

**ASSESSMENT OF THE PREVALENCE OF PATHOGENIC BACTERIA IN STREET
VENDED READY-TO-EAT MEATS IN WINDHOEK, NAMIBIA**

A THESIS

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ABSTRACT

While street vended ready-to-eat meats provide a source of readily available and nutritious meals for the consumers, there is a concern for their safety and microbiological quality. The prevalence of *E. coli*, *Salmonella*, *Shigella*, *L. monocytogenes*, *S. aureus* and *Enterobacteriaceae* was assessed in a total of 96 street vended ready-to-eat meat samples collected from Windhoek locations, namely, Katutura, Prosperita and Dorado Park. Selective media was used to assess aerobic plate count, *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Shigella*, *Salmonella* and *Enterobacteriaceae*. Biochemical confirmation tests were employed using the conventional biochemical tests and the VITEK[®] 2 system. The data showed that 42%, 15%, 6%, 52% and 83% of the samples were positive for *E. coli*, *L. monocytogenes*, *Shigella*, *S.aureus* and *Enterobacteriaceae* respectively. The highest bacteria counts obtained for aerobic plate count, *E. coli*, *S. aureus*, *L. monocytogenes*, *Shigella* and *Enterobacteriaceae* were 7.74 log cfu g⁻¹, 5.67 log cfu g⁻¹, 5.12 log cfu g⁻¹, 4.56 log cfu g⁻¹, 3.3 log cfu g⁻¹, 5.75 log cfu g⁻¹ respectively. Unsatisfactory microbial levels were 32% for aerobic plate count, 26% for *Enterobacteriaceae*, 35% for *E. coli*, 11% for *L. monocytogenes*, 7% for *S. aureus* and 6% for *Shigella*. *Salmonella* was only detected after enrichment of culture media in 11% and 40% of samples from two Katutura suburbs, Wanaheda and Havana respectively, which made the samples potentially hazardous. The Tukey's multiple comparison test showed that the prevalence of *E. coli* was significantly higher in samples purchased from Havana than other locations sampled ($p < 0.05$). Pearson's correlation tests showed significant positive correlation between the prevalence of *E. coli* and the collection time of meat samples ($r=0.449$, $p=0.000$). None of the samples was found to be positive for enteropathogenic *E. coli*. This study has helped to bridge a gap in knowledge by establishing the prevalence of pathogenic bacteria of public health concern in street vended ready-to-eat chicken and beef in Windhoek, Namibia. The unsatisfactory microbiological quality of some ready to eat meats

determined in this study may be due to inadequate processing and poor handling practices, necessitating the provision of training on food safety and hygiene for street vendors for consumer protection. The relationship between the sampling locations, type of meat, preparation methods, serving temperature and the time of purchase and the prevalence of pathogenic bacteria has also been established. Further studies may include assessment of prevalence of food poisoning resulting from consumption of ready-to-eat meats. Moreover, assessment of microbiological quality of other popular street vended ready-to-eat foods in Namibia such as fruits, fat cakes and salads, as well as the microbiological quality of some meat ingredients like spices, onions and tomatoes and their effect on the microbiological quality of ready-to-eat meats.

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Abbreviations

ANOVA- Analysis of Variance

APC- Aerobic Plate Count

API - Analytical Profile Index

ATCC- American Type Culture Collection

BPA- Baird Parker Agar

BPB - Butterfield's Phosphate Buffer

BPW – Buffered Peptone Water

CDC – Center for Disease Control and Prevention

CFU- Colony Forming Units

CVL – Central Veterinary Laboratory

DNA- Deoxyribonucleic acid

EFSA – European Food Safety Authority

FDA- Food and Drug Administration

FHG SOPs- Food Hygiene Standard Operation Procedures

GN- Gram negative

GP-Gram positive

HACCP – Hazard Analysis Critical Control Point

ICMSF- International Commission on Microbiological Specifications for foods

ISO - International Standards Organization

LT- Lauryl Tryptose broth

MKKTn - Mueller Kauffmann Tetrathionate Novobiocin

NORS -National Outbreaks Reporting System

PCA- Plate Count Agar

PCR- Polymerase Chain Reaction

RTE- Ready-to-eat foods

RVS - Rappaport Vassiliadis medium with soya

SD -Standard Deviation

SOPs- Standard Operational Procedures

SFP- Staphylococcal food poisoning

SPSS - Statistical Package for Social Sciences

SSA-*Salmonella-Shigella* agar

TBX- Tryptone Bile X-glucuronide agar

TSI - Triple Sugar Iron

UK - United Kingdom

VP - Voges Proskauer

VRBG - Violet Red Bile Dextrose Agar

WHO- World Health Organisation

XLD - Xylose Lysine Desoxycholate agar

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Dedication

This thesis is dedicated to my family. To my lovely mother Mrs Hileni Shiningeni for all the love, support, sacrifices, motivation, and encouragement and for believing in me. To my father Mr David Shiningeni and my granny Mrs Petrina Hamunyela for all the guidance. To my four siblings, Daven, Davelina, Daphley and Dabby for always looking up to me as your elder sister. May the Almighty God let this work to be the constant source of inspiration and motivation.

Declaration

I, **Daphney Shiningeni**, declare hereby that this study is a true reflection of my own research, and that this work, or part thereof has not been submitted for a degree in any other institution of higher education.

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Date.....

Daphney Shiningeni

CHAPTER 1: INTRODUCTION

1.1. Orientation of the study

Street vended ready-to eat meats are those that are prepared and sold by vendors on the street and in other public places for immediate consumption or for consumption at a later time without further processing or preparation (Muaduke, Awe and Jonah, 2014). Street vended ready-to-eat meats represent a significant portion of the diet of many inhabitants in many major cities. It is estimated that about 2.5 billion people world-wide consumed street vended ready-to-eat meats every day (Yah, Obinna and Shalom, 2009; Muaduke et al., 2014). Generally, there are many several benefits of street vended foods. This sector provides a regular source of income for millions of people especially women and contributes to local and national growth. Street foods represent a source of inexpensive, convenient and often nutritious food for urban and rural people. They also provide an opportunity to develop business skills with low capital investment while giving a chance for self-employment at the same time (World Health Organisation (WHO), 2011).

Moreover, the street vending continues to increase worldwide because it is very profitable, accessible and requires very low capitalization, however controlling the number of street vended ready-to-eat meat vendors and the quality of meat that they offer is becoming a challenge (Manguait and Fang, 2013). Contamination of street vended ready-to-eat meats may occur during preparation, transportation and marketing. Marketing is performed at open areas such as industrial areas, streets, public places and open markets (Muaduke et al., 2014; Manguiat and Fang, 2013).

In a survey conducted by WHO (2011), almost all countries have reported a large variety of street vended ready-to-eat meats, types of preparation, facilities and infrastructure. Some key findings of the survey were that, diverse type of meats such as beef, pork, chicken and poultry were sold in

the street. There were different methods of preparation varying from foods without any preparation, (65%) fried meat, (97%) roasted meat and meat cooked on site (82%). The majority of the countries reported contamination of meat coming from raw meat, infected handlers and inadequate clean equipment. The surfaces where the meat is prepared sometimes have remains of meat prepared earlier which can become source of contamination (Yah et al., 2009; Cabeza et al., 2009).

Sale of ready-to-eat meats in the streets is very controversial from a health standpoint. This is because ready-to-eat meats have been implicated in foodborne disease outbreaks (Kotzekidou, 2013). The hygienic behavior and numbers of people involved in handling the meat is unknown (Kotzekidou, 2013). According to Magwedere et al. (2013) the main contaminating meat pathogens (disease-causing microorganisms) are *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella*, *Shigella* and *E. coli* 0157:H7. It has been clearly indicated that people who consume street vended ready-to-eat meats may be putting their health at risk (Manguiat and Fang, 2013). Moreover, the prevalence of multi-drug resistance among pathogenic bacteria, such as *Salmonella*, *E. coli* and *S. aureus* has been increasing and poses a real threat to the public health because street vended ready-to-eat meats could possibly become a medium by which these pathogenic bacteria are transmitted to people (Manguiat and Fang, 2013; Rodriguez et al., 2010).

