prerequisites programmes like good manufacturing practices (GMPs) are essential foundations for the development and implementation of successful HACCP plans. Food safety

systems based on the HACCP systems have been successfully applied in food processing plants, retail food stores and food service operations. It should be emphasized that HACCP is a preventive approach, and not reactive (WHO, 2002). So as to verify that the procedures are being implemented, inspection schedules, review plans, records and sampling should be incorporated into the methods, procedures and tests of the whole preparation process. Todd (1996) estimated that 5% of all food- borne illnesses may be traced to abusive industrial practices. Ninety five percent are associated with abusive practices in food service, restaurants or home preparation of foods. HACCP principles can be applied in food service establishments as implied by Bernard (2002), and can reduce the number of outbreaks of food-borne illness. The first CCP of product is at the receiving area where those responsible must examine the condition of each item as it is unloaded, from known and approved suppliers who should have functional temperature indicators which should be checked to monitor abuse (Firestone, 1992). Food fried in badly abused oils may absorb the degraded fat, causing gastrointestinal distress. Complaints of this nature and studies on oil quality led to the development of regulations governing restaurants frying oils in developed countries like Europe (WHO.2005). (Flyers, 2008) says, the benefit underlying this system for all food sectors and consumers alike to the government include among others improved public health, more efficient and targeted food control, reduced public health costs, trade facilitation and increased confidence of the community in the food industry. To the industry, there will be increased consumer and government confidence, reduced legal and insurance costs, increased market access, reduction in production costs, improved staff-management commitment to the food safety and decreased business risks. To the consumer, there will be reduced risks of food-borne diseases, increased awareness of basic hygiene, increased confidence in the food supply chain and improved quality of life.

Some of the barriers to the implementation of the HACCP systems in food establishment are external conditions which increase the pressure on the strategies for its implementation like regulatory market forces, promotion by public health and food control authorities (WHO, 2002). Others could be internal factors like the level of knowledge or resources available and luck of government or industry support. Management should be commitment to the system and need to change attitude and organizational culture towards the system approaches.

Adequate training is important for overcoming barriers related to human resources. This should include both employees and enforcement officials and should lead to behavioural changes,

enhance competency along with assessment thereafter. The application of HACCP in restaurants should be mandatory (Stuart, 2002). This is to change the traditional role of food safety agencies and food inspectors since the system is making headways in the food industries. Educating food handlers to adhere to good personal hygiene and proper handling of food is an essential component of National Food Safety Programme and especially handling of fish (Owaga, 2004).

1.7.2. HACCP Study-Setting Priorities in the Restaurants

A complete HACCP study cannot be done for every type of restaurant in Thika town. If possible, epidemiological data should be used to set or establish priorities. Foods that are commonly implicated as sources of food-borne diseases should be given first priority; however, Kenya does not have food-borne surveillance programmes which could provide data (GoK, 2005). Therefore, priorities can be based on the following risk factors: Intrinsic properties of the foods involved, preparation and handling, volume of food prepared and susceptibility of consumers.

The HACCP system consists of seven principal activities which should be considered during the HACCP process but in implementing the process, each step should be applied in a manner consistent with the needs and resources of the restaurants. The steps in the HACCP process can be outlined as follows (WHO, 2005):

a) Hazard analysis – This will consist of pre-visits to the restaurants, description of the products and their intended use, construct flow charts and on site confirmation and finally listing all potential hazards associated with each step.

b) Determine Critical Control Points- This is the heart of the HACCP study and the success is on flexibility and common sense.

c) Establish critical limits – Critical limits must be specified for each control measure, so as to monitor the CCPs. This will include characteristics like temperature, time, moisture level and parameters which are organoleptic such as visual and texture e.g. clear running of juices in meats and boiling of liquids which are an indication of thorough cooking.

d) Establish monitoring procedures – Monitoring is the scheduled measurement or observation at a CCP of the compliance with the critical limits set out for each control measure. Physical, chemical and sensory monitoring methods are preferred because of their speed of response. To

monitor the critical control point, make observations, use of senses to evaluate characteristics of foods or measure physical or chemical attributes of foods.

e) Establish corrective action procedures – Each deviation has two types of action needed. Corrective actions are those that will bring the CCP back under control and disposition actions are those actions to be taken with the food that has been produced in the time period when CCP was out of control. This might include increase cooking temperature, time, adjusting quantities of some ingredients, adjusting preparation or storage at a later stage, decreasing holding time, increasing holding temperature, reheating, re-washing and sanitizing, rejecting incoming goods and finally disposal of products. Disposition actions will require judgments based on the hazards and their assessed severity and risks.

f) Establish verification procedures – This should be done by health personnel who are experienced in HACCP and knowledgeable about preparing the foods of concern.

g) Establish documentation procedures – This calls for maintenance of log or record forms in which to put results of monitoring. This is essential for food processing operations and prudent in marketing of food service operations in the restaurants.

1.7.3. Background in Food Safety and HACCP System

Hundreds of centuries ago, people those lived long ago observed that ingestion of soured or contaminated food made people sick. Throughout history, to keep food safe and to decrease hazard of food borne cases different methods were applied such as refrigeration and pasteurization technology which participate in food preservation practices. In the modern world, the food industry was successfully developed from raw material production, procurement and handling, to manufacturing, distribution, and consumption of food products. Almost every person relies on the national and international food supply system nationally or internationally (Roberts, 2001).

However, these developments increased the risk of food borne illnesses. Negligence at any stage in food plants causing public healthy disaster, which makes food safety one of the hottest topics in the 21st Century. (HACCP) is a management system extensively used in advanced food companies to analyze and control biological, chemical and physical hazards through the whole food production process to achieve food safety (Ying Zhen, 2011).

HACCP is an internationally recognized, science-based, food safety system that is used to help ensure the manufacture of safe food products. It is designed to prevent, reduce or eliminate potential biological, chemical and physical food safety hazards, including those caused by cross contamination. During the development of a HACCP system, potential hazards are identified and control measures are implemented at specific points in the manufacturing process (Roberts, 2001). To ensure the health and food safety in the space flights, Pillsbury Corporation with NASA in 1960's was developed HACCP system. After a decade this system applied in the industry, and then it was established for the US meat and poultry industries. The National Advisory Committee on Microbiological Criteria for Foods (NACMCF, 1997) provided information for international standards on the development and implementation of HACCP principles. The General Accounting Office (GAO) endorsed HACCP as a scientific, risk-based system to protect public health. The Food Safety and Inspection Service (1996) (FSIS) of the US published a final rule of HACCP. ISO issued (2005) ISO 22000 "Food Safety Management System- Requirements for Organizations in Food Chain", which is a complete food safety and quality management system that included all HACCP principles and incorporated the prerequisite programs, such as, Good Manufacturing Practice (GMP), and Sanitation Standard Operation Procedures (SSOP) (Ying Zhen, 2011).

1.8. Significance of Food Safety

The World Health Organization (WHO) claims that food safety is an increasing important public health issue. Food-borne diseases are widespread, not only threaten public health, but also significantly reduce the economic productivity (WHO, 2011).

Food-borne and water borne diarrheal diseases are very have a very big impact and humans' lives and kill approximately 2.2 million people annually (WHO, 2011). About 13 million children under the age of five die each year from infections and malnutrition most oftenly attributed to contaminated food (WHO, 2007). According to Centers for Disease Control and Prevention (CDC's) research and analysis based on the information from multiple surveillance systems and other sources, foodborne diseases cause approximately 76 million illnesses, 325.000 hospitalizations, and 5.000 deaths in the United States every year (Mead, 2000).

The costs of the food contamination are a social and economic burden to the community. In the United States, the estimated annual medical costs/productivity losses due to the seven major foodborne pathogens range from $6.6 billion to $37.1 billion (Daniell, 2000).

More than 200 known diseases are transmitted through food. For example, *Escherichia coli* (*E.coli*), which is one of the most common foodborne pathogens, will normally cause problems. A food poisoning outbreak in 2011, at a daycare facility resulted in that an infant and a toddler had tested positive for *E. coli* (Haglund, 2011). *E. coli* related disease causes diarrhea and stomach cramping, sometimes even kidney failure or death especially for young children and elderly. In the year 2011 once more, 310.248 pounds of ground beef products had been recalled due to *E. coli* contamination. It is a direct threat to public health and a survival challenge to the food processing companies (Drew, 2011).

1.9. Food Safety Related Hazards

Food industry is different from other industries. It needs an excellent understanding of the characteristics of products being handled to efficiently prevent the development of potential hazards and to control the ones that exist. Three categories of hazards are related to food safety, including: 1) biological hazards, 2) chemical hazards and 3) physical hazards (Ying Zhen, 2011).

Bacterial pathogens, viruses, and parasites are biological food hazards include. Typical hazardous microorganisms frequently cause foodborne illnesses including *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Salmonella typhi*, and so on. *Listeria monocytogene* is major harmful food-borne pathogens (Roberts, 2001). It causes the highest mortality rate compare with other food borne bacterial pathogens. The organism mainly causes nerves signs including septicemia, meningitis, encephalitis, and many other illnesses. Primary sources of the organism are raw milk, ice cream, raw meat, and sea food. It can survive at temperatures as low as 0°C (Roberts, 2001).

E. coli O157:H7 cause serious clinical signs such as hemorrhagic diarrhea, abdominal cramp, and even kidney failure, particularly in young children and elderly. It is transmitted via the fecal-oral route and people basically infection due to ingestion of undercooked food, such as ground beef, unpasteurized milk, vegetables, and water (Roberts, 2001). *Salmonella typhi* always

causes diarrhea, extremely dangerous infections in kids and the overage. The essential sources of infection are meats, poultry, eggs, and milk (Roberts, 2001).

Chemical food hazards are food that contaminated with chemical substances or compounds that might injurious to human. These chemical cause serious health problems when deposited in body tissues. Physical food hazards are including: glass fragments, wood, stone, metal fragments, insulation, bone, plastic, and many others. Which cause injuries or illnesses, physical hazard usually not harmful as others, but can made serious problem for young children and overage (Roberts, 2001).

1.10. The HACCP System

The HACCP system, which is science based and systematic, identifies specific hazards and measures for their control to ensure the safety of food. HACCP is a tool to assess hazards and establish control systems that focus on prevention rather than relying mainly on end-product testing. Any HACCP system is capable of accommodating change, such as advances in equipment design, processing procedures or technological developments. HACCP can be applied throughout the food chain from the primary producer to final consumer and its implementation should be guided by scientific evidence of risks to human health. As well as enhancing food safety, implementation of HACCP can provide other significant benefits. In addition, the application of HACCP systems can aid inspection by regulatory authorities and promote international trade by increasing confidence in food safety. The successful application of HACCP requires the full commitment and involvement of management and the workforce. It also requires a multidisciplinary approach; this multidiscipline approach should include, when appropriate, expertise in agronomy, veterinary health, production, microbiology, medicine, public health, food technology, environmental health, chemistry, and engineering according to the particular study. The application of HACCP is compatible with the implementation of quality management systems, such as the ISO 9000 series, and is the system of choice in the management of food safety within such systems. While the application of HACCP to food safety was considered here, the concept can be applied to other aspects of food quality (Donald, 1998).

1.10.1. Principles of the HACCP System

HACCP has Seven Principles that **1)** To conduct a hazard analysis, **2)** To identify critical control points, **3)** To establish critical limits for each critical control point, **4)** To establish critical control point monitoring requirements food safety, **5)** To establish corrective actions, **6)** establish record keeping procedures and 7**)** To Establish procedures for ensuring the HACCP system is working as intended (Ying Zhen, 2011).

1.10.2. Common Benefits of HACCP

Although the adoption of HACCP systems worldwide is due primarily to the added food safety protection provided to the consumer, a number of other benefits to the food industry, including your company, can be realized by implementing a successful HACCP system (Troy *et al.*, 2007).

1.10.3. Increased Focus and Ownership of Food Safety

Food safety is the responsibility of everyone in the food supply chain. Through the process of developing and implementing a HACCP system, your company's employees will become more aware of food safety and their roles in maintaining and contributing to food safety. This increased awareness may lead to increased ownership and pride in the production of a safe product (Troy *et al.*, 2007).