The contribution of the food street vending industry to socio-economic growth is enormous. This emphasizes the importance of placing a priority on assisting street meat vendors in understanding the importance and the requirements of food safety (Manguiat and Fang, 2013). In Windhoek, street meat vending is a popular practice and majority of residents consume street vended ready-

to-eat meats. Chicken and beef are street vended ready-to-eat meats commonly sold in Windhoek. There is very little knowledge documented on the subject in the Namibian context, hence the importance of this study. In a study investigating the occurrence of *L. monocytogenes* in ready to eat foods sourced from retail outlets and university cafeterias in Namibia, *L. monocytogenes* loads were higher in hotdogs and polony than in other foods such as apples, meat salads and egg salads, although loads were too low to warrant fears of possible outbreaks of human listeriosis (Mogomotsi and Chinsebu, 2012). The study was however limited to one pathogenic micro-organism and it is necessary to investigate the prevalence of other pathogens of public health concern. Shilangale, Kaaya and Chimwamurombe (2015) reported a prevalence of 0.85 % for *Salmonella* in raw beef samples from abattoirs in Namibia.

1.2. Statement of the problem

The global threat of foodborne diseases especially those associated with *Salmonella*, *E. coli*, *S. aureus* and *L. monocytogenes* such as diarrhoea, headache, muscle pain, nausea and vomiting has been on the increase (Matsheka et al., 2014). In Windhoek, various street vended ready-to-eat meats such as pork, beef and chicken are sold at most public places and industrial areas. However, they might be the contamination vehicles of different pathogenic bacteria, posing a health risk. There is limited information on the prevalence of foodborne pathogens in street vended ready-to-eat meats in Namibia. This study is useful for documenting the prevalence of pathogenic bacteria of public health concern in street vended ready-to-eat chicken and beef in Windhoek, Namibia.

1.3. Objective of the study

The main objective of this study was to assess the prevalence of pathogenic bacteria in street vended ready-to-eat meats in Windhoek, Namibia.

1.3.1. Specific Objectives

1. To assess the prevalence of *E. coli*, *Salmonella*, *Enterobacteriaceae*, *S. aureus*, *L. monocytogenes*, *Shigella* and *Salmonella* in street vended ready-to-eat meats such as beef and chicken in Katutura (Wanaheda, Single Quarters, Havana, Greenwell, and Okuryangava), Prosperita and Dorado Park locations in Windhoek, Namibia.
2. To assess the relationship between the preparation methods (roasting, frying, stewing) and serving temperature (hot, cold) and the prevalence of *E. coli*, *Salmonella*, *Enterobacteriaceae*, *S. aureus*, *L. monocytogenes*, *Shigella* and *Salmonella* in ready-to-eat meats.
3. To investigate the relationship between the type of meat (beef and chicken), sampling locations and the prevalence of *E. coli*, *Salmonella*, *Enterobacteriaceae*, *S. aureus*, *L. monocytogenes*, *Shigella* and *Salmonella*.

1.4. Research questions

1. What is the prevalence of *E. coli*, *Salmonella*, *Enterobacteriaceae*, *S. aureus*, *L. monocytogenes*, *Shigella* and *Salmonella* in street vended ready-to-eat meats such as beef and chicken in Katutura (Wanaheda, Single Quarters, Havana, Greenwell, and Okuryangava), Prosperita and Dorado Park locations in Windhoek, Namibia?

2. What is the relationship between preparation methods (roasting, frying and stewing) and the serving temperature (hot and cold) and the prevalence of *E. coli*, *Salmonella*, *Enterobacteriaceae*, *S. aureus*, *L. monocytogenes*, *Shigella* and *Salmonella* in the meat?

3. What is the relationship between the type of meat (beef and chicken) and the prevalence of *E. coli*, *Salmonella*, *Enterobacteriaceae*, *S. aureus*, *L. monocytogenes*, *Shigella* and *Salmonella*?

1.5. Significance of the study

The study will contribute to the knowledge of the safety status of commonly consumed street vended ready-to-eat meats in Windhoek, Namibia. Data on the prevalence of pathogenic bacteria in street vended ready-to-eat meats in Windhoek will provide information on the microbial hazards present in the street vended ready-to-eat meats category and can be used as inputs to microbiological risk assessments. Additionally, the results of the study could provide the basis for the development of science-based interventions to control the hazards and improve food hygiene and safety management systems for the street vending industry. Information obtained from this study could be used to increase public health knowledge and consumer awareness of the importance of food safety as well as contributing to the development of food vending policies.

1.6. Limitation of the study

1. Ideally, the study would have included a larger number of samples within different locations in Windhoek where ready-to-eat meats are sold. This was not possible in this study as the study sites accessible within the research duration were limited.

2. The study was based on Culture dependent methods.

CHAPTER 2: LITERATURE REVIEW

2.1. Microbiological quality of street vended ready-to-eat meats

Insufficient knowledge on food safety, poor hygienic practices and operation in unsanitary environments are considered as the major risk factors leading to the production of microbiologically unsafe street vended ready-to-eat meats (Kotzekidou, 2013). Various studies have identified that the main risk associated with consuming street vended ready-to-eat meats is microbial contamination. Manguiat and Fang (2013) explained that most street vended ready-to-eat meats have high levels of coliform bacteria and pathogenic bacteria such as *Escherichia coli* 0157:H7, *Salmonella*, *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Clostridium perfringens* and *Vibrio cholerae*. Similar results were obtained by (Kotzekidou, 2013) in ready-to-eat baked frozen pastries. According to the Center for Disease Control and Prevention (CDC) 31 pathogens are known to cause food borne illness (CDC, 2013). Among the pathogens found in street vended ready-to-eat meats, *B. cereus*, *C. perfringens*, *Salmonella* and *S. aureus* were the most common one (CDC, 2013).

In a study carried out in Brazil, foodborne pathogens and high microbial counts have been found in different street vended ready-to-eat meats in the country, approximately 35% of the street meat samples were inappropriate for consumption and in 12.5%, 2.5% and 22.5% of street meat samples, *B. cereus*, *S. aureus* and *E. coli* were present respectively (Mamun et al., 2013).

In another study conducted in South Africa, *B. cereus* was the most prevalent bacteria detected in 23 (17%) out of 132 street food samples (Mosupye and Holy, 2010). In the same study, *C. perfringens* was detected in one raw chicken sample, *S. aureus* in two raw beef and two stew samples of street vended foods.

In a more recent study that was done in Cameroon, of 200 samples of ready-to-eat meats collected, 60 (30%) were contaminated with *E. coli*, 46 (23%) were contaminated with *B. cereus*, 38 (19%) with *S. aureus*, 30 (15%) with *Salmonella* spp. and 10 (5%) with yeast and moulds. Seven (26.92%) with coagulase positive *S. aureus*, however, *L. monocytogenes*, *V. cholerae* and *Y. enterocolitica* were not detected (Djoulde, James and Bakary, 2015).

Mashak et al. (2015) carried out a study in Iran to examine the bacteriological quality of ready-to-eat meats and reported that, 62% of semi cooked samples contained more than 10^2 cfu/g coliform, while *S. aureus* was more than 10^2 cfu/g in 35% and 40% of samples, respectively. Also 28% of cooked samples and 44% of semi cooked samples contained *E. coli*, 14% of all samples were contaminated by *Salmonella*, hence were microbiologically unsatisfactory.

2.1.1 Methods for determination of microbiological quality

According to the Center for Food Safety (2014), there are different tests that can be used to determine the microbiological quality of ready-to-eat foods. Ready-to-eat foods vary widely, the decision on what test to use may vary from product to product. Generally, the decision on what method to use may depend on; the type of ingredients in the finished product, status of ingredients (for example, cooked vs. raw), type of cooking/processing undertaken, level of handling after cooking or processing, presence of preservatives, including acids and salt, presence and type of packaging as well as the distribution and storage of finished product. For ready-to-eat meats, the following tests may be used to determine the microbiological quality (Hocking, 2003).

Enumeration of Standard Plate Count (SPC) also known as Aerobic Plate Count (APC) in foods is usually carried out by using conventional methods based on the use of media such as Plate Count Agar. SPC can provide a general indication of the microbiological quality of ready-to-eat meats (for example, a high SPC count may indicate that the product may have been prepared unhygienically) however, a standard plate count will not differentiate between the natural microflora or spoilage microorganisms (Gilbert et al., 2000).

Likewise, the routine identification and enumeration of foodborne pathogens including *S. aureus*, *E. coli*, *L. monocytogenes*, *Salmonella*, *Shigella*, *Enterobacteriaceae* and *B. cereus* in foods are usually carried out using conventional methods based on the use of selective media. The common used selective media are; Baird-Parker agar, MacConkey agar, RAPID *Listeria* agar, Xylose Desoxylate Agar (XLD), *Salmonella-Shigella* agar, Violet Red Bile Dextrose Agar (VRBG) and *B. cereus* agar respectively. These is usually followed by the identification of suspicious colonies by gram- staining and specific various biochemical tests (Food Standards Australia New Zealand, 2009).