1.10.4. Increased purchasing power with consumer confidence

There is an increasing trend for purchasing power to request HACCP from their suppliers. Food processors who have implemented a HACCP system provide buyers and consumers with a greater degree of confidence that the facility is producing a safe food product (Troy *et al.*, 2007).

1.10.5. Maintaining or Increasing Market Access

Market forces continue to drive food safety awareness and HACCP implementation throughout the food processing sector. As food safety systems, particularly HACCP, become more common, market access is limited for processors who do not implement them. In many cases, buyer demands require HACCP implementation to maintain market share and/or gain access to previously inaccessible markets. HACCP implementation may also permit reentry into a market that had been lost. Considering the economic implications, HACCP implementation may be a necessary cost of business (Troy *et al.*, 2007).

1.10.6. Business Liability Protection

Implementation of a HACCP system can provide your facility with some degree of increased business liability protection and may lead to reduced insurance premiums (Troy *et al.*, 2007).

1.10.7. Reduced Operational Costs

The process of developing and implementing a HACCP system requires that the entire manufacturing process be reviewed and analyzed, and written procedures developed. This process often reveals areas where operational costs can be streamlined. For example, developing a sanitation program may identify that excessive chemical concentrations are being used. Reducing chemicals to the correct concentration may decrease sanitation costs (Troy *et al.*, 2007).

1.10.8. Efficient Oversight

Similarly, HACCP implementation can provide your company with ongoing efficient oversight. It can be cost effective to implement HACCP in spite of the associated costs. Activities that are performed on a regular basis, such as product and process monitoring, employee training and review of procedures, allow your company to maintain control over the facility and product. You may find there are certain areas of the process that can be made more efficient and productive (Troy *et al.*, 2007).

1.10.9. Improved Product Quality and Consistency

The implementation of a HACCP system may indirectly enhance product quality. Procedures that minimize the presence and growth of pathogenic microorganisms can also minimize the presence and growth of spoilage microorganisms, leading to an increased product shelf life. In addition, the attention given to standardized procedures can improve product consistency (Troy *et al.*, 2007).

1.10.10. Reduced Wastage

The preventative nature of HACCP allows a company to control costs by minimizing the amount of product requiring rework or rejection, and focusing resources on areas that have been identified as critical in the manufacture of a safe food product. You will find that many problems are addressed before they escalate and before products are shipped from your facility; you will not simply be waiting for the results of end-product testing. With the regular monitoring inherent in a HACCP system, you can become aware of problems earlier, and your costs of wastage can be reduced (Troy *et al.*, 2007).

1.10.11. HACCP Plan

A document prepared in accordance with the principles of HACCP to ensure control of hazards which are significant for food safety in the segment of the food chain under consideration (David, 2004). The application of the HACCP plan in food manufacturing is recommended by FDA because it is considered the most effective and efficient management system to prevent and control food hazards, and to produce safe products. HACCP provides a scientific safety assurance theory that prevents the safety hazards before they occur instead of evaluating the products by end-testing (USFDA, 1997).

HACCP plan covering each product produced by that establishment whenever a hazard analysis reveals one or more food safety hazards that are reasonably likely to occur, based on the hazard analysis conducted in accordance with paragraph (a) of this section, including products in the following processing In order to select the model or models that will be most useful for the activities performed in any specific plant, the following steps should be taken:

1) for slaughtering operations, select the model for the appropriate species, 2) for processed products, make a list of all products produced in the plant, 3) examine the list and group like

products, considering common processing steps and equipment used and4) Compare the grouped products with the list of processes in the regulations; this step should reveal how many and which of the generic models might be useful (USDA, 1999).

1.10.12. Critical Control Points

Critical Control Points (CCPs) are located at any step in the process at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level (Donald, 1998).

1.10.13. Identify Critical Control Points

In a HACCP plan, CCP identification is the foundation of the whole plan. CCP is a point or a step in the food processing where controls can be taken to prevent, eliminate, or reduce the occurrence or the severity of food hazards. The identification process is based on the knowledge of the production process, characteristics of the food products, and the potential food hazards. The number of critical control points depends on the presence of PRP, the nature of the product, the complexity of the process and the accepted risk (Ying Zhen, 2011). A company can choose to indicate each point, process or process step that influences a certain hazard, as a CCP. This makes sense only when the company is able to control each point and this is most often not the case. Furthermore, in this system, the most important critical control points are not getting the needed attention. For this reason, there is a trend in the identification of CCP's to consider as a CCP only these steps, points or processes where loss of control results in an unacceptable risk for public health and where by means of concrete measures an efficient and quick control is possible. The other point, where loss of control doesn't result in an unacceptable risk for public health and where no immediate adjustment of the product happens, is considered as a control point (CP). However, at these control points, inspection is still needed and at regular times, control should be performed (Pieternel *et al.*, 2006). Furthermore, the probability of the occurrence of a serious hazard can only be kept under control, when at these points good preventive measures are present, such as a detailed cleaning and disinfection plan, rules for hygiene, clear work instructions (Pieternel *et al.*, 2006).

1.10.14. CCPs versus CCs

CCP's are points where continuous control is necessary to eliminate or reduce the hazard to an acceptable level. When control of these points is lost, (1) there is a high probability that the products are a risk for public health or are of no good quality or (2) the effect of a certain hazard is serious. The performed controls should be demonstrable by means of registrations. Points, which are only controlled once a month, are no real CCP's (Pieternel *et al.*, 2006).

CP's are points, which need continuous attention, but the risks can be controlled by general preventive measures, belonging to basic rules for hygienic and safe operation in a food company. When the observation of these preventive measures is controlled frequently, the risks are considered as being sufficiently under control (Pieternel *et al*., 2006). The identification of CCP's is a complex and critical process. Some production lines are rather extended and are processing a high number of ingredients. However, the number of CCP's should be limited to 5-10. At a higher number, control becomes too complex. Different companies, producing the same product, can differ in their hazards, risks and also in their CCP's (as a consequence of different layout, equipment, ingredients, and work condition). A general HACCP plan can be used as a guide. However, it is still necessary to consider the specific conditions belonging to a specific production line and that each company identifies its own CCP’s (Pieternel *et al.*, 2006).

1.10.15. Determination of CCPs

A CCP decision tree was developed to incorporate with step directions and facilitates the identification process. For each procedure, food hazards are evaluated. The first thing that needs to be considered is Question 1, which is, if there are any control measures for the identified hazard (Ying Zhen, 2011). If yes, the efficiency of the measurement should be evaluated by Question 2. Is the occurrence of the hazard eliminated or reduced to an acceptable level? If the answer is positive, then it is a CCP. If the answer is negative, then the severity of the hazards will be evaluated. If no health threat exists from this food hazards, it is not a CCP and the process stops. If the contamination is serious enough to risk human's health, then consider the subsequent step. If there is no efficient subsequent step, it is a CCP; otherwise, it is not. For this process, if the Question 1 preventive measure does not exist for the identified hazard, then the necessity of the control will be questioned. If there is no necessity, it is not a CCP. If the control is necessary,

this step needs to be modified with a preventive measure, and brought into the evaluation cycle discussed before (Ying Zhen, 2011).

1.11. Microbial Critical and Predisposing Factors

FATTOM is a mnemonic device that is used in the food service industry to describe the six favorable conditions required for the growth of food-borne pathogens. It is an acronym for food, acidity, time, temperature, oxygen and moisture (National Restaurant Association, 2008) Each of the six conditions that foster the growth food-borne pathogens are defined in set ranges.

1.12. Microbial Water Quality

Water is a natural resource and is essential to sustain life. Accessibility and availability of fresh clean water does not only play a fundamental role in economic development and social welfare, but also it is an essential element in health, food production and poverty reduction (Ashbolt *et al.*, 2001).

However, safe drinking-water remains inaccessible for about 1.1 billion people in the world and the annual deaths due to biologically contaminated drinking-water is estimated to be around 400 children under five years old (Gadgil, 1998). Safe drinking-water is important to (1) maintain the moisture of internal organs of the body and the normal volume and consistency of fluids such as blood and lymph, (2) regulate body temperature, (3) remove poisons or toxins from the body through urine, sweat and breathing, and (4) regulate the normal structure and functions of the skin (Dooge, 2001). The microbiological quality of drinking-water is a concern to consumers, water suppliers, regulators and public health authorities. The potential of drinking-water to transport microbial pathogens to great number of people, causing subsequent illness is well studied and investigated in countries at all levels of economic development (Payment, 1997). Several researchers have attempted to estimate the total burden of waterborne diseases world-wide. Waterborne disease might account for one-third of the intestinal infections world-wide, while it is estimated that water, sanitation and hygiene were responsible for 40.0% of all deaths and 5.70% of the total disease burden occurring worldwide (Pruss *et al.*, 2002). Human, livestock and wild animals are all sources of faecal contamination; in general, human faecal

waste gives rise to the highest risk of waterborne disease (Craun, 1996). A wide spectrum of pathogenic agents can be found in water and monitoring for their presence on a routine basis is impractical. Traditionally, microbial safety of drinking-water has been confirmed by monitoring for absence of micro-organisms from faecal origin (Pruss *et al.*, 2002).

1.12.1. Development of indicators: the Coliforms

The use of bacteria as indicators of the sanitary quality of water probably dates back to 1880 when Von Fritsch described *Klebsiella pneumonia* and *K. rhinoscleromatis* as microorganisms characteristically found in human faeces (Odonkor and Ampofo, 2013). In 1885, Percy and Grace Frankland started the first routine bacteriological examination of water in London, using Robert Koch's solid gelatin media to count bacteria. Also in 1885, Escherich described *Bacillus coli* and renamed it *Escherichia coli.* In 1891, the Franklands came up with the concept that organism's characteristic sewage must be identified to provide evidence of potentially dangerous pollution. By 1893, the Wurtz method of enumerating *E. coli* by direct plating of water samples on litmus lactose agar was being used by sanitary bacteriologists, using the concept of acid from lactose as a diagnostic feature. This was followed by gas production, with the introduction of the Durham tube. The concept of *coliform* bacteria those bacteria resembling *E. coli,* was in use in Britain in 1901 (Odonkor and Ampofo, 2013). The colony count of bacteria in water, however, was not formally introduced until the first report. Therefore, the sanitary significance of findi various Coliforms along with streptococci and *C. perfringens* was recognized by bacteriologists by the start of the twentieth century. It was not until 1905, however, that MacConkay described his now famous MacConkay's broth, which was diagnostic for lactose-fermenting bacteria tolerant of bile salts. Nonetheless, *coli-forms* were still considered to be a heterogeneous group of organisms, many of which were not of faecal origin. The origins of the critical observation that *E. coli* was largely faecal in origin while other Coliforms were not could be claimed (Odonkor and Ampofo, 2013).

1.12.2. Use of Escherichia coli as indicator organism

Escherichia coli are the predominant member of the facultative anaerobic portion of the human colonic normal flora. The bacterium's only natural habitat is the large intestine of warm-

blooded animals and since *E. coli,* with some exceptions, generally does not survive well outside of the intestinal tract, its presence in environmental samples, food, or water usually indicates recent faecal contamination or poor sanitation practices in food-processing facilities. The population of *E. coli* in these samples is influenced by the extent of faecal pollution, lack of hygienic practices, and storage conditions (Odonkor and Ampofo, 2013). The mere presence of *E. coli* in food or water does not indicate directly that pathogenic microorganisms are in the sample, but it does indicate that there is a heightened risk of the presence of other faecal-borne bacteria and viruses, many of which, such as Salmonella spp. or hepatitis A virus, are pathogenic (Brüssow *et al.*, 2004; Odonkor and Ampofo, 2013). For this reason, *E. coli* is widely used as an indicator organism to identify food and water samples that may contain unacceptable levels of fecal contamination. *E. coli* is considered a more specific indicator of fecal contamination than fecal coliforms since the more general test for fecal coliforms also detects thermotolerant non-fecal coliform bacteria. The *E. coli* test recommended by the United States Environmental Protection Agency (EPA) confirms presumptive fecal coliforms by testing for the lack of an enzyme which is selective for the *E. coli* organism. This test separates *E. coli* from non-fecal thermotolerant coliforms (Brüssow *et al.*, 2004; Odonkor and Ampofo, 2013).

1.12.3. Challenges of using E. coli as an indicator organism

As soon as the coliform test came into widespread acceptance, complications with its use and interpretation began to emerge. One concern was the discovery that a variety of microorganisms that read positive in the coliform test were not of fecal origin. As a result, the test method has evolved continually to become more specific. Some of the more significant developments were the so-called fecal coliform test which selects for coliforms of fecal origin by using a higher incubation temperature (Eckner, 1998; Hoffmann *et al.*, 2006; Odonkor and Ampofo, 2013). Though, disease-causing strains of E. coli species have been isolated from tap water, drinking water sources and mountain streams, examination of pathogenic *E. coli* is not easy due to the uncertainty in determining the pathogenic nature of isolated *E. coli* strains. There is no biochemical marker that can separate pathogenic from non-pathogenic strains and the relationship between serotype and pathogenicity is questionable. The use of *E. coli* as an indicator organism is somewhat restricted by the fact that *E. coli* is not a single species; certain genera of the coliform group such as *Proteus* and *Aerobacter* are normally found outside the

human intestinal tract in soil; other organisms found in water that do not represent fecal pollution possess some of the characteristics attributed to *E. coli* and *E. coli* identical to that found in humans is also found in the intestinal tract of other warm-blooded animals (Eckner, 1998). However, primarily, studies have shown that *E. coli* is a much better indicator of disease risk than is faecal coliform, EPA has therefore, recommended that *E. coli* be used as a criteria for classifying waters for fresh water contact recreation. Another weakness of the faecal coliform test and perhaps any indicator organism test geared to human waste is that there are some bacterial pathogens which are unrelated to human wastes (Hoffmann *et al.*, 2006). To the degree that naturally occurring microbial pathogens become a significant public health concern, completely new test procedures may have to be developed. Furthermore, while *E. coli* is specific for faecal contamination, there are three inherent problems of using *E. coli* as a confirmation of faecal contamination: i) it is outnumbered by other types of fecal bacteria making it more difficult to find; ii) it does not survive for long outside of the gut; iii) it can be found in pristine environments in the tropics. Therefore, the absence or presence of *E. coli* via a culture test does not absolutely confirm the absence or presence of faecal contamination. The *E. coli* tests used today as an indication of fecal contamination are commonly culture tests although there are PCR tests for the pathogenic strain *E.coli O157:H7* and for enterotoxigenic strains (Odonkor and Ampofo, 2013). In addition to the inherent differences in the ecology of the above mentioned indicator organism, there is also the problem using culturable tests. All culture tests have an inherent bias in that they always underestimate the number of *E. coli* present in the sample. This occurrence happens for a number of reasons, but in the instance of recovering faecal indicators, the bias is primarily for two reasons: i) *some healthy* coliforms are viable but will not grow in the media prescribed for them; and ii) coliforms found in the environment are often stressed thereby making recovery very difficult despite the growth media used (Eckner, 1998; Hoffmann *et al.*, 2006; Odonkor and Ampofo, 2013).

1.12.4. Current trends of E. coli as indicator organism

While the faecal coliform test has its limitations and problems, it also has many attributes (Feng and Hartman, 1982; Frampton and Restaino, 1993). Perhaps, the most significant attribute is that: as a regulatory tool, it has worked long and well. In the case of water quality regulation, coliform testing has been used successfully for well over fifty years. For the foreseeable future,

the faecal coliform test will continue to be the basis for much of the regulatory decision making regarding both quality water harvesting and contact recreation. The primary bias of using culturable tests in isolating *E. coli* as an indicator organism, has being overcome by using PCR, which detects both live and dead bacteria. The PCR is a rapid and reliable tool for the molecular-based diagnosis of a variety of infectious diseases. PCR analysis for screening drinking water and environmental samples has been reported, and has been utilized to identify *E. coli* in primary water specimens, stool specimens and outbreaks (; Frampton and Restaino, 1993; Odonkor and Ampofo, 2013).

Chapter Two

Materials and Methods

2.1. Sampling of the Restaurants

A cross-sectional study was conducted for a period of six months, from June to December 2012, in Salalah Municipality, Sultanate of Oman. A total number of 142 samples were collected from 21 restaurants from 7 different areas (3 restaurants in each), namely; Al-Haafah, Al-Sinaat Al-Jadeedah, Al-Goof, Awgaad, Al-Saadah North and South and Salalal Al-Wustaa. Samples were collected randomly as follow: 41 ready to eat food samples and 38 water

samples, in addition to 63 swab samples. The food samples were collected in sterile bags and included cooked meats (chicken, fish and beef) and beans (lentil) and vegetables (potatoes and others). The water samples were collected in sterile 100-ml glass bottles from the drinking water and from the water used for cleaning and washing the utensils used in food preparation in the restaurants. Furthermore, the swab samples were collected from the hands worker , surfaces and kitchenware (knives, and cutting boards) used for food preparation. The collected food samples and the swabs were marked, numbered and transported promptly on ice to the Food and Water Laboratory, Directorate General of Salalah State Municipality, where they were examined.

2.2. Isolation and Identification Procedures

2.2.1. Testing of the Water Samples

The water samples were transported on ice to the municipal laboratory within 2 hours and **were each inoculated into one Colilert-18 Quantitray. Undiluted freshwater samples were a+*ssayed directly in accordance with the manufacturer's instructions. The inoculated Quantitrays were subsequently sealed and incubated at 35°C for 18 to 20 hours. Following incubation, the Quantitray wells were read for Y color, indicating ONPG hydrolysis, and fluorescence, indicating MUG cleavage. A handheld UV light (366 nm) was used to identify F wells. The number and types of well reactions in each Quantitray were translated into MPN estimates for fecal coliforms and *Escherichia coli* according to the manufacturer's instructions. Following incubation, the backing material of each Quantitray was disinfected by application of 70% ethanol with a sterile swab. After the residual ethanol evaporated, sterile pipette tips were used to pierce the backing material of two MUG-positive, ONPG-positive wells; two MUG-positive, ONPG-negative wells; and one MUG-negative, ONPG-positive well per tray. One tray was processed per water sample. One hundred microliters of fluid was withdrawn from each well and added to a separate tube containing 5 ml of EC broth (Difco) and to a Durham tube. The samples from the Colilert wells, an *E. coli*-positive control, and an uninoculate control were incubated at 44.5°C in a water bath. After 24 hours, all of the tubes were examined for turbidity and the Durham tubes were examined for gas. At the same time the EC tubes were inoculated, fluid from each well was used to inoculate selective-differential media. One drop (approximately 20 µl) of well content was streaked for isolation on MacConkey agar and Trypticase soy agar

(TSA) amended with 10 mg of MUG/liter (TSA plus MUG). Following incubation at 35.0°C for 24 hours, colonies were examined for lactose utilization on MacConkey agar and for MUG activity on TSA plus MUG.

2.2.2. Testing of the Food Samples

a. Total Bacterial and *Enterobacteriace* Counts

The Pouring Plate Method (PPM) was used for the Total Bacterial Count (TBC) and *Enterobacteriace* Enumeration (EE). The standard plate count agar was used in the total plate count while the violet red bile glucose agar was used for enumerating the *Enterobacteriace* as described by Barrow and Feltham (2003).

The total bacterial count of the isolated microorganism was carried out by making of a serial dilution of each sample (form 10^{-1} up to 10^{-5}). Ten-fold increments were done by preparing five sterile and labelled test tubes from (1) to (5). From the test tube (1), a solution of 1 ml was pipette into the test tube (2) which contains 9 ml of distilled water to yield a total volume of 10 ml to form 1. The process continued until serial dilution of original bacterial suspension in the test tube (5) was made. Each dilution was spread out on a disposable Petri-dish contained a solidified agar medium. Then 0.1-0.2 ml of the dilution were taken out, this was done by sterile bent spreader. Then all plates incubated upside down at 37°C. After 24 hours the number of all colonies on the plate, between 30 and 300, was counted for each dilution and the mean count was determined. Each colony forming unit represented a bacterium that was present in the diluted sample, therefore the concentration of viable bacteria per Millilitre in initial sample was calculated and expressed in cfu /ml.

The Enterobacteriace Enumeration was carried out by using a duplicate 1 ml pour plates with a Violet Red Bile Agar (VRBA) overlay which were prepared using dilutions of each analytical unit. Plates were incubated at 35-37ºC for 24-48 hrs. The ones that did not develop colonies were computed as count 1 colony forming unit (cfu) multiplied by the smallest used dilution factor (Anon. 1992). The higher limit of detection was 1 X $l0^4$ cfu g^{-1} and the lower was 10 cfu g^{-1}.

a.a. Methodology of viable bacterial cell count

Serial dilutions were used; plating and counting of live bacteria to determine the number of bacteria in a given population were used. Serial dilutions of a solution containing an unknown number of bacteria were made.

The total number of bacteria in the original solution was determined by counting the number of colony forming units and comparing them to the dilution factor. Each colony forming unit represented a bacterium that was present in the diluted sample. The numbers of colony forming units (CFU's) were divided by the product of the dilution factor and the volume of the plated diluted suspension to determine the number of bacteria per mL that were present in the original solution.

a.a.a. Serial Dilutions

Five small, sterile test tubes were prepared labeled 1 through 10 and then 4.5 ml of M9 salts was added to each test tube. M9 salts are a physiological buffered minimal medium that contains inorganic salts but no carbon source. Bacteria do not grow in this media but remain in a state of stasis until the diluted cells are plated on media containing a carbon source.

0.5 ml of the original solution was pipetted into test tube 1. Bacterial suspension was mixed thoroughly (using the vortexes on each bench) before proceeding to the next step. 0.5 ml of the diluted bacterial suspension was withdrawn using from the first test tube a clean pipette and pipettes that into the second test tube. Continual in this fashion until serial dilution of original bacterial suspension into test tube 5 was made. In test tube the bacteria was diluted 10 fold, a 1:10 or 1 x 10-1 dilution, in test tube 5 was the bacteria diluted from the original tube to obtain a 1 x 10-5 dilution.

a.a.b. Plating the serially diluted cells

The following dilutions were made: 1 x 10-1, 1 x 10-2, 1 x 10-3, 1 x 10-4, 1 x 10-5. Were cultured in TSA plates and incubated at 37 ^{0}C. After sterilizing the stick, the hockey stick was used to spread the bacterial suspension evenly over the entire surface of the plate. The plate was allowed to dry. This process was done with the remainder of the bacterial dilutions. All the plates were taped together and incubated, upside down, at 37 ^{0}C for 24 hours.