2.1.1.1 Confirmation methods

Biochemical tests such as, Motility test, Coagulase test, Indole test, Glucose test, Citrate test, Triple Sugar Iron test, Catalase test and Serological tests are among the confirmation tests that are usually used to identify bacteria isolates. However, these traditional methods are cumbersome and time consuming. Furthermore, they frequently lead to ambiguous results due to the history of field isolates in some tests (Food Standards Australia New Zealand, 2009).

According to Hendricksen (2003), the Analytical Profile Index (API) kits system is a manual, standardized, miniaturized version of conventional procedures for the identification of gram-positive, yeast, anaerobic bacteria, gram-negative bacteria and other microorganisms to their species level. It is a microtube system designed for the performance of about 23 standard biochemical tests from isolated colonies on plating medium (Lindquist, 2001). Test results are entered into an online database to determine the bacterial identity. The API kit may be selected after presumptive organism identification using gram's staining, morphological features and other simple biochemical tests. API strips give accurate identifications based on extensive databases and are standardized easy-to-use test systems (Reynold, 2009).

The manual biochemical tests may require some additional incubation hours for identification; these delays have prompted the development of several automated and rapid identification and susceptibility testing systems that are used by some clinical laboratories (Wallet et al., 2005). These include the VITEK[®] 2 automated identification, which automates all mandatory steps for identification and antimicrobial susceptibility testing after a standardized inoculum has been loaded into the system (Wallet et al., 2005). The results are available within 3 hours for identification and 3–18 hours for susceptibility testing. It uses reagent cards that have 64 wells that can each contain an individual test substrate. Substrates measure various metabolic activities such as acidification, alkalization, enzyme hydrolysis and growth in the presence of inhibitory substances (Pincus, 2001). There are currently four reagent cards available for the identification of different organism classes as follows: GN-Gram-negative fermenting and non-fermenting bacilli, GP-Gram-positive cocci and non-spore-forming bacilli, YST-yeasts and yeast-like organisms, BCL-Gram-positive spore-forming bacilli (Nimer, Saada and Abuelaish, 2016). According to Pincus (2001), the VITEK 2 identification products databases are constructed with

reference strains (for example American Type Culture Collection (ATCC) and the test data from an unknown organism are compared to the respective database to determine a quantitative value for proximity to each of the database taxa. An unknown biopattern is compared to the database of reactions for each taxon and a numerical probability calculation is performed. Various qualitative levels of identification are assigned based on the numerical probability calculation, these are; excellent, very good, good, acceptable, low discrimination and unidentified organism. However, in case of a low discrimination and unidentified organism, supplemental testing should be done and gram-staining as well as purity should be re-checked (Pincus, 2001). Various studies have evaluated the performance of the VITEK[®] 2 systems for identification of gram-negative and gram-positive bacteria, but the results vary across studies (Wallet et al., 2005; Nimer et al., 2016).

Fast and sensitive methods for identification of foodborne pathogens are important for microbiological safety throughout the food production chain. In the last 30 years, a considerable number of detection methods using molecular tools have been proposed. The Polymerase Chain Reaction (PCR) method which was invented by Kary B. Mullis in 1985 is based on 16Sr RNA gene and it is a sensitive and fast method for the detection and identification of pathogenic bacteria in foods (Okafor, 2007). The PCR is a technology used to amplify segments of DNA by using a set of primers that are designed to hybridize to a segment of the bacterial genome previously identified as a good diagnostic marker for that bacterial species, therefore, if a DNA segment corresponding to the size and base sequence is produced in the amplification, it can be proved that the specific bacteria is present in the sample tested (Lackie, 2007).

2.1.2 Criteria for the assessment of microbiological quality

Four categories of microbiological quality have been assigned based on Aerobic Plate Counts, levels of indicator organisms and the number or presence of pathogens. These are satisfactory, marginal, unsatisfactory and potentially hazardous (Centre for Food Safety, 2014).

Centre for Food Safety (2014) explained that, **Satisfactory**; results indicate good microbiological quality and no actions are required. **Marginal**; results are borderline in that they are within limits of acceptable microbiological quality but may indicate possible hygiene problems in the preparation of the food, thus, re-sampling may be appropriate. Premises that regularly yield borderline results should have their food handling controls investigated. **Unsatisfactory**; results are outside of acceptable microbiological limits and are indicative of poor hygiene or food handling practices. Further sampling, including the sampling of other foods from the food premises may be required and an investigation undertaken to determine whether food handling controls and hygiene practices are adequate. **Potentially hazardous**; the levels in this range may cause food borne illness and immediate remedial action should be initiated, that is, consideration should be given to the withdrawal of any of the food still available for sale or distribution. An investigation of food production or handling practices should be investigated to determine the source/cause of the problem so that remedial actions can commence (WHO, 2014; Center for Food Safety, 2014; International Commission on Microbiological Specifications for foods, 2001).

Table 1. Guideline levels for determining the microbiological quality of ready-to-eat meats
(ICMSF, 2001)

Test	Microbiological Quality (cfu per gram)			
	Satisfactory	Marginal	Unsatisfactory	Potentially Hazardous
Standard Plate Count	<10 ⁴	<10 ⁵	≥10 ⁵	
Indicators				
<i>Enterobacteriaceae</i>	<10 ²	10 ² -10 ⁴	≥10 ⁴	
<i>E. coli</i>	<3	3-100	≥100	
Pathogens				
<i>S. aureus</i> & other coagulase positive Staphylococci	<10 ²	10 ² -10 ³	10 ³ -10 ⁴	≥10 ⁴
<i>Campylobacter</i> spp	not detected in 25g	N/A	Detected in 25g	
<i>Salmonella</i> spp	not detected in 25g	N/A	Detected in 25g	

<i>L. monocytogenes</i>	not detected in 25g	detected but <10 ²	≥10 ²	
<i>E. coli</i> O157:H7 and other Shiga toxin-producing <i>E. coli</i>	not detected in 25g	N/A	Detected in 25g	
<i>Shigella</i> spp.	not detected in 25g	N/A	Detected in 25g	

2.2. Epidemiology of foodborne diseases associated with ready-to-eat meats

Foodborne diseases are increasingly being recognized as a major cause of morbidity in both industrialized and developing countries. However, the full extent of the social and economic effects is hard to measure due to underreporting of cases (Ntanga, 2013). Nweze (2010) explained that although epidemiological data on the incidence of foodborne diseases are inadequate and the outbreak often not investigated, the recurrent episodes of food borne illnesses with symptoms of gastrointestinal distress like diarrhea, vomiting, abdominal cramp and nausea has remained a major cause of mortality and morbidity worldwide.

Studies done by various researchers indicated that, the foods of animal origin are the main vehicles of pathogens in human foods (Ntanga, 2013; Nweze, 2010). These animal products, mainly beef and poultry have been implicated in foodborne diseases as the live animals are exposed to a variety of potential sources of microorganisms at various rearing points (Abdullahi et al., 2006). There

has been an increased safety concern with meat (including ready-to-eat meats) and meat products as there seem to be an increased occurrence of foodborne diseases with the consumption of these products (Abdullahi et al., 2006).

Moreover, in a survey done by Yodav et al. (2011) in India indicated that microorganisms particularly, *Salmonella*, *Staphylococcus aureus*, *Clostridium perfringens*, *Listeria monocytogenes* and *Bacillus cereus* were the main causative agents of foodborne diseases and the foods that were mainly involved were ready-to-eat meats which had a higher number of cases than other street vended foods such as cheese and sausages.

The microorganism sources are diverse and include soil, traditional foods and beverages, water, feed, air and other animals. In healthy animals, the microorganisms are confined primarily to the gastrointestinal tract and exterior surfaces such as hooves and hair, however, during slaughtering the meat usually becomes contaminated with these microorganisms and the level of contamination depends on the handling practices and materials / equipment used (Anasthecian, 2008).

Worldwide, despite lack of pathological reports, work done by various researchers has shown the possibility of contamination of meat by various pathogens which may contribute to the occurrence of foodborne diseases. For instance, Forster et al. (2007) reported that South African meat can be contaminated with bacterial pathogens during processing, though not at high levels, but could easily pose a health risk to immune-compromised individuals. Kotzekidou (2013) indicated that Greece ready-to-eat meats and frozen pastries can be contaminated during preparation as a result of improper handling. In a similar study that was done in Namibia, Mogomotsi and Chinsembu (2013) showed that ready-to-eat meat products such as hotdogs, polony and meat salads can be easily cross-contaminated by pathogens through animal faeces or during meat processing.

Foodborne diseases are more likely to affect the extremes of age (new born and elderly) as well as immunocompromised patients and pregnant women. These groups suffer higher incidences of morbidity and mortality. The effect of foodborne disease may extend beyond the immediate illness. This has been shown by a Danish study, which demonstrated a greater than threefold risk of dying in the year after contracting a foodborne illness (Gould et al., 2013).