To calculate the number of bacteria per mL of diluted sample one should use the following equation:

$$\frac{\text{Number of CFU}}{\text{Volume plated (mL) x total dilution used}} \longrightarrow \frac{\text{Number of CFU}}{\text{mL}}$$

For cell suspension and counted 200 bacteria, then the calculation would be:

200/0.1 mL x 10-5 or 200/10-4 or 2.0 x 1011 bacteria per ml

b. Bacterial isolation and identification

The isolation and identification of the bacteria were done as described by Barrow and Feltham (2003). The swab and food samples were cultured using prepared Nutrient Agar, Nutrient Broth, Buffered Peptone Water and Selenite Cystine Broth, Tryptone Broth, VRBA, MCA, Brilliant Green Agar, Xylose Lysine Deoxycholate Agar (XLDA), Blood Agar, Mannitol Salt Agar (MSA) and Baird Parker Agar. The plates were incubated at 37°C for 24 hrs but some steps needed to be incubated at 44°C. The morphology of colonies on agar media were examined microscopically, smears were made from clean slides which werer fixed with heat and subjected to Gram stain and examined under oil immersion. In addition to that, the identification was also based mainly on biochemical tests (Barrow and Feltham, 2003).

b.a. Liquid cultural media

b.a.a. Peptone water

Peptone water was prepared according to Cruikshnk *et al.* (1975). Ten gram peptone and five grams NaCl were dissolved by heating in 1000 ml distilled water. The pH was adjusted to 7.2 and the medium was distributed in five amounts in the test tubes and sterilized by autoclaving at 1150 C for 15 minutes under pressure 15lb per square inch. The stock was preserved in the refrigerator.

b.a.b. Nutrient broth

Nutrient broth contained lab-lemco powder one gram yeast extract two grams peptone five grams and sodium chloride five gram. The pH was adjusted to 7.4 approximately. An amount of 13 grams of the dehydrated medium was added to one liter of distilled water. The reconstituted medium was mixed well and distributed in five ml amounts and sterilized by autoclaving at 121^0C for 15 minutes under pressure 15 lb per inch.

b.b. Solid cultural media

b.b.a. plate count agar

Plate Count Agar (PCA), also called Standard Methods Agar (SMA), is a microbiological growth medium commonly used to assess or to monitor "total" or viable bacterial growth of a sample. PCA is not a selective medium. The composition of plate count agar may vary, but typically it contains (w/v):[1] 0.5% peptone 0.25% yeast extract 0.1% glucose 1.5% agar pH adjusted to neutral at 25 C.

b.b.b. Violet Red Bile Glucose Agar (VRBGA)

VRBGA is a glucose-containing selective medium for the detection and enumeration of *Enterobacteriaceae* in food products. Results from tests that may be applied to water to detect coli-aerogenes organisms as possible indicators of faecal contamination possess far less significance when applied to raw foods. In the examination of foodstuffs, detection of a more defined group of organisms, the *Enterobacteriaceae* that ferment glucose to produce acid and/or gas has been recommended.

b.b.b.a Technique

A series of dilutions of the samples were prepared so that at least one will be included that will yield 100-200 colonies from a 1 ml aliquot. 1 ml aliquots was transfered of each dilution to 9 cm Petri dishes using 2 plates foreach dilution. 15 ml of medium were added and cooled to 47° C. The plates were gently swirled 3 times clockwise and 3 times anti-clockwise. After the medium has solidified, an overlay with 10 ml of the same medium was made and left to solidify. The dishes were inverted and incubated at > 42°C for 18 hours, 32°C for 24-48 hours or 4°C for 10 days depending on the groups of *Enterobacteriaceae* to be recovered. The agar overlay ensured anaerobic conditions which suppressed the growth of non-fermentative Gram-negative

bacteria. It also encouraged the fermentation of glucose which favors the formation of clearlyvisible purple colonies, surrounded by a purple-pink halo.

b.b.b.b. Characteristic appearance of colonies

Round, purple-pink, 1-2mm diameter surrounded by purple haloes. Although colony size is generally 1-2 mm, size can be affected by a number of influences and all purple pink colonies should be counted. Confirmation of the identity of these colonies must be made by further test.

b.b.c. Violet Red Bile Agar (VRBA)

VRBA is a medium used for the enumeration of coliforms in food and dairy product conforms to American Public Health Association (APHA).

b.b.c.a. Test procedure

Presumptive test for coliforms using solid medium: 1) a 1 ml aliquot of test sample was transfered to a petri dish, 2) 10 ml of VRBA were added (at 48°C) and swirled to mix, 3) the medium was allowed to solidify before incubating at 35°C for 18 - 24 hours and 4) finally the medium was examined for purple-red colonies, 0.5 mm in diameter (or larger), surrounded by a zone of precipitate bile acids.

Lactose fermenters were purple-red, with or without a zone of precipitate around the colonies. Lactose non-fermenters were colorless to transparent colonies. Gram-positive cocci were colorless, pin-point colonies. For *E. coli* confirmation was done by brilliant green + truptone water at 44 ºC.

b.b.d. Mannitol salt agar (MSA)

MSA is used for the isolation of staphylococci. Chapman formulated MSA to isolate staphylococci by inhibiting growth of most other bacteria with a high salt concentration. Chapman added 7.5% Sodium Chloride to Phenol Red Mannitol Agar, and noted pathogenic strains of staphylococci (coagulase-positive staphylococci) grew abundantly and produced yellow colonies with yellow zones. Nonpathogenic staphylococci produced small red colonies with no color change to the surrounding medium. However, MSA is highly selective, and specimens from heavily contaminated sources may be streaked onto this medium without danger

of overgrowth. It is recommended for isolating pathogenic staphylococci from clinical specimens, cosmetics, and microbial limit tests.

b.b.d.a. Principles of the procedure

Enzymatic digest of Casein, animal tissue, and beef extract provide the nitrogen, vitamins, and carbon in Mannitol Salt Agar. D-Mannitol is the carbohydrate source. In high concentrations, Sodium Chloride inhibits most bacteria other than staphylococci. Phenol Red is the pH indicator. Agar is the solidifying agent. Bacteria that grow in the presence of a high salt concentration and ferment mannitol produce acid products, turning the Phenol Red pH indicator from red to yellow. Typical pathogenic staphylococci bacteria ferment mannitol and form yellow colonies with yellow zones. Typical non-pathogenic staphylococci bacteria do not ferment mannitol and form red colonies.

b.b.d.b. Test procedure

Specimens were inoculated on medium as a primary isolation or inoculated isolated colonies onto medium for differentiation.

Staphylococci grew on this medium, while the growth of most other bacteria was inhibited. Coagulase-positive staphylococci produced luxuriant growth of yellow colonies and may have a yellow halo around the colony. Coagulase-negative staphylococci produced small colorless to pink colonies with no color change to the medium.

b.b.e. Baird Parker agar (BPA)

BPA is a medium used for detection and enumeration of *Staphylococcus aureus* in foods. It was first described in 1962. It is a selective medium for the isolation and presumptive identification of coagulase-positive staphylococci. They can grow and reproduce in cosmetic products. These products are tested for the presence of coagulase-positive staphylococci using standard microbiological methods.

b.b.e.a. Test procedure

1) dilutions of test samples were prepared, 2) 1 ml of the sample was transfered to each of 3 Baird Parker Agar plates, distributed over the surface using a sterile, bent glass rod, 3) the inoculum was allowed to be absorbed by the medium before inverting the plates, 4) the plates were then incubated at 35 - 37°C for 45 - 48 hours and 5) finally examined for 20 - 200 colonies, counting colonies typical of *Staphylococcus aureus*.

Coagulase-positive staphylococci produced black, shiny, convex colonies with entire margins and clear zones, with or without an opaque zone. Coagulase-negative staphylococci produced poor or no growth. If growth occurs, colonies are black; clear or opaque zones were rare. The majorities of other organisms were inhibited or grew poorly. If growth appeared, colonies were light to brown-black, with no clear or opaque zones.

b.b.f. Thiosulfate-Citrate-Bile-Sucrose Agar (TCBSA)

TCBSA, also called Vibrio Selective Agar, is a medium used for the selective isolation of *Vibrio cholera* and other enteropathogenic vibrios. All pathogenic Vibrio species, except *Vibrio hollisae*, will grow on TCBSA. This highly selective agar meets the nutritional requirements of Vibrio species, and allows them to compete with intestinal flora. Vibrio species are able to grow in media containing increased salt concentrations, and some species are halophilic**.** Infections have been associated with ingestion of contaminated water and consumption of contaminated shellfish or seafood. Vibrio species are natural inhabitants of seawater.

After 18 – 48 hours of incubation at 35 ± 2ºC, sucrose-fermentating vibrios (*V. cholerae*, *V. alginolyticus*, *V. hareyi*, *V. cincinnatiensis*, *V. fluvialis*, *V. furnissii*, *V. metschnikovii*, and some *V. vulnificus* strains) appear smooth, opaque, thin-edged yellow colonies on TCBSA.

b.b.g Selenite cystine broth base

It is recommended as a selective enrichment media for Salmonella and possibly *Shigella sonnei* from faeces, urine, water and foodstuffs. Suspend 19.01 grams in 1000 ml distilled water. 4 grams of sodium hydrogen selenite (M1079B) were added and warmed to dissolve the medium completely and then distributed in sterile test tubes. Sterilization in a boiling water bath or free flowing steam for 10 minutes was made. Large amount of selenite was reduced (indicated by red precipitate at the bottom of tube/bottle).

b.b.h Xylose lysine agar (XLA)

XLA is a medium used for the isolation and differentiation of enteric pathogens. XLA base was supplemented with sodium thiosulfate, ferric ammonium citrate, and sodium deoxycholate to develop a more selective medium, XLD Agar. XLD Agar was developed principally for isolating Shigella species and Providencia species, and shown to be an effective differential media.

b.b.i. Brilliance bacillus cereus agar

Brillianc Bacillus cereus Agar (formerly Chromogenic Bacillus cereus Agar) is a chromogenic medium for the isolation and differentiation of Bacillus cereus from food samples Suspend 20.5g in 500ml of distilled water. Mix well and bring to the boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. Cool the medium to 50°C and aseptically add 1 vial of Brilliance Bacillus cereus Selective Supplement. Mix well and pour into sterile Petri dishes.

b.b.j. Oxford listeria agar base

is used with antimicrobics for the selective isolation of *Listeria* spp.

Listeria monocytogenes, described first in 1926 by Murray, Webb, and Swann, is an extensive problem in public health and food industries. This organism has the ability to cause human illness and death, particularly in immunocompromised individuals. Epidemiological evidence from outbreaks of listeriosis has indicated that the principle route of transmission is via the consumption of foodstuffs contaminated with *Listeria monocytogenes*. Implicated vehicles of transmission included turkey frankfurters, coleslaw, pasteurized milk, Mexican style cheese, and pate. *Listeria* spp. are ubiquitous in nature, being present in a wide range of unprocessed foods as well as in soil, sewage, and river water.

Oxford Listeria Agar Base is prepared according to the formulation of Curtis et al.6 *Listeria* spp. grow over a pH range of 5.0 - 9.6, and survive in food products with pH levels outside these parameters.

b.b.j.a Oxford Medium Base

1. Suspend 57.5 g of the medium in one liter of purified water.

2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.

3. Autoclave at 121°C for 10 – 15 minutes. Cool to 45 - 50°C.

b.b.j.b Oxford Medium

Aseptically add a filtered sterilized aqueous solution of 5 mg acriflavin, 2 mg cefotetan, 20 mg colistin sulfate, 400 mg cycloheximide*, and 10 mg phosphomycin. Note:*Natamycin may be used in place of cycloheximide at 25 mg.

b.b.j.c Test Procedure

adding 25 mL of liquid or 25 g of solid material to 225 mL Listeria Enrichment Broth and incubating at 30°C for two days. After enrichment, the broth is plated onto Oxford Medium.

2.3. Statistical Analyses

The data were analyzed with Statistical Package for the Social Sciences (SPSS) version 20.0, IBM/SPSS. Descriptive statistics were used to analyze the data. In addition, all TVCs bacteria were converted to $\log_{10}$ cfu/cm^2 for analysis and ANOVA was performed to compare the recorded means. Statistical significance was set at *p- value* of ≤0.5.

Chapter Three

Results

3.1. Microbial Quality of Water

3.1.1. Coliforms and E. coli in Drinking Water

13, 50 and 14 coliform bacteria were detected in one of the investigated three sites at Al-Haafah, Al-Sinaat Al-Jadeedah and Al-Goof areas respectively. However, 50 and 1 coliform bacteria were counted in two sites at Awgaad and 50 and 51 at Al-Saadah North as well. Coliforms were not found in Al-Saadah South and Salalal Al-Wustaa. However, *E. coli* was not detected in any drinking water of the investigated 21 sites. Furthermore, 12 (57.1%) of the studied sites had safe drinking water while 7 (33.3%) had unsafe drinking water. Besides, the quality of the water of 2 (9.6%) sites was not evaluated (Table 1).

Table 1: Number of Coliform and *E. coli* Bacteria in the Drinking Water by Area in Restaurants of Salalah Municipality (From June to December/2012).