Salmonellosis is an infection of the intestine caused by *Salmonella* bacteria. WHO (2014) explained that, it is mainly caused by eating foods (including ready-to-eat meats) or drinking water contaminated with human or animal feces containing the bacteria. Additionally, salmonellosis may spread from person-to person by fecal-oral contact that can occur when taking care of, or eating foods prepared by someone with diarrhea caused by *Salmonella*. Some people may be infected with *Salmonella* and can spread it to others without showing any symptoms of illness. Most people with salmonellosis will recover without treatment. However, a doctor can prescribe antibiotics to treat severe cases of the disease (Bhunia, 2008).

According to Bhunia (2008), *E. coli* O157:H7 can live in the intestines of healthy cattle and other animals; hence, meat may become contaminated during slaughtering. Eating undercooked ready-to-eat meats can be a major cause of infection. The bacteria are also found in the stools of infected person and can be passed from person to person if good hand washing habits are not followed. Antibiotics appear to play no role in treatment, because the illness is the result of a Shiga toxin produced by the bacteria (Bhunia, 2008).

WHO (2014) discussed listeriosis as an illness that can result from eating foods contaminated by *L. monocytogenes*, especially by eating contaminated ready-to-eat meats, drinking or eating raw (unpasteurized) milk or milk products, or from consuming contaminated raw vegetables. A

pregnant woman infected with the bacteria can transmit the disease to her unborn child which can result in the death of the fetus, premature birth of the child, or infection of the child after it is born. Antibiotics especially ampicillin may be used to reduce the symptoms (Cleveland Clinic Foundation, 2015).

Shigellosis is an infection of the intestine caused by a group of bacteria called *Shigella*. Most people with shigellosis will recover without treatment. However, a doctor can prescribe antibiotics to treat severe cases of the disease (Cleveland Clinic Foundation, 2015). The incidence of shigellosis in Australia in 2012 was 2.4 cases per 100,000 population (549 cases), which includes both foodborne and non-foodborne cases (CDC, 2012). In the US, 4.82 cases of shigellosis were notified per 100,000 populations in 2010 (CDC, 2012). In the European Union there was three strong evidence foodborne outbreaks of shigellosis in 2011 and one outbreak reported in 2010 (European Food Safety Authority, 2012).

In the USA, data for foodborne disease outbreaks for the years, 2000-2003 showed that most outbreaks were caused by bacteria. Aetiological agents were confirmed for 38% of outbreaks, of which *Salmonella* constituted 28% of outbreaks and 45% of the cases. Moreover, 13% of outbreaks were caused by *Staphylococcus aureus*, 8.1% by *Clostridium botulinum*, 4.1% by *Shigella* and 2% by *Campylobacter*. Most deaths were caused by *Salmonella* species and *C. botulinum*, with an average of 29 and 8 deaths per year, respectively. Reported outbreaks of *E. coli* O157:H7 increased from 4 in 2002 to 30 in 2004 (Boyce, Swerdlow and Griffin, 2005).

In the UK in 2002 and 2003, *Salmonella* species and *Clostridium perfringens* were responsible for three-quarters of all foodborne disease outbreaks recorded. *Salmonella enteritidis* phage type 4

accounted for 41% of all outbreaks and 71% of outbreaks of *Salmonella* (Cowden, Lynch and Joseph, 2005). The number of outbreaks attributed to *E. coli* O157:H7 in the UK increased from 7 in 1999-2001 to 18 in 2002-2004; 76 and 173 people were infected respectively. Of the cases from 2002-2004, there were 5 deaths, 38% of cases were admitted to hospital and 21% of cases developed diarrhea (Cowden et al., 2005).

According to De Boer and Beuner (2011), analyzing foods for the presence of both pathogenic and spoilage bacteria is a standard way of ensuring food safety and quality. If microorganisms are able to survive and grow on foods which are sold and consumed by people, then the risk of foodborne illness is increased in the society (De Boer and Beuner, 2011).

2.3. Factors that may contribute to microbial contamination of street vended foods

Poor personal hygiene, Abuse of the time temperature relationship, Cross contaminating raw and cooked meats are among the major factors contributing to microbial contamination of street vended foods (Munide and Kuria, 2005).

2.3.1. Poor Personal Hygiene

Different studies have discussed that poor personal hygiene can result in food contamination, for example, when a food personnel fail to wash hands properly after using the toilet, there is a serious risk of fecal contamination (WHO, 2014; Anasthecian, 2008; Githaiga, 2012). WHO (2014) stated that everyone has bacteria on the skin, mouth, hands and so many other organisms on various parts of the body like hair. Food service personnel can contaminate food and cause food-borne illness. Food workers may transmit pathogens to food from a contaminated surface, from one food to

another or from hands contaminated with organisms from the gastrointestinal tracts (Munide and Kuria, 2005).

2.3.2. Abuse of the time-temperature relationship

Food and Drug Administration (2014) clearly explained that the abuse of time-temperature relationship is a factor that can cause food contamination that may result in foodborne illness. To prevent the risk of food-borne illness, it is important to control the time that food is kept in the temperature danger zone. This means hot foods should be kept at 60°C or above and cold foods at 5°C or below (FDA, 2014; Kendall, 2012). Cooked or refrigerated foods, such as salads should not be left at room temperature for more than two hours (FDA, 2014; Kendall, 2012). Time temperature relationship problems occur because food is not stored, prepared or held at the required temperature; food is not cooked or reheated to temperature high enough to kill harmful microorganisms (FDA, 2004).

2.3.3. Cross-contaminating raw and cooked Foods

Kendall (2012) discussed that, cross-contaminating raw and cooked food is transferring of harmful microorganisms from a surface to food or from one food to another food. Cross-contamination can occur when food contact surfaces are not cleaned or sanitized as necessary for food safety (Kendall, 2012). To prevent cross- contamination, it is important to wash hands with soap and warm water before preparing food or before handling a different food (for example, after handling raw chicken, hands should be washed before preparing a salad) and after using the bathroom. Sneezing or coughing on food should be avoided. Organisms can move from raw to cooked food, thus, raw food should not be left in contact with cooked food (FDA, 2014; Kendall,

2.4. Common foodborne bacterial pathogens

2.4.1. *Salmonella*

Salmonella are gram negative, non-sporing, facultative anaerobic rods and are a major cause of foodborne outbreaks in humans throughout the world. They are widely distributed in nature, with humans and animals being their primary reservoirs and can be mostly found in the intestinal tract of animals (Callaway et al., 2008; FDA, 2014). *Salmonella* can frequently be isolated from raw and cooked foods of animal origin, including ready-to-eat meats. Environmental contaminations can also result in *Salmonella* being present in a wide variety of foods, although generally at lower numbers. Their presence in ready-to-eat meats may be a result of undercooking, poor handling practices and cross contamination (Center for Food Safety, 2014).

Salmonellosis is grossly underreported because it is generally self-limiting gastroenteritis which may be misdiagnosed as intestinal influenza by patient or the physician (Anasthecian, 2008). Busby (1995) indicated that over a period of 5 years from 1983 to 1987 beef was the major contributor to foodborne diseases from *Salmonella* in the United States. However, Ntaate (2010) reported that currently the incidence rates of *Salmonella* on raw beef are generally low (5%).

More recently, Shilangale et al. (2015) reported a prevalence of 0.85 % for *Salmonella* in raw beef samples from abattoirs in Namibia. However, Mann (2006) and Ntaate (2010) stated that the levels can be relative and can be fairly high depending on the health or handling of the animals during slaughtering. Ntaate (2010) further explained that, there are various environmental sources of *Salmonella* that include water, soil, kitchen surfaces and animal feces that may help in the transmission of this pathogen. *Salmonella* are transmitted through the fecal matter of people or animals and are usually transmitted to humans by eating foods that have been contaminated with fecal matter through cross-contaminations (Ntaate, 2010). *Salmonella* can be destroyed by cooking, but contamination of cooked foods may occur from contact with utensils that were not

properly washed after use with raw products. If *salmonella* is present in raw or cooked foods, its growth can be controlled by refrigeration below 40°C (Kendall, 2012).