Area	Site	No. of Coliform	95% CI		No. of *E. coli*	95% CI		Result
			Lower	Higher		Lower	Higher	
Al-Haafah	a	13	8.8	25.7	0	0	3.70	0
	b	0	0	3.70	0	0	3.70	1
	c	0	0	3.70	0	0	3.70	1
Al-Sinaat	d	50	146	~	0	0	3.70	0
	e	0	0	3.7	0	0	3.70	1
	f	-	-	-	-	-	-	-
Al-Goof	g	0	0	3.70	0	0	3.70	1
	h	14	9.8	27.5	0	0	3.70	0

	i	0	0	3.70	0	0	3.70	1
Awgaad	j	50	146	~	0	0	3.70	0
	k	1	0	3.70	0	0	3.70	0
	l	-	-	-	-	-	-	-
Al-Saadah North	m	0	0	3.70	0	0	3.70	1
	n	51	146	~	0	0	3.70	0
	o	50	146	~	0	0	3.70	0
Al-Saadah South	p	0	0	3.70	0	0	3.70	1
	q	0	0	3.70	0	0	3.70	1
	r	0	0	3.70	0	0	3.70	1
Salalal Al-Wustaa	s	0	0	3.70	0	0	3.70	1
	t	0	0	3.70	0	0	3.70	1
	u	0	0	3.70	0	0	3.70	1

Alphabets indicate sampled sites = 21, - = not done, ~ = unlimited & results: 1 = safe, 0 = not safe

3.1.2. Coliforms and E. coli in Washing Water

1, 45 and 10 coliforms and 2 *E. coli* bacteria were detected, respectively, in the three investigated sites at Al-Haafah area. At Al-Sinaat Al-Jadeedah 50 coliforms and 8 *E. coli* colonized one site. However, coliforms (1, 10 and 3 bacteria) and *E. coli* (0 bacteria) colonized the three sites at Al-Goof. But only 1 coliform and 0 *E. coli* was detected at Awgaad area. 0, 0, 51, 51, 2 and 3 coliforms and 0 *E. coli* were computed in the three sites of Al-Saadah North and South, respectively. 0 coliforms and 0 *E. coli* were seen in the three sites of Salalal Al-Wustaa. Furthermore, one third (n = 7, 33.3%) of the sites had safe and less than two thirds (n = 12, 57.1%) had unsafe washing water and the quality of the water of 9.6% (n = 2) sites was not measured (Table 2).

Table 2: Number of Coliform and *E. coli* Bacteria in the Washing Water by Area in Restaurants of Salalah Municipality (From June to December/2012).

Area	**Site**	**No. of Coliform**	**95 % CI**		**No. of *E. coli***	**95 % CI**		**Result**
			Lower	**Higher**		**Lower**	**Higher**	
Al-Haafah	a	1	0.30	5.6	0	0	3.70	0
	b	45	78.6	185.7	0	0	3.70	0
	c	10	6.1	20.5	2	0.6	7.3	0
Al-Sinaat	d	0	0	3.70	0	0	3.70	1
	e	0	0	3.70	0	0	3.70	1
	f	50	146	~	8	4.5	17.1	0
Al-Goof	g	1	0	3.70	0	0	3.70	0
	h	10	6.1	20.5	0	0	3.70	0
	i	3	1.7	9	0	0	3.70	0
Awgaad	j	-	-	-	-	-	-	-

	k	-	-	-	-	-	-	-
	l	1	0	3.70	0	0	3.70	0
Al-Saadah North	m	0	0	3.70	0	0	3.70	1
	n	0	0	3.70	0	0	3.70	1
	o	51	146	~	0	0	3.70	0
Al-Saadah South	p	51	146	~	0	0	3.70	0
	q	2	0.6	7.3	0	0	3.70	0
	r	3	1.1	9	0	0	3.70	0
Salalal Al-Wustaa	s	0	0	3.70	0	0	3.70	1
	t	0	0	3.70	0	0	3.70	1
	u	0	0	3.70	0	0	3.70	1

Alphabets indicate sampled sites = 21, - = not done, ~ = unlimited & results: 1 = safe, 0 = not safe

3.2. Microbial Quality of Food

3.2.1. Prevalence of Bacterial Species in Restaurants

Isolation and identification of bacteria from the 7 different areas under investigation revealed two prevailing species of bacteria as shown in Table 3. *E. coli* and *Staphylococcus aureus* were detected in the samples collected from meat, vegetables, utensils used for food preparation, surfaces and hands of workers in the restaurants. *E. coli* was prevalent in 5.0% (95% CI, 0.71 - 9.29) and *Staphylococcus aureus* was in 3.0% (95% CI, -1.08 - 7.08) of the investigated samples. However, no any *Salmonella* species, *Bacillus* species, *Listeria* species and Yeast and molds were detected in any of the studied sites/restaurants; prevalence of 0.0% (95% CI, 0.0 - 0.0).

Table 3: Prevalence of Isolated Bacteria from the different investigated Restaurants of Salalah Municipality (From June to December/2012).

Bacteria	**Number of tested**	**Number of positive**	**Percentage of Positive**	**95% CI**	
				Lower	**Upper**
E. coli	99	5	5.0	0.71 - 9.29	
Salmonella species	50	0	0.0	0 - 0	
Staphylococcus aureus	67	2	3.0	-1.08 - 7.08	
Bacillus	50	0	0.0	0 - 0	
Listeria	50	0	0.0	0 - 0	
Yeast and molds	50	0	0.0	0 - 0	

3.2.2. Total Bacterial Count

The study revealed no statistical significant difference, at *p-value* ($p \leq 0.05$), observed in the Total Bacterial Count (TBC) estimated between the samples collected from meat, vegetables, utensils used in food preparation, surfaces and hands of workers in restaurants of Al-Haafah, Al-Sinaat Al-Jadeedah, Al-Goof, Awgaad, Al-Saadah North and South and Salalal Al-Wustaa. As shown in Table 2 and Fig. 1, the TBC revealed the highest contamination levels in meat (3.3×10^5 cfu/cm^{-2}) in Al-Sinaat area, in vegetables (1.3×10^4 cfu/cm^{-2}) in Al-Saadah South area, in utensils (1.0×10^5 cfu/ml) in Al-Haafah and Al-Saadah North areas, in surfaces (2.0×10^5 cfu/ml) in Al-Haafah area and on hands of workers (1.6×10^5 cfu/ml) in Al-Saadah South area.

Table 4: Comparison of Mean Total Bacterial Count ($\log_{10}$ cfu/cm^{-2} or $\log_{10}$ cfu/ml) by Area and Kind of Food and the other investigated Critical Control Points in Restaurants of Salalah Municipality (From June to December/2012).

Area	**Food and Critical points**					**Significance**
	Meat	**Vegetable**	**Utensils**	**Surfaces**	**Hands**	
Al-Haafah	1.4×10^5	3.7×10^3	1.0×10^5	2.0×10^5	1.0×10^5	NS
Al-Sinaat	3.3×10^5	1.0×10^4	2.7×10^4	4.2×10^4	1.0×10^5	NS
Al-Goof	1.0×10^3	1.4×10^2	3.8×10^3	1.0×10^5	1.4×10^5	NS
Awgaad	1.6×10^5	1.3×10^3	1.5×10^3	1.1×10^3	1.0×10^5	NS
Al-Saadah North	7.6×10^2	2.7×10^3	1.0×10^5	5.2×10^4	4.9×10^3	NS
Al-Saadah South	1.0×10^5	1.3×10^4	2.5×10^4	7.3×10^4	1.6×10^5	NS
Salalal Al-Wustaa	1.0×10^2	6.6×10^3	1.0×10^3	1.0×10^5	3.8×10^4	NS

* = significant difference at ($P < 0.05$); ND = not done and NS = not significant ($P > 0.05$)

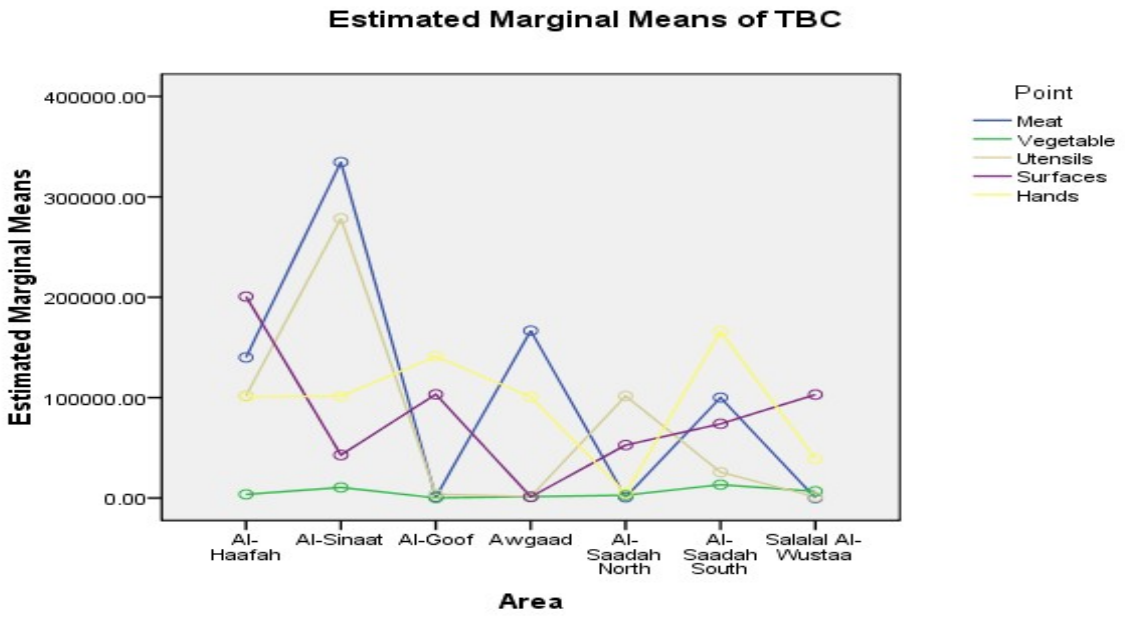

Fig 1: Comparison of Mean Total Bacterial Count (log_{10} cfu/cm^{-2} or log_{10} cfu/ml) by Area and Kind of Food and the other investigated Critical Control Points in Restaurants of Salalah Municipality (From June to December/2012).

3.2.3. Enterobacteriace Enumeration

The study revealed no statistical significant difference, at *p-value* ($p \leq 0.05$), observed in the *Enterobacteriace* Enumeration estimated between the samples collected from meat, vegetables, utensils used in food preparation, surfaces and hands of workers in restaurants of Al-Haafah, Al-Sinaat Al-Jadeedah, Al-Goof, Awgaad, Al-Saadah North and South and Salalal Al-Wustaa. As shown in Table 2 and Fig. 1, the EE revealed the highest contamination levels in meat (0.037×10^3) in Al-haafah and Al-Sinaat area, 1in vegetables (1.000×10^3) in Salalaha Al-Wustaa area, in utensils (3.400×10^3) in Al-Saadah North area, in surfaces (1.500×10^3) in Salalaha Al-Wustaa area and on hands of workers (0.267×10^3) in Al-Saadah North area.

Table 5: Comparison of Mean *Enterobacteriace* Enumeration (log_{10} cfu/cm^{-2} or log_{10} cfu/ml) by Area and Kind of Food and the other investigated Critical Control Points in Restaurants of Salalah Municipality (From June to December/2012).