2.4.2. Coagulase positive *Staphylococcus aureus* (CPS)

S. aureus is a gram-positive, facultative anaerobe. *S. aureus* can be routinely isolated from humans and associated environments. Approximately 40% of adults carry *S. aureus* in the respiratory passage, skins and superficial wounds (Anasthessian, 2008). Therefore, coughs and sneezes may carry droplet infections which can easily spread not only to the environment but also to the food being handled (Anasthessian, 2008). The presence of Coagulase Positive Staphylococci (a subgroup of *S. aureus*), is an indication of human contact. Some CPS strains produce a toxin which can cause ready-to-eat meats poisoning. Even minimal handling of ready-to-eat meats can result in coagulase positive staphylococci being present in ready-to-eat meats at low levels (Food Standards Australia New Zealand, 2009). Furthermore, Carmo et al. (2004) discussed that the growth of these bacteria is favored by protein rich foods with high salt content. Foods that are frequently incriminated in staphylococcal food intoxication include beef and beef products, poultry and egg products, fish and fish products, salads, milk and milk products, and cream-filled bakery products. The researchers added that, if the contaminated food is stored at temperatures that encourage the growth of these organisms, production of enterotoxins may occur in the food and after ingestion of this food it will spread rapidly in the body. *S. aureus* is able to grow in a wide range of temperatures (7°C to 48.5°C with an optimum of 30°C to 37°C), pH (4.2 to 9.3 with an optimum of 7 to 7.5) and sodium chloride concentrations up to 15% (Carmo et al., 2004). Although cooking destroys the bacteria, the toxin produced is heat stable and may not be destroyed. Sometimes foods are left at room temperature for periods of time, allowing the bacteria to grow and produce the

toxin. The symptoms of *S. aureus* food poisoning develop within about 8 hours of ingestion of contaminated food and the symptoms include nausea, vomiting, abdominal cramps, diarrhea and sometimes a fall in body temperature (Colombari et al., 2007). *S. aureus* causes food intoxication or poisoning in various countries of the world (Bergdoll, 2000). Epidemic outbreaks of staphylococcal food poisoning (SFP) are relatively rare in developed countries. SFP accounts for only 1.3% of the total estimated cases of food-borne illnesses caused by known agents in United States of America (Sousa, 2008; Genigeorgis, 1989). In the early 1980's, SFP was reported to account for 14% of total food borne outbreaks in USA (Mead, 1999). Similar decrease in frequency has been reported in Japan. Before 1984, 25-35% of all cases of bacterial food-borne illness in Japan involved SFP, whereas in the late 1990's, only 2-5% of incidents involved SFP (Colombari et al., 2007). Although the disease caused by this pathogen is characterized by low mortality and relatively short duration, the frequency of poisoning and severity of the symptoms marks *S. aureus* food poisoning as an important foodborne disease (Anasthessian, 2008). Good personal hygiene when handling foods will keep *S. aureus* out of foods and refrigeration of raw and cooked foods will prevent the growth of these bacteria if any is present (Wagner, 2001).

2.4.3. *Shigella*

Shigella is a genus of gram-negative, non-spore forming, non-motile, non-encapsulated, non-lactose fermenting, facultative anaerobes and rod-shaped bacteria closely related to *E. coli* and *Salmonella* found in most surface-waters, sewage and food. Shigellosis, or bacillary dysentery, as it is commonly known, is caused by bacteria of this genus, which include *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii* and *Shigella sonnei* (Bryan, 1998). The normal habitat for *Shigella* is the intestinal tract of humans and other primates. Primary mode of transmission appears

to be person to person by the fecal-oral route. *Shigella* is mostly associated with chicken, raw vegetables, dairy products and poultry (Niyogi, 2005). Contamination of these foods is usually through the fecal-oral route and is most commonly due to faecally contaminated water and unsanitary handling by food handlers (Niyogi, 2005). *Shigella* is transmitted through the fecal matter of people or animals and is usually transmitted to humans by eating foods that have been contaminated with fecal matter through cross contamination. Most infections that occur are the result of the bacteria passing from stools or of soiled fingers of one person to the mouth or finger of another person (Niyogi, 2005). *Shigella dysenteriae* type cause deadly epidemics in developing countries (CDC, 2004). Common presenting features of shigellosis can include diarrhea that is bloody or watery, with or without mucus, fever, abdominal cramps & tenesmus. The annual number of *Shigella* episodes throughout the world is estimated to be 165 million, with 69% of all deaths attributable to Shigellosis involving children less than five years of age (Hamhata and Chinsebu, 2012). Shigellosis is a current health burden which is endemic and is estimated to affect 80-165 million individuals annually. The study that was conducted by WHO (2011), showed that 99% of infections caused by *Shigella* occur in developing countries.

2.4.4. *E. coli*

E. coli is a gram-negative, flagellated, rod-shaped and lactose-fermenting and part of the normal microflora of the intestinal tract of humans and warm-blooded animals. As such, their presence in ready-to-eat meats (fully cooked or semi cooked) can be an indication of poor hygiene and sanitation or inadequate heat treatment (indicator organisms) (Sousa, 2008; Taega, 2010). *E. coli* is usually not harmful but some strains may cause gastrointestinal infections, for example, Ingestion of the pathogenic serotype *E. coli* 0157:H7 and other Shiga toxin-producing *E. coli* (STEC) derived from infected meat can cause colitis with watery or bloody diarrhea, which may

give rise to the complications of hemolytic uremic syndrome (Elizabeth and Martin, 2003). *E. coli* is a significant cause of diarrhea in developing countries and localities with poor sanitation. The major source of the bacteria in the environment is probably the feces of infected human, animal reservoirs and untreated water which are most likely sources for contamination of food with *E. coli* 0157: H7 (Elizabeth and Martin, 2003). Buzby (2001) stated that its link to foodborne illness became well known to the public as a result of the outbreak of *E. coli* 0157:H7 food poisoning in 1993 which was caused by contaminated hamburgers in Finland. Over 700 people became ill from this outbreak and four children died (Buzby, 2001). *E. coli* 0157:H7 may be acquired through consumption of meat that has not been sufficiently cooked, and person-to-person transmission can occur through the fecal oral route. *E. coli* 0157: H7 can be found in the diarrhea stool of infected persons. The pathogens can be spread if personal hygiene and hand washing procedures are inadequate (Buzby, 2001).

2.4.5 *Enterobacteriaceae*

Members of *Enterobacteriaceae* are gram-negative, straight rods which are facultatively anaerobic and oxidase negative, for example, *Citrobacter* spp, *Salmonella* spp, *Klebsiella* spp, *Serratia* spp, *Proteus* spp, *Enterobacter* spp, *Shigella* spp, *E. coli* spp, *Yersinia* spp etc (Center for Food Safety, 2014). They can be found in many environments and are mainly found in the animal intestines, soil and plants from where they contaminate the food chain. They are regarded as indicators of fecal contamination when present in foods and are commonly found in hooves and hides of cattle. Furthermore, *Enterobacteriaceae* generally make up a greater proportion of the intestine and their presence in meat is usually a result of fecal contamination (Madigan et al., 2009). These organisms

may cause foodborne gastroenteritis. Control is mainly assured by proper slaughtering techniques, hygiene during slaughtering and adequate cooling (Kendall, 2012).

2.4.6. *L. monocytogenes*

L. monocytogenes is a gram-positive and facultative intracellular pathogen that causes listeriosis. It is found mainly in the soil, water, air or the intestinal tract (Gilmour et al., 2010). It was recognized as a human pathogen in 1929 (Gray and Killinger, 1966). The vast majority of human listeriosis cases are foodborne and the most commonly implicated sources of infection are ready-to-eat food products such as meat, dairy, seafood, and fresh produce that are contaminated with *L. monocytogenes* during processing (Gilmour et al., 2010). Most cases of human listeriosis are caused by the consumption of contaminated ready-to-eat foodstuffs which may occur due to cross-contamination with environmental sources or from the faeces of food production animals such as poultry, sheep and cattle (Esteban et al., 2009).

The incidence of *L. monocytogenes* is approximately 30-50% or greater in raw meat although levels are usually <100 cfu/g. Interest in *Listeria* has increased because it is capable of growing at refrigeration storage temperatures. The duration of storage may also give it extra time to grow to potentially dangerous levels. Ntaate (2010) also reported *L. monocytogenes* to be more pathogenic when grown at low temperature as it produces a toxin. *L. monocytogenes* causes an infection called listeriosis which is characterized by clinical symptoms such as gastrointestinal disorders (non-bloody diarrhea, nausea, and vomiting), influenza-like sickness (high fever, headache, and myalgia), and serious septicemia and meningitis (Indrawattana et al., 2011). Persons susceptible to symptomatic listeriosis include the elderly, infants, pregnant women, and individuals with

impaired immune systems due to HIV/AIDS, major surgery, malnutrition, gastric acidity, and lack of physical fitness (Swaminathan and Gerner-Smidt, 2007).

Table 2. Common food-borne bacteria and associated foods (Anasthedian, 2008)

Bacteria	Associated foods	Disease	Symptoms
<i>Bacillus Cereus</i>	Meats, milk, rice, potato and cheese products	<i>B. cereus</i> Food poisoning	Diarrhea, abdominal cramps, nausea.
<i>Clostridium Jejuni</i>	Raw chicken, unpasteurized milk, non-chlorinated water	Campylobacteriosis	Diarrhea, abdominal cramps, nausea and fever, headache and muscle pain
<i>Clostridium botulinum</i>	Canned foods including vegetables meats and soups.	Food-borne Botulism	Weakness, double vision, difficulty in speaking, swallowing & constipation.

<i>Clostridium perfringens</i>	Non-refrigerated prepared foods meats & meat products		Cramps, diarrhea
<i>Escherichia coli</i>	Undercooked meals, raw ground Beef	Hemorrhagic colitis	Severe abdominal pain, watery and bloody diarrhea and vomiting.
<i>Salmonella species</i>	Poultry and eggs milk and dairy products, raw meats, fish shrimp, peanut butter	Salmonellosis	Diarrhea abdominal pain, fever, headache diarrhea, vomiting, blood or mucus in stool.
<i>Shigella species</i>	Poultry milk, dairy products, raw vegetables, fecally	Shigellosis	Diarrhea, abdominal pain, fever, vomiting,

	contaminated water, salads, fish etc		blood or mucus in stool.
<i>Staphylococcus aureus</i>	Sandwich, salads, poultry and egg products, meat products, dairy products.	Staphyloenterotoxigenesis & Staphyloenterotoxemia	Abdominal cramping, Nausea and vomiting
<i>Vibrio cholerae</i>	Contaminated water and fish.	Cholera	Watery diarrhea, abdominal pain, dehydration, vomiting, shock.
<i>Listeria monocytogenes</i>	Meat, dairy, seafood, and fresh produce	Listeriosis	Non-bloody diarrhea, nausea, vomiting, high fever, headache, myalgia, serious septicemia and meningitis

2.5. Prevention of food-borne diseases

2.5.1. Good personal hygiene

According to CDC (2015), the most important practice one can do to prevent foodborne illness is to wash hands with soap and warm water frequently, especially after using the toilet, changing a diaper, petting an animal, and before preparing or eating foods. Diarrhea sick people should not prepare food for others because a high number of infectious viruses or bacteria can be in fecal matter and the infectious dose is low for many of these pathogens (Medeiros, Hillers and Kendall, 2000). Furthermore, cuts and burns on hands should be covered while preparing foods because infected cuts can be a source of *Staphylococcus* species and other germs. Hands should be washed after any contact with raw poultry, beef or seafood and food handlers should always make sure that their hair, fingernails and clothing are clean. If clean utensils can be used to mix foods, using hands should be avoided (Medeiros et al., 2000). Moreover, hands should be kept away from the mouth, nose, and hair when handling food. When coughing or sneezing, the nose and the mouth should be covered with disposable tissues and hands should be washed with soap afterwards. A clean spoon should be used each time when tasting food while preparing, cooking or serving (CDC, 2015; Kendall, 2012).

2.5.2. Avoiding cross-contamination

Cross-contamination or the moving of pathogens from one food to another food should be avoided; this is an especially critical consideration when dealing with food that is ready-to-eat. An excellent general rule to avoid cross contamination is to always keep cooked and ready-to-eat foods separate from raw foods. Food preparation surfaces should be cleaned before and after preparing food. Hands, knives, cutting boards, food preparation surfaces and sink should be washed thoroughly

after any contact with raw poultry, beef, seafood, or any other potentially hazardous foods (WHO, 2008).

Additionally, raw and ready-to-eat meats should not be stored together in the refrigerator. Kitchen cloths and sponges should be kept clean and dry. It is best to have 2 cutting boards, one for raw meats and another for cooked meats. The cutting board and utensils can be washed with hot water and sanitized with a kitchen sanitizer to detract the pathogens (CDC, 2015).

2.5.3. Keeping foods at safe temperatures

Like most other living things, bacteria need food, warmth, moisture, time to grow and multiply. In order to prevent bacteria from growing in foods, hot foods should be kept hot (above 60°C) and cold foods cold (below 4°C) (Medeiros et al., 2000). All prepared and leftover foods should be refrigerated within 2–3 hours. Foods can become unsafe if held for too long in the 16–52°C range, the zone where bacteria grow most rapidly, thus it is advised to not hold food for too long at 16–52°C range (WHO, 2008). In most cases, prompt cooling and proper refrigeration of foods can keep the number of bacteria at a safe level, the colder the food is kept, the less chance bacteria have to grow. The refrigerator temperature should be between 2°C and 4°C. Foods shouldn't be prepared too far in advance of serving without plans for proper cooling or reheating and all perishable foods such as eggs, meat, and dairy products should be stored at or below 4°C (CDC, 2015).

2.5.4. Avoiding foods and water from unsafe sources

Medeiros et al. (2000) explained that, some foods have such a high probability of being contaminated with pathogens or toxins and their consumption should be avoided. The risk is

highest for people susceptible to foodborne illness like pregnant women. Many of these foods are known as “ready-to-eat” but have been produced or processed in a way that does not kill pathogens. Foods and beverages frequently linked to foodborne illness include: Unpasteurized milk and milk products, raw or undercooked beefs and poultry, unpasteurized fruit juice, raw sprouts of all types, raw seafood and raw fish or undercooked eggs and contaminated water (Kendall, 2012; Medeiros et al., 2000).

2.5.5. Cooking foods adequately

Cooking is an essential part of making foods safe to eat because almost all pathogens are killed when food is heated to 71°C for a few seconds. However, keeping foods at lower temperatures for longer periods of time may also kill pathogens. The best way to be certain that food has been cooked to proper temperatures is to check it with a thermometer (FDA, 2014). Many pathogens live naturally in the intestinal tracts of animal, for example, *Salmonella spp.* and *Campylobacter spp.* A survey conducted by WHO (2014) on raw and ready-to-eat meats sold in various retail food stores indicated that between ¼ and ¾ of meat may be contaminated with one or more of these pathogens. Lastly, any foods likely to be contaminated with pathogens should be heated to 71°C because at this temperature, most pathogens can be killed very quickly (Kendall, 2012).

CHAPTER 3: MATERIALS AND METHODS

3.1 Research design

The research design consisted of experiments that produced both quantitative and qualitative data. Detection, biochemical confirmation and microscopic confirmation of the isolates related experiments yielded qualitative data. Data from the enumeration of microorganisms per sample was quantitative.