Area	Food and Critical points	Significanc

	Meat	Vegetable	Utensils	Surfaces	Hands	e	
Al-Haafah	0.037×10^3	0.033×10^3	0.033×10^3	0.067×10^3	0.033×10^3		NS
Al-Sinaat	0.037×10^3	0.067×10^3	0.029×10^3	0.037×10^3	0.000×10^3		NS
Al-Goof	0.000×10^3	0.000×10^3	0.000×10^3	0.133×10^3	0.033×10^3		NS
Awgaad	0.033×10^3	0.133×10^3	0.000×10^3	0.000×10^3	0.000×10^3		NS
Al-Saadah North	0.000×10^3	0.667×10^3	3.400×10^3	1.100×10^3	0.267×10^3		NS
Al-Saadah South	0.033×10^3	0.033×10^3	0.000×10^3	0.400×10^3	0.000×10^3		NS
Salalal Al-Wustaa	0.000×10^3	1.000×10^3	0.100×10^3	1.500×10^3	0.000×10^3		NS

* = significant difference at (P ≤0.05); ND = not done and NS = not significant (P > 0.05)

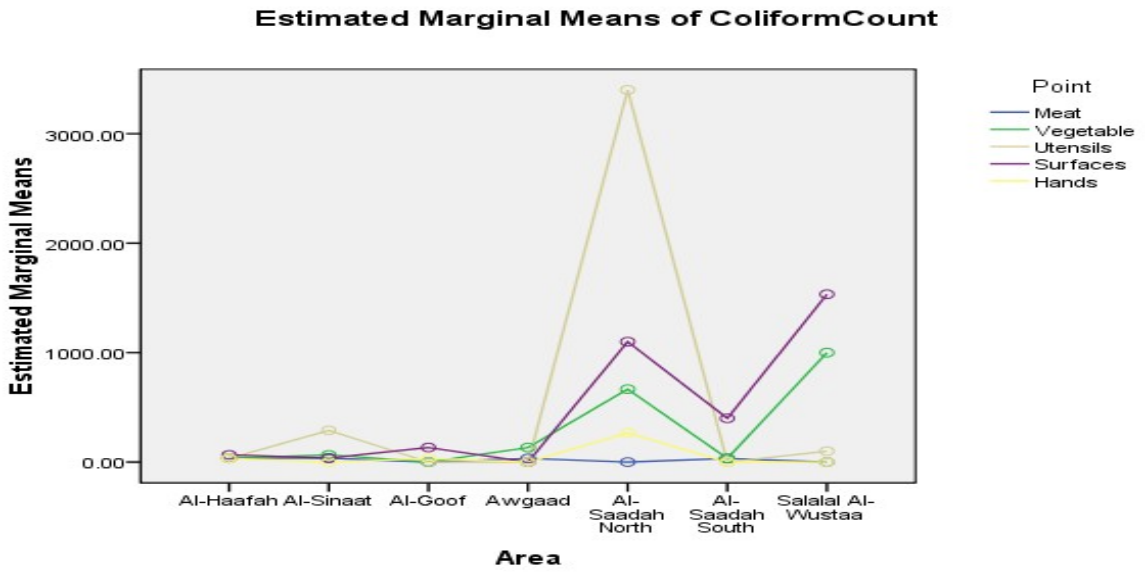

Fig 2: Comparison of Mean *Enterobacteriace* Enumeration ($\log_{10}$ cfu/cm^{-2} or $\log_{10}$ cfu/ml) by Area and Kind of Food and the other investigated Critical Control Points in Restaurants of Salalah Municipality (From June to December/2012).

3.3. Food Safety Knowledge of the Restaurants Workers

All the respondents (n = 21, 100%) were sure that washing hands before starting food preparation, putting on or using gloves during food preparation, as well as, proper cleaning and handing of food preparation instruments reduce the risk of food contamination. Moreover, all of them (n = 21, 100%) were once again sure that eating and drinking at the work place increase the risk of food contaminationand food-borne illnesses impact the society. All persons, including: children, adults, pregnant women and elderly are at equal risk for food poisoning were perceived true by 14.2% (n = 3) while the rest (n = 18, 85.8%) of the respondents did not agree (Table 6).

Table 6: Responses of the Restaurants Workers in Salalah Municipality regarding Risk of Food Contamination (From June to December/2012)

Statement	A	%	B	%	C	%

Washing hands reduces risk	21	100	0	0.0	0	0.0
Wearing gloves reduces risk	21	100	0	0.0	0	0.0
Cleaning instruments reduces risk	21	100	0	0.0	0	0.0
Ingestion at work place increases risk	21	100	0	0.0	0	0.0
All persons are at risk for food poisoning	03	14.2	18	85.8	0	0.0
Food-borne illnesses impact the society	21	100	0	0.0	0	0.0

A- True, B- false, C- do not know

As presented in Table 7, Typhoid (n = 11, 52.3%), jaundice(n = 8, 38.0%), diarrhea (n = 21, 100%), brucellosis (n = 9, 42.8%) and bloody diarrhea (n = 19, 90.4%) were surely thought to be transmitted by food while all of the respondents (n = 21, 100%) answering the questionnaire indicated that Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome (HIV/AIDS) is not transmissible by food.

Table 7: Responses of the Restaurants Workers in Salalah Municipality regarding Diseases Transmitted by Food (From June to December/2012)

Diseases Transmitted by Food	**A**	**%**	**B**	**%**	**C**	**%**
Typhoid	11	52.3	10	47.7	0	0.0
Jaundice	08	38	13	62	0	0.0
Diarrhea	21	100	0	0.0	0	0.0
HIV/AIDS	00	0	21	100	0	0.0
Brucellosis	09	42.8	12	57.2	0	0.0
Bloody diarrhea	19	90.4	02	9.6	0	0.0

A- True, B- false, C- do not know

57.2, 52.3%, 52.3%, 0.0, and 71.4% indicated that *Salmonella* species, *Staphylococcus* species, *Clostridium* species and Hepatitis A and B viruses are among the bacterial and viral food-borne pathogens (Table 8).

Table 8: Responses of the Restaurants Workers in Salalah Municipality regarding Food-borne Pathogens (From June to December/2012)

Food-borne Pathogens	**A**	**%**	**B**	**%**	**C**	**%**
Salmonella species	12	57.2	9	42.8	0	0.0
Hepatitis A virus	11	52.3	10	49.7	0	0.0
Hepatitis B virus	0	0.0	21	100	0	0.0
Staphylococcus species	11	52.3	10	49.7	0	0.0
Clostridium species	15	71.4	6	28.6	0	0.0

A- True, B- false, C- do not know

Regarding infectious diseases, all the respondents (n = 21, 100%) thought it is very necessary to take a leave from work when a worker has an infectious disease of skin or eyes but only 9.5% and 19% knew the correct temperature of the refrigerator is 4 ºC and thought for sure abortion is a food-borne disease (Table 9).

Table 9: Responses of the Restaurants Workers in Salalah Municipality regarding infectious diseases (From June to December/2012)

Infectious disease	**A**	**%**	**B**	**%**	**C**	**%**
Refrigerator temp is 4 ºC	02	9.5	19	90.5	0	0.0
Infectious disease of skin	21	100	0	0.0	0	0.0
Infectious disease of eye	21	100	0	0.0	0	0.0
Abortion by food-borne disease	04	19	17	81	0	0.0

A- True, B- false, C- do not know

To ensure the reduction of micro-organisms to the least possible number in the served food the following food-safety measures: washing hands, putting on/wearing or using gloves, an apron and caps are thought to be very important by all (n = 21, 100%) of the respondents responsibilities of the food handler. Moreover, food handlers who have abrasion or cut on hands should not touch foods without gloves, raw and cooked foods should be stored separately, food hygiene training for workers, checking the temperature of the refrigerator periodically and evaluation of the health status of the workers before employing are other important food-safety measures, when applied for sure will result in reducing the risk of food contamination as claimed and perceived by 100% (n = 21) of the respondents. Also, 100% (n = 21) of the respondents were certain that food-borne illnesses can have deleterious health and economic effect on the society. 18 (85.7%) and 3 (14.3%) of the respondents think that putting on masks is important in reducing risk of food contamination is true and false, respectively (Table 10).

Table 10: Responses of the Restaurants Workers in Salalah Municipality regarding infectious diseases (From June to December/2012)

Food Safety Measures	A	%	B	%	C	%
Washing hands	21	100	0	0.0	0	0.0
Wearing gloves	21	100	0	0.0	0	0.0
Wearing apron	21	100	0	0.0	0	0.0
Wearing masks	18	85.7	3	14.3	0	0.0
Wearing caps	21	100	0	0.0	0	0.0
Cuts must wear gloves	21	100	0	0.0	0	0.0
Raw & cooked foods stored separately	21	100	0	0.0	0	0.0
Training of workers	21	100	0	0.0	0	0.0
Check fridge temp	21	100	0	0.0	0	0.0
Evaluating health status of workers	21	100	0	0.0	0	0.0

A- True, B- false, C- do not know

3.4. General Behaviors of the Workers

The respondents were asked if they wear gloves when working, wash hands before putting on the gloves, wear an apron and a mask and put on a cap when working, wash hands before and after touching raw meat, wash hands after the rest time when coming back to work, eat and/or drink and smoke at work place. They were also asked how often do they use the products of their working plants and how often do they recommend the products of your working plants to others. The answers were somehow diverse but never had the vast majority frequency (Table 11).

Table 11: Responses of the Restaurants Workers in Salalah Municipality regarding their General Behaviors (From June to December/2012)

Do you?	Always	Often	Sometimes	Rarely	Never
wear gloves	10 (47.6)	0 (0.0)	11 (52.4)	0 (0.0)	0 (0.0)
wash hands before gloves	20 (95.2)	0 (0.0)	1 (4.8)	0 (0.0)	0 (0.0)
wear apron	20 (95.2)	0 (0.0)	1 (4.8)	0 (0.0)	0 (0.0)
wear mask	2 (9.5)	0 (0.0)	5 (23.8)	0 (0.0)	14 (66.7)
put on cap	16 (76.2)	0 (0.0)	4 (19.0)	0 (0.0)	1 (4.8)
wash hands before touch meat	20 (95.2)	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.8)
wash hands after touch meat	21 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
wash hands after rest	21 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
ingest at work place	0 (0.0)	1 (4.76)	0 (0.0)	0 (0.0)	20 (95.2)
smoke at work place	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	21 (100)
use products of your plant	20 (95.2)	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.8)
advise products to others	21 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Chapter Four

Discussion

4.1. Presence of bacteria in water

57.1% (n = 12) of the studied sites had safe while 33.3% (n = 7) had unsafe drinking water. But 33.3% (n = 7) of the sites had safe and 57.1% (n = 12) had unsafe utensils washing and cleaning water. This could be explained by that fining, refining and desalination of the drinking water is much more effort is given, stressed more and has the priority upon the fining and refining of the utensils washing and cleaning water. However, Nawas *et al*. (2012) found colifrms in 33.33%, *Salmonella* species in 46.67% and *Vibrio* species in 53.33% of restaurants water in Chittagong, Bangladesh.

13, 50 and 14 coliform bacteria were detected in one of the investigated three sites at Al-Haafah, Al-Sinaat Al-Jadeedah and Al-Goof areas. However, 50 and 1 coliform bacteria were counted in two sites at Awgaad and 50 and 51 at Al-Saadah North as well. Coliforms were not found in Al-Saadah South and Salalal Al-Wustaa. However, *E. coli* was not detected in any drinking water of the investigated 21 sites. 1, 45 and 10 coliforms and 2 *E. coli* bacteria were detected, respectively, in the three investigated sites at Al-Haafah area. At Al-Sinaat Al-Jadeedah 50 coliforms and 8 *E. coli* colonized one site. However, coliforms (1, 10 and 3 bacteria) and *E. coli* (0 bacteria) colonized the three sites at Al-Goof. But only 1 coliform and 0 *E. coli* was detected at Awgaad area. 3, 5, 51, 51, 2 and 3 coliforms and 0 *E. coli* were computed in the three sites of Al-Saadah North and South, respectively. 0 coliforms and 0 *E. coli* were seen in the three sites of Salalal Al-Wustaa. Total Viable Count was 1.60×104 cfu/ml to 4.38×105 cfu/ml for water. Total colifrm count of > 1100 cfu/100 ml was found in water samples (Nawas *et al*., 2012). Furthermore, Christensen *et al*. (2013) indicated that E. coli and other coliform bacteria were present in the investigated ponds in the drinking water distribution systems in the Netherlands. Relatively high concentrations of *E. coli* and total coliforms in water were found; two *E. coli*/mL−1 and five total coliforms/mL−1) and sediment (200 *E. coli* /mL−1 and > 240 total coliforms /mL−1). Schets *et al.* (2002) investigated average total coliform and *E. coli* counts in a set of samples (a total of 179) per 100 ml in three different laboratories in the Netherlands. The recorded the following results: total coliforms of 70.2, 50.1 and 30.3 and *E. coli* of 14.0, 14.5 and 4.1. Coliform bacteria and *E. coli* generally originate from the intestines of

mammals. Their presence could be related to improper disposal of sanitary waste. Their presence in water indicates a strong likelihood that human or animal wastes are entering the water system. Total coliform bacteria are not likely to cause illness, but their presence indicates that your water supply may be vulnerable to contamination by more harmful microorganisms. E. coli is the only member of the total coliform group of bacteria that is found only in the intestines of mammals, including humans. The presence of E.coli in water indicates recent fecal contamination and may indicate the possible presence of disease-causing pathogens, such as bacteria, viruses, and parasites. Although most strains of E.coli bacteria are harmless, certain strains, such as E.coli 0157:H7, may cause illness. The main source of pathogens in drinking water is through recent contamination from human or animal waste, from improperly treated septic and sewage discharges, leaching of animal manure, storm water runoff and domestic animals or wildlife.