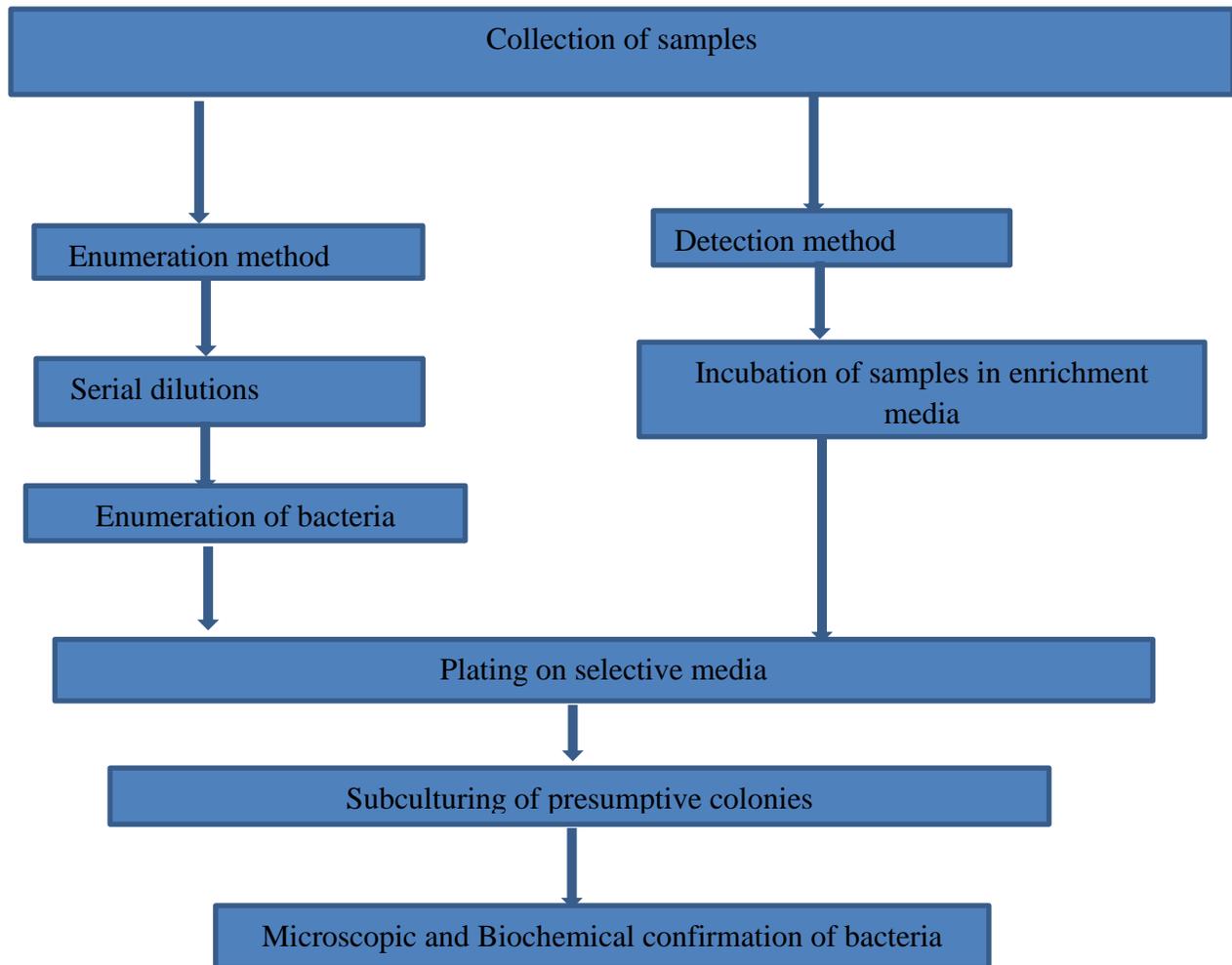


Figure 1. Schematic flow chart of research methods that were used in this study.

3.2. Data Collection

A preliminary survey was conducted in Windhoek to identify different locations where street-vending of ready-to-eat meats takes place and identified. The selected locations were Katutura (Single Quarters, Havana, Okuryangava, Greenwell and Wanaheda), Dorado Park and Prosperita in Windhoek, Namibia.

3.3 Sample size

Based on the prevalence of *Salmonella* (8%) in a study that was done by Manguiat and Fang (2013) in Taiwan, the following formula was used to estimate the sample size of this study. For a 95% confidence interval and a margin of error of 0.05, the following formula was used: $n = p \cdot (1-p) \cdot z^2 / d^2$, $p =$ percentage prevalence of the previous study, $z = 1.96$, appropriate value for a 95% confidence interval, $d = 0.05$, the margin of error. Therefore, $n = 0.06 \cdot (1-0.06) \cdot (1.96 \cdot 1.96) / 0.05 \cdot 0.05$ $n = 86.7$. For statistical reliability, a sample size of ≥ 87 was required, 96 samples of ready-to-eat meats samples were purchased in this study.

3.4 Sample collection and storage

Samples of ready-to-eat meats including chicken and beef were collected from Katutura (Single Quarters, Havana, Okuryangava, Greenwell and Wanaheda), Dorado Park and Prosperita in Windhoek, Namibia. Samples were selected using a simple random sampling, the purchased ready-to-eat meat samples were placed into sterile lunch boxes at the point of purchase, just as a consumer would do. Samples were then aseptically transferred to sterile stomacher bags. The packed samples were placed in a cooler box and immediately transported to the Department of Food Microbiology at the Central Veterinary Laboratory of the Ministry of Agriculture, Water and Forestry in

Windhoek, Namibia. Microbiological analysis of all samples was carried out within 4 hours of purchase.

3.5 Microbiological Analysis

3.5.1 Enumeration of bacteria in each sample

The conventional methods for the microbiological analysis were performed according to the Central Veterinary Laboratory (Food Hygiene section)'s Standard Operation Procedures (SOPs). Samples were analysed for Aerobic Plate Count (APC), *E. coli*, *Enterobacteriaceae*, *S. aureus*, *Salmonella* spp., *Shigella* spp. and *L. monocytogenes*. The sample (25g) was aseptically transferred and mixed with 225ml of sterile butterfield's phosphate buffer (BPB) (Merck, Germany) in a sterile stomacher bag. The sample and the phosphate buffer mixture were blended for 2 minutes. Ten-fold serial dilutions (10^{-1} - 10^{-4}) were prepared in 2% sterile Butterfield 's phosphate buffer (Merck, Germany) and the sample suspension (1 ml) was inoculated on Plate Count Agar plates for Aerobic Plate Count (APC) (Merck, Germany), on Violet Red Bile Dextrose Agar (VRBG) (Biolab, Merck, Germany) for *Enterobacteriaceae* and on Tryptone Bile X-glucuronide (TBX) agar (Merck, Germany) plates for *E. coli*, Subsequently, 0.1 ml of the sample suspension was inoculated on Xylose Desoxylate Agar (XLD) (Merck, Wadeville, South Africa) for *Salmonella*, on *Salmonella-Shigella* agar (Biolab, Merck, Germany) selective media for *Shigella*, on Baired Parker Agar (BPA) (Scharlau, Chemie SA, Spain) for *S. aureus* and on Oxford agar (Biolab, Merck, Germany) for the detection of *L. monocytogenes*. The spread plating (for *L. monocytogenes*, *Salmonella* and *Shigella*) and the pour plating (for APC, *Enterobacteriaceae* and *E. coli*) methods were used. The plates were then incubated at their specific temperatures for 30-72 hours, that is, APC at $30\pm 1^{\circ}\text{C}$ for 24-72 hours, *Enterobacteriaceae*, *Salmonella*, *Shigella*, *L.*

monocytogenes and *S. aureus* at $37\pm 1^\circ\text{C}$ for 18 to 24 h, *E. coli* at $44 \pm 1^\circ\text{C}$ for 18 to 24 h. After incubation, Results were expressed in Log cfu/g (Colony Forming Units/g). Representative colonies were picked from each agar plate and were purified by subculturing on respective agar using the streaking method. Presumptive colonies of *S. aureus* on BPA appear black clear zones and *E. coli* colonies on TBX appear blue.

3.5.2 Isolation and Confirmation of *Salmonella* bacteria

The conventional methods for the isolation of *Salmonella* was performed according to the International for Standards Organisation (ISO) (6579: 2002) for Microbiology of food and animal feeding stuffs horizontal method for the detection of *Salmonella*. The procedure has four main stages; pre-enrichment, enrichment, plating and confirmation. The pre-enrichment stage for all types of ready-to-eat meat samples was done using Buffered Peptone Water (BPW).

3.5.2.1 Non-selective pre-enrichment

Twenty-five grams of ready-to-eat meat or chicken sample was weighed and transferred into a sterile stomacher bag. About 225 ml of buffered peptone water (BPW) (Merck, Darmstadt, Germany) was added into the sample. The sample was then homogenized with a stomacher machine (Merck, Darmstadt, Germany) for about 2 minutes, followed by incubation at $37 \pm 1^\circ\text{C}$ for approximately 18 to 24 hours.

3.5.2.2 Selective enrichment

After the incubation time, samples from the pre-enrichment broth were mixed and then inoculated into a selective broth. Approximately 0.1 ml of the pre-enrichment broth was transferred into a tube containing 10 ml of Rappaport-Vassiliadis medium with soya (RVS) broth (Scharlau Chemie S.A. Barcelona, Spain). Another 1 ml of the pre-enrichment broth was transferred into a tube containing 10 ml of Mueller Kauffmann Tetrathionate Novobiocin (MKTTn) broth (Merck,

Darmstadt, Germany). Inoculated RVS broth (Scharlau Chemie S.A. Barcelona, Spain) was incubated at $41.5 \pm 1^\circ\text{C}$ for 18 to 24 hours while inoculated MKKTn broth was incubated at $37 \pm 1^\circ\text{C}$ for 18 to 24 h.

3.5.2.3 Culture and identification

After incubation, a loopful of the enriched cultures of RVS broth and MKKTn broth was streaked separately onto two selective agar plates: Xylose Lysine Desoxycholate (XLD) (Merck, Wadeville) and Rambach agar (Scharlau, Chemie SA, Barcelona, Spain). These plates were incubated in an inverted position at $37 \pm 1^\circ\text{C}$ for 18 to 24 h. After incubation, the black and pink colonies with or without black center on XLD agar, and the pink-red on Rambach agar were identified as presumptive *Salmonella* spp. Those colonies were selected and sub-cultured on nutrient agar (Merck, Wadeville, South Africa) and incubated at $37 \pm 1^\circ\text{C}$ for approximately 18 to 24 hours. Two or more colonies of typical suspicious *Salmonella* were selected from the Nutrient agar using a sterile inoculating wire loop. The selected colonies were used to perform biochemical confirmation tests. Gram staining was first done to indicate a gram reaction.

3.5.2.4 Gram staining

Identification of isolates through gram staining was according to the following steps; a thin smear on a clean slide was done by air drying, heat fixing and allowed to cool down for 1 min, the slide was then flooded completely with carbol gentian violet for 3 minutes and the stain was poured off. After, the slide was decolorized with alcohol until the smear appeared grayish blue. The slide was then counterstained with dilute fuschin for 15 seconds, rinsed with water and air dried. Lastly, the slide was counterstained with lugol's iodine for 2 minutes. Slides were viewed using an optical microscope (Olympus BX51, Japan). Gram reaction as gram positive (+) was indicated by a purple

or blue colour while, gram negative (-) indicated by a red or pink colour (Bartholomew and Finkelstein, 1958). Gram negative (-) isolates were selected for *Salmonella* identification.

3.5.2.5 Biochemical tests

Urea agar test; the urea agar slant surface was inoculated by streaking the agar slope surface and stabbing the butt with pure culture of typical suspicious *Salmonella* from the Nutrient agar. The Urea agar slants were then incubated at $37 \pm 1^\circ\text{C}$ for approximately 18 to 24 hours, followed by results interpretation. The positive reaction showed splitting of urea which liberated ammonia, with changes of the colour from phenol red to rose pink, and later to deep cerise (moderate red). The reaction is often apparent after 2 to 4 hours. For a negative reaction, the color of the Urea media remained unchanged. Triple sugar iron agar (TSI agar); the TSI agar slant was inoculated by streaking slant and stabbing the butt with pure culture of typical suspicious *Salmonella* from the Nutrient agar. After inoculation the TSI agar was incubated at $37 \pm 1^\circ\text{C}$ for 18 to 24 hours. The inoculated tubes were capped loosely to maintain aerobic conditions while incubating in order to prevent excessive Hydrogen Sulphide (H_2S) production. For interpretation of the TSI results, typical *Salmonella* cultures show alkaline (red) slants and acid (yellow) butts with gas formation (bubbles) and (in about 90% of the cases) formation of hydrogen sulfide (blackening of the agar). When lactose-positive *Salmonella* is isolated the TSI agar slant is yellow. Indole test was done by inoculating pure culture of typical suspicious *Salmonella* from the nutrient agar onto test tubes containing 9ml of Tryptone broth and incubated at 37°C for 24 hours. The positive reaction showed a purple ring after the addition of the Kovac's reagent which shows that the bacteria contain tryptophanase and can hydrolyse tryptophan to indole, pyruvic acid and ammonia. For a negative reaction, the colour of the ring is yellow after the addition of the Kovac's reagent. Serological

confirmation; omnivalent anti-sera (Siemens, Marburg) was used for serological confirmation. A portion of the colony to be tested was dispersed in the drop in order to obtain a homogeneous and turbid suspension. The slide was then gently shaken for approximately 30 to 60 seconds and the result was observed in contrast to a dark background. If the bacteria are clumped, the strain was considered auto agglutinable. Gram negative, Indole negative, Urea negative, TSI positive colonies were selected for further identification. For each test, a positive control *Salmonella typhimurium* ATCC 14028 and *Salmonella paratyphi* ATCC 9150 and a negative control (*L. monocytogenes*) was used.

3.5.3. Isolation and Confirmation of *Shigella* bacteria

3.5.3.1 Non-selective pre-enrichment

A Sample of 25g was enriched into 225 ml of buffered peptone water (Merck, Darmstadt, Germany) and incubated at $37 \pm 1^\circ\text{C}$ for 18 to 24 hours. The culture was then streaked onto the *Salmonella-Shigella* agar (SSA) (Biolab, Merck, Germany). On SSA, presumptive *Shigella* colonies appear cream, while, the *Salmonella* colonies appear black with or without black center. Those colonies were selected and sub-cultured on nutrient agar (Merck, Wadeville, South Africa) and incubated at $37 \pm 1^\circ\text{C}$ for approximately 18 to 24 hours. Two or more colonies of typical suspicious *Shigella* were selected from the Nutrient agar using a sterile inoculating wire loop. The selected colonies were used to perform biochemical and serological confirmation tests.

3.5.3.2 Gram staining

Gram staining was done using (same procedures in 3.5.2.4) was first done to indicate a gram reaction. Slides were viewed using an optical microscope. Gram reaction as gram positive (+) was indicated by a purple or blue colour or gram negative (-) indicated by a red or pink color

(Bartholomew and Finkelstein, 1958). Gram negative (-) isolates was selected for *Shigella* identification.

3.5.3.3 Serological confirmation

Shigella antisera's was used for serological confirmation. Two separate drops (40 µl) of saline were put on a sterile glass slide, *Shigella* culture was emulsified with a sterile loop in each drop of saline to give a smooth, fairly dense suspension. To one suspension, 40ul of saline was added as a control and mixed. To the other suspension, one drop (40µl) of undiluted antiserum was added and mixed. The slide was rocked for one minute and observed for agglutination. Biochemical tests: Urea agar test was done by inoculating the pure colonies from nutrient agar into test tubes containing the Urea Stuarts's agar slant test tubes and were incubated at $37\pm 1^{\circ}\text{C}$ for 18 to 24 hours. Urea negative was indicated by no colour change and positive results by changes from yellow to red or pink. Triple Sugar Iron (TSI) test was done by inoculating pure colonies on test tubes containing the TSI agar slant and were incubated at 37°C for 24 hours. Indole test was done by inoculating pure colonies of *Shigella* onto test tubes containing 9ml of Tryptone broth and incubated at 37°C for 24 hours. At the end of the incubation period, 0.3 ml of the Indole Kovac's reagent was added and the color changes were observed. Gram negative, Indole negative, Urea negative, TSI negative colonies were selected for further identification.

3.5.4. Isolation and confirmation of *L. monocytogenes*

The conventional methods for the isolation of *L. monocytogenes* were performed according to the ISO 11290-1: 1996 for Microbiology of food and animal feeding stuffs horizontal method for the detection and enumeration of *L. monocytogenes*. The procedure has four main stages; primary and secondary enrichment, plating and confirmation.

3.5.4.1 Primary and secondary enrichment

Primary and secondary enrichment was done using the *L. monocytogenes* half fraser broth and Mops BLEB broth respectively. For primary enrichment, an amount of 9 ml of the *L. monocytogenes* enrichment broth (half fraser broth) was poured into test tubes. An amount of 1g was blended and added into different test tubes containing 9 ml of the enrichment broth. These test tubes were then incubated at 37 °C ±1 for 18 to 24 hours. For secondary enrichment, 0.1ml of the culture was added to 10ml of the *L. monocytogenes* Mops BLEB broth. The culture was then streaked onto to selective agar: Oxford agar (Biolab, Merck, Germany) and PALCAM agar (Biolab, Merck, Germany) respectively and incubated at 37 °C ±1 for 48 hours. After the incubation period, the appearance of colourless-shiny and opaque colonies on Oxford agar and small greyish green or olive green was indicative of *L. monocytogenes*. Identification of isolates through gram stain to indicate a gram reaction was done using the same procedures in 3.5.2. Slides were viewed using an optical microscope. Gram reaction as gram positive (+) was indicated by a purple or blue colour or gram negative (-) indicated by a red or pink colour (Bartholomew and Finkelstein, 1958). Grams positive (+) isolates were selected for *L. monocytogenes* identification.

3.5.4.2 Biochemical confirmation tests

Catalase test, Oxidase and Citrate test were performed. Catalase test was done by placing a drop of 3% hydrogen peroxide on a clean slide and a colony was spread from each of the selective media. Gas bubble production was observed, indicating catalase positive reaction and no gas bubble indicated catalase negative reaction. Citrate test was done by inoculating the test tubes containing the Simmons' citrate agar slant and incubated at 37 °C for 18 to 24 hours. Test tubes indicating a change from green to blue indicated positive results (contain citrase enzyme that can metabolise citrate to produce alkaline end products that can raise pH causing bromthymol blue to turn to blue), while those that remained green indicated negative results (Stiles and Laiking, 2000). Oxidase test was performed by inoculating a colony on the oxidase test-strips. Colour observation was done after 20-60 seconds. Yellow to red colour indicated negative results and blue to purple colour indicated positive results. Gram positive, Oxidase negative, Citrate negative and Catalase positive colonies were kept for further *L. monocytogenes* identification.

3.5.5 Confirmation of *S. aureus*

The presumptive colonies of *S. aureus* on BPA are black with clear halos around the colonies or opaque zones due to the egg yolk that is added in the BPA media (SABS ISO 6888-1: 1999). Identification of isolates through gram stain to indicate a gram reaction was done using the same procedures in 3.5.2. Slides were viewed using an optical microscope. Gram reaction as gram positive (+) was indicated by a purple or blue color or gram negative