4.2. Prevalence of bacterial species in restaurants

Results of this study showed that *E. coli* and *Staphylococcus aureus* were present in the samples collected from meat, vegetables, utensils used for food preparation, surfaces and hands of workers in the restaurants of Salalah, Sultanate of Oman. However, *E. coli* was prevalent in 5.0%, with 95% CI from 0.71 to 9.29, of the investigated restaurants. This finding was lower than the reported by Castro *et al*. (2012) from restaurants of three categories in Mexico: a) national chain restaurants and b) local restaurants, both with the H distinctive (a recognition that the Secretary of Tourism grants to restaurants that manage supplies with high levels of hygiene); and c) local small inexpensive restaurants without H distinctive. Castro *et al*. (2012) found that 99.0% (129/130) of the restaurants were contaminated with Coliforms; 85.0% (110/129) were colonized by *E. coli* and 7.0% (8/110) by diarrheagenic *E. coli* pathotypes. Moreover, higher than our findings once more, Stagnitta *et al*. (2006) found that 58.3% of the samples of meat foods, mostly hamburgers and fresh sausages, were infected with coliforms and *E. coli* in San Luis, Argentina. Saeed *et al.* (2013) detected that 32.0% (18/55) of the vegetable salads investigated from restaurants and cafeteria in Iraq as *E. coli* positive, with 80.0% being detected in the salads of cafeteria and 22.2% in the salads of restaurants, all these findings were higher than the findings of this study. Further higher reports than the one of this study were observed in the USA where a total of 350 outbreaks in the period from 1982 to 2002 were documented (Rangel *et al.*, 2005). Among these outbreaks, transmission routes for 183 (52.0%) and 10

(3.0%) were food-borne and drinking water besides to other transmission routes. Food-borne outbreaks occurred in 28.0% restaurants and other food serving facilities. Ground beef, other beef, dairy products and other foods including poultry products were the most colonized by *E. coli*. Furthermore, in Bangladesh and Nigeria Nawas *et al*. (2012) and Salihu *et al*. (2010) found *E. coli* in 73.33% of the restaurants' salad and water and in 36.6% of the samples ot traditionally cooked meat.

In the present study, *Staphylococcus aureus* was in 3.0% (95% CI, -1.08 - 7.08) of the investigated samples. This finding did confirm the observations of Kadariya *et al*. (2014) who indicated that *Staphylococcus aureus* has been detected in commercially-distributed meats from farms to restaurants and food serving centers in different parts of the world. Not very different from the findings of this study, Kadariya *et al*. (2014) reported *Staphylococcus aureus* in 4.0% the retail beef meat in the US. Another US study testing retail meat in Louisiana isolated MRSA from 5.0% (6/120) of meat samples while 39.2% (47/120) of samples were positive for other types of *S. aureus* (Pu *et al.*, 2009). Higher prevalences of *S. aureus* of 16.4% (27/165) and MRSA in 1.2% (2/165) of the investigated red meat samples were also seen in the US (Hanson *et al.*, 2011), besides to, multidrug resistant (MDR) *S. aureus* in 52.0% (71/136) of the meat and poultry samples (Waters *et al.*, 2011), and any *S. aureus* in 22.5% (65/289) and MRSA in 2% (6/289) of meat and poultry samples (Bhargava *et al.*, 2011). However, higher than the finding of this study, Kadariya *et al*. (2014) indicated that in Asia like Japan and South Korea and in Europe like the Netherlands, *Staphylococcus aureus* has been found in raw retail meat products with diverse prevalences that reaches up to 11.9%. Furthermore, being a very much higher than the report of the present study, 63.0% (63/100) of beef meat products were found positive for *S. aureus* in Georgia (Kadariya *et al*., 2014). Far higher than the findings of this study, Salihu *et al*. (2010) found aerobic bacteria were 100% of the tested samples of the traditionally prepared fried ground beef (*Dambun nama*) in Sokoto, Nigeria, besides to 49.5% (109/216) of the samples had detectable feacal coliforms. Salihu *et al*. (2010) also reported *S. aureus* in 69.9% (151/216) of the samples.

However, no any *Salmonella* species, *Bacillus* species, *Listeria* species and Yeast and molds were detected in any of the studied sites/restaurants in the present study; prevalence of 0.0% (95% CI, 0.0 - 0.0). This is contrary to the findings of Cheung *et al*. (2007) and Nawas *et al*. (2012). Cheung *et al*. (2007) indicated that presence of *Salmonella* in 25 g of the samples

examined is regarded as potentially hazardous to consumers, and is unacceptable for consumption and 13.33% salad sample were found unsatisfactory while Nawas *et al*. (2012) found *Salmonella* species in 46.67% of restaurants' salad. Nawas *et al*. (2012) also detected *Vibrio* species and other bacteria including: *Proteus* species, *Enterobacter* species, *Hafnia* species, *Serrtia* species, and *Citrobacter* species. Furthermore, unlike the results of this study Rahimi and Shakerian (2013) detected *Listeria* species in 8.5% in oloveyh salad, yogurt stew, vegetable salad, macaroni salad and meat salad in restaurants in Shahrekord, Iran. The highest were isolated from vegetable salad (17.3%) and the lowest from macaroni salad (4.2%). *Listeria monocytogenes* (3.0%), *L. innocua* (4.7%) and *L. seeligeri* (0.9%) were the isolates. Dissimilar to Stagnitta *et al*. (2006) who found a count ranging from 10^3 to 10^5 cfu/g molds and yeasts in meat foods in San Luis, Argentina.

4.3. Bacterial counts in restaurants

The evaluation of bacterial agents can be a good indicator of the bacterial quality of meat food products, as aerobic flora has been used as criteria to predict the mean life of products. The microorganisms can be used as indicators of inadequate product manufacturing and /or handling (Salihu *et al*., 2010). The study revealed no statistical significant differences, at *p-value* ($p \leq 0.05$), between the TBCs estimated from the samples collected of meat, vegetables, utensils used in food preparation, surfaces and hands of workers in restaurants of Al-Haafah, Al-Sinaat Al-Jadeedah, Al-Goof, Awgaad, Al-Saadah North and South and Salalal Al-Wustaa. The TBC revealed the highest contamination levels in meat (3.3×10^5 cfu/cm^{-2}) in Al-Sinaat area, in vegetables (1.3×10^4 cfu/cm^{-2}) in Al-Saadah South area which was lower than the TVC estimated by Nawas *et al*. (2012) who found 1.86×10^4 to 7.28×10^5 cfu/g and 1.60×10^4 cfu/ml to 4.38×10^5 cfu/ml for salad and water, respectively in different 15 restaurants located in Chittagong, Bangladesh. However, the TBC in utensils (1.0×10^5 cfu/ml) was found in Al-Haafah and Al-Saadah North areas, on surfaces (2.0×10^5 cfu/ml) was in Al-Haafah area and on hands of workers (1.6×10^5 cfu/ml) in Al-Saadah South area. The study also revealed no statistical significant differences between the EE estimated from the samples collected from meat, vegetables, utensils used in food preparation, surfaces and hands of workers in restaurants in the study area. The EE revealed the highest contamination levels in meat (3.3×10^5 cfu/cm^{-2}) in Al-Sinaat area, in vegetables (1.3×10^4 cfu/cm^{-2}) in Al-Saadah South area, in utensils (1.0×10^5

cfu/ml) in Al-Haafah and Al-Saadah North areas, in surfaces (2.0×10^5 cfu/ml) in Al-Haafah area and on hands of workers (1.6×10^5 cfu/ml) in Al-Saadah South area. There are no available regulatory standards for the microbiological safety criteria for locally (non nonindustrial) prepared ready to eat foods in Oman. According to the Moroccan regulatory standards for microbiological safety criteria for foods (Moroccan Department order, 2004), the aerobic plate counts, feacal coliforms and *S. aureus*, should not go beyond 5.7, 2 and 2 log cfu/g^{-1} respectively in raw ground meats. However, not very diverse from the findings of this study Salihu *et al*. (2010) found a total mesophilic aerobic counts that ranged between 6.70×10^8 and 9.30×10^9 cfu/g^{-1}, with a mean count of 4.5×10 cfu/g^{-1}. On the other hand, the counts of feacal coliforms ranged between 10×10^3 and 10×10^5 cfu/g^{-1} while *E. coli* count ranged between 10×10^2 and 10×10^5 cfu/g^{-1}. *S. aureus* count ranging between 10×10^5 and 10×10^7 cfu/g^{-1}. The average count of 2.2×10^4 cfu/g^{-1} recorded was higher than 1.4 ± 0.6 $\log_{10}$ cfu/g^{-1} previously reported by Scanga *et al*. (2000).

In a study of meat foods carried out in Johannesburg, *E. coli* count was 10×10^3 cfu/g (Stagnitta *et al.*, 2006). In Australia, counts above 10×10^6 cfu/g have been reported for meat foods (Vanderlinde *et al.*, 1998). In this work samples were found to have counts of total coliforms and *E. coli* above 10×10^2 cfu/g. Low microbiological quality is associated with storage above 8ºC, presliced meats, infrequent cleaning of slicing equipments and poor control of practices that likely lead to cross contamination **(**Elson *et al.*, 2004). Personal hygiene precautions can prevent possible risk transmission, but poor restaurant hygiene in most developing countries continues to create an insurmountable risk of acquiring traveler's diarrhea (Shlim, 2005). This study provides very useful information about the microbiological quality of foods consumed in Oman, and could help caterers, retailers, enforcement officers and policy retailers understand the role played by food safety practices on the microbiological quality of food.

The bacteria counts in the investigated restaurants in this study could probably be attributed to the filthy environment, poor personal hygiene of the processors, retailers and the use of contaminated utensils during processing, packaging. There could be possible cross contamination of the finished product from adjacent raw meat through unclean hands of the handlers and workers.

4.4. Food Safety knowledge of the restaurants workers

In the present study all the respondents were sure that washing hands before starting food preparation and putting on or using gloves during food preparation decrease the risk of food contamination. These findings resembled the results of Ko (2011) and Rosnani *et al.* (2014). Ko (2011) found out that the vast majority of the restaurants employees of Fu-Jen University in China believe that hands washing before touching food and wearing disinfected water proof gloves for processing uncooked foods can decrease the risk of contamination. While Rosnani *et al.* (2014) concluded that touching food which was not wrapped up with bare hands is a bad practice with an average score of 78.9±25.611 as thought by restaurant workers in Putrajaya, Malaysia. Furthermore, Latif *et al*., (2014) found that 93.3% (n = 28) and 90.0% (n = 27) of abattoir workers in Khartoum state, the Sudan were sure that washing hands before starting food preparation and putting on or using gloves during food preparation decrease the risk of food contamination. In food workers-associated foodborne outbreaks, the most frequently reported route of transmission involved poor hand hygiene or bare hand contact with food (Todd *et al.*, 2007). Azanza (2005) found that the knowledge and application of the basic principles of the hygiene like washing hands during preparation of food and serving it, has led to a significant reduction in the level of microbial contamination in Philippines. Van-Campen (1998) found out that lack of hand washers and the low level of the peoples' knowledge led to the preparation of unhealthy and risky food in Jakarta. Furthermore, 33.4% of the samples collected from the hands of the food workers were having a higher level of bacterial contaminants than the recommended level. Previous epidemiological studies have shown that *E. coli*, *Salmonella* species and *Staphylococcus aureus* can survive on the hands for a certain period of time if the hands were not washed or even sometimes when they are washed, thus wearing gloves during food preparation is advisable as they significantly reduce the food contamination (Pether and Gilbert, 1971; WHO, 1989). Bas (2006), Santos (2008) and Sani (2011) found out that most of the food workers and handlers have a firm knowledge on the hygienic measure to prepare safe food like cleanness. However, disagreeing with Ko (2011) all the respondents were sure that proper cleaning and handing of food preparation instruments reduce the risk of food contamination. Ko (2011) observed not more than 4 point scales in response to the following questions: I did not need to clean the drainage each day, When I washed the dishes, I would use the three sinks method, and If there were cracks on the dishes I would still use them. Vice versa, Latif *et al*.,

(2014) found that 93.3% (n = 28) of the respondents had in mind that cleaning and disinfecting instruments is one of the most important practices for reducing the risks of meat contamination. However, regarding eating and drinking at the work place and that it increase the risk of food contamination, results of this study agreed with those of Latif *et al*., (2014) who when asked the respondents 20.0% (n = 6) of them did not really know if eating and drinking at the work place is a wrong habit while 76.7% (n = 23) of them agreed this is a wrong habit and only 3.3% (n = 1) disagreed. All persons, including: children, adults, pregnant women and elderly are not at equal risk for food poisoning were perceived by the respondents. This was similar to the findings of Latif *et al*., (2014) who found only one half (n = 15) of the respondents think all persons are at the same risk of food poisoning. All respondents thought food-borne illnesses impact the society as did by Latif *et al*., (2014) where 86.7% (n = 26) of the respondents were sure that foodborne illnesses can have deleterious health and economic effects on the society.

All of the respondents thought that Typhoid and some other diseases like jaundice and brucellosis were without doubt transmitted by food but HIV/AIDS is not. This finding showed the lack of enough knowledge about transmission routes of infectious and poisonous agents that can be forwarded by foods as did by Ko (2011). However, Latif *et al*., (2014) found diverse thoughts with 56.7% (n = 17), 40.0% (n = 12) and 80.0% (n = 24) of the respondents had no doubt that Typhoid, HIV/AIDS and brucellosis, respectively, are transmitted by food. While 13.3% (n = 4) think totally the reverse concerning the same diseases. Nevertheless, 30.0% (n = 9), 46.7% (n = 14) and 6.7% (n = 2) of the respondents failed to develop an idea whether Typhoid, HIV/AIDS and brucellosis are transmitted by food or not (Latif *et al*., 2014). This could be elaborated that the media is stressing very much on HIV this is why the respondents knew is is not transmitted by foods.

With exception of Hepatitis B virus, between half and two thirds of the respondents perceived that *Salmonella* species, *Staphylococcus* species, *Clostridium* species, and and Hepatitis A virus are for sure among the bacterial and viral food-borne pathogens. This result was contradicting the results of Ko (2011) who found that the least correctly answered question was "*Salmonella* is easily contracted from seafood products" with only a 28.8% correct rate. However, this study and Ko (2011) agreed that most restaurant employees were not familiar enough with food poisoning agents and the types of food poisoning. 73.3% (n = 22), 58.6% (n = 17), 63.3% (n = 19) and 73.3% (n = 22) of the respondents in the study of Latif *et al*., (2014)

strongly supported the idea of *Salmonella* species, *Staphylococcus* species, *Clostridium* species and Hepatitis A and B viruses are for sure among the bacterial and viral food-borne pathogens. Moreover, 13.3% (n = 4), 17.2% (n = 5) and 24.2% (n = 8); 13.3% (n = 4) and 23.3% (n = 7); and 6.7% (n = 2) and 20.0% (n = 6) of the respondents were either against or did not develop an idea that *Salmonella* species, *Staphylococcus* species, *Clostridium* species and Hepatitis A and B viruses are for sure among the bacterial and viral food-borne pathogens.

Regarding infectious diseases, all the respondents thought it is very necessary to take a leave from work when a worker has an infectious disease of skin or eyes or other illness symptoms. This is in agreement with what Carpenter *et al*. (2013) recommended; workers with symptoms of illness, especially foodborne ones, should be excluded from work. Furthermore, Carpenter *et al*. (2013) observed that 60.0% (n = 491) of workers recalled working while ill in 9 different locations in the US. 20.0% of them indicated that they had worked while vomiting or having diarrhea for at least one shift in the year 2012 (Carpenter *et al*., 2013), although they known that handling of food by an infected person or a carrier of pathogens is a contributing factor in up to two-thirds of restaurant-related foodborne outbreaks as has been found out by Hedberg *et al.* (2006). Carpenter *et al.* (2013) found that most workers' decisions to work or not while ill was influenced the possibility of spreading illness and this suggested that the food workers were aware of their potential role in the spread of infection. But the likelihood of spreading the infection was not always a primary factor in the decision-making process.

In the current study most of the respondents were against the idea "abortion is a food-borne disease" agreeing with Latif *et al*., (2014) who found around half of the respondents believed that abortion is a food-borne disease while the other half were either disagreeing or failed to develop an idea. Contrary to the findings of this study, Latif *et al*., (2014) found that only 80.0% (n = 24) However, the majority of the respondents did not know the correct temperature of the refrigerator is 4 ºC and it is necessary to check the temperature of the refrigerator periodically to reduce risk of food contamination. However, Rosnani *et al.* (2014) noted that there was lack of knowledge regarding reheating of food (75.1±25.662) and safe temperature of cooked food (71.9± 33.548) and 77.2% (n = 98) of the respondents agreed that defrosted food should not be refrozen. Ko (2011) noted that the least correctly answered question pertained to "Freezing had a better sterilizing effect than heating". One observed problem is improper food storage. The ready-to-eat food is often left at room temperature for long time.

Also slow cooling and improper cooking without letting the temperature reaches the required degree were also seen to be practiced by the food workers. Wrong thawing of frozen meat, poultry and fish could result in some food poisoning cases in the consumers (Robert, 1982; WHO, 1989 and Abdalla, 2008). Bas (2006), Santos (2008) and Sani (2011) found out that most of the food workers and handlers lack the knowledge on the other very important issues for preparing and serving a safe food like the proper temperature for food storage; only 9.5% of the food workers who were answering the questionnaire knew the right temperature for food storage.

Raw and cooked foods should be stored separately, food hygiene and safety trainings for workers and evaluation of the health status of the workers before employing are generally important food-safety measures, when applied for sure will result in reducing the risk of food contamination as claimed and perceived by all of the respondents. Also, all of the respondents were certain that food-borne illnesses can have deleterious health and economic effect on the society. 85.7% (n = 18) of the respondents think that putting on masks is important in reducing risk of food contamination is true. However, Rosnani *et al*. (2014) found that 80.3% (n = 102) were not supporting the suggestion of storing raw and cooked foods separately. Other general food-safety measures stated by Rosnani *et al.* (2014) included: workers should not rub hands or face and hair and should not smoke while working and separate kitchen utensils used to serve and prepare cooked and raw foods, respectively. The findings of this study did confirm the findings of Ko (2011) who found that among the questions that had the highest scores was, “I think raw food and cooked food must be handled separately”.

Although the results of the questionnaire showed that all of the food workers and handlers know the importance of washing hands before work and proper cleaning and handing of instruments and kitchen utensils and their role in reducing the risk of food contamination, in addition to their knowledge that eating and drinking at the work place increases the risk of food contamination, we observed that these workers do not practice that as a part of their routine work.

Conclusions

Result of the study showed that *E. coli* and *Staphylococcus aureus* qwere present in restaurant of Salalah State Municipality, Sultanate Of Oman. According to the results of the study and the detective that was displayed, we could conclude that there is a real need to develop practical side through improved hygiene practices for employees of restaurants to ensure food safety and raise the level of public health, where the researcher found that there is a weakness, noting in the preparation and handling of food due to the weakness of the cognitive and cultural level of the workers .`restaurant workers should be adequately educated on the role of food in disease transmission as well as on rule of personal hygine and approved practice in preparation of food . and must be monitoring and controlling the water sources to ensure safe water..

Recommendations

According to this study recommendation may include the followings :-

• Attention to the educational aspect of the health workers restaurants.

• Intensification of inspection and control of preparation areas, food processing through sampling and laboratory tests periodically.

• Provision and use of screening devices and portable analysis for the detection of Contaminants in food, water, tools and equipment food processing.

• raise the technical level of health inspectors through advanced training courses.

• Statement of the legal implications of all health violations restaurants and rigor in the

application.

• Classification of restaurants and health according to their level to facilitate the role of the inspectors.

• ensure the safety of water and disposal systems in places of preparation and processing of foods.

• Media side entry in the food safety system and educate the consumer.

• ensure the safety of water in restaurants.

• Linking extraction health card for employees to reflect the outcome of an interview of knowledge level of the worker.

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Annexes

Annex 1: Operational Terms

Control: To manage the conditions of an operation to maintain compliance with established criteria or the state where correct procedures, criteria are being followed.

Critical control point: A step at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level.

Good hygiene practices: All practices regarding the conditions and measures necessary to ensure the safety and suitability of food at all stages of food chain.

HACCP: Hazard Analyses Critical Control Point, a system approach to the identification, evaluation and control of food safety hazards.

Hazard: A biological, chemical, or physical agent that is reasonably likely to cause illness or injury when used in the manner and quantity proposed.

Food-service establishment: Establishments for the preparation and serving of meals and other edible products to clients/customers.

Hazard analysis: The process of collecting and evaluating information on hazards associated with food under consideration to decide which are significant to affect food safety.

Risk: A function of the likelihood and severity of an adverse health effect on the consumer as a result of exposure to a hazard.

Sanitation: As applied to food industry, it is the creation and maintenance of hygienic and healthful conditions/environment**.**

Severity: The seriousness of the effect(s) of a hazard.

Prerequisite programs: Procedures, including GMPs that address operational conditions providing the foundation for the HACCP system.

Food safety – Assurance of food products against hazard, which may expose the consumer to a health problem when used in the manner and quantity proposed.

Analyze hazards – Potential hazards associated with food and measures to control those hazards are identified. The hazards could be biological, such as a microbe or chemical such as toxin or physical such as ground glass or metal fragments.

Identify critical control points – These are points in a food production from its raw material state through processing and dispatch to consumption by the customer at which the potential hazard can be controlled or eliminated as in cooking, cooling, packaging and metal detection.

Establish preventive measures with critical limits for each control point – For cooked food, this will include setting the minimum cooking temperature and time required to ensure the elimination of any harmful microbes.

Establish procedures to monitor the critical control points - This will include determining how and by whom cooking time and temperature should be monitored.

Establish corrective actions to be taken when monitoring shows that a critical parameter has not been met – This could be reprocessing or disposing of food if the minimum cooking temperature is not met.

Establish procedures to verify that the system is working properly – testing time and temperature recording devices to verify that a cooking unit is working properly.

Establish effective record keeping documenting the HACCP system – this includes records of hazards and their control methods, the monitoring of safety requirements and action taken to correct potential problems.

HACCP looks at the flow of food through the restaurant, from the time it is delivered to the time it is served to the customer. This can be appertained to the restaurant as follows:

The Delivery – all the deliveries should be in good condition. Frozen foods must be received frozen (-18˚C). Produce should be 4.4˚C and dry goods intact. Check dates of expiry and refuse any products that do not meet these standards.

The Storage of products – Rotate, remember FIFO rule. Refrigerated products should be stored below 4.4˚C and frozen foods must be stored at -18˚C with enough room for circulation.

Food Preparation – Use clean and sanitized equipment and utensils. Thaw all frozen foods in the refrigerator and keep them cold until you work with them. All hot foods should be prepared quickly and should reach the right temperature (73.9˚C) and be held at (60.8˚C.) Never mix old products with new. Proper hygiene habits are a must for all staff with proper hand washing. Prepare only the food you plan to use in one day and date all the food prepared.

Serving customers – All employees must have high personal hygiene habits because they can transmit diseases/illness, (Weber, 1994). They must have clean hands, hair in place, clean uniform and thoroughly trained in proper hand washing techniques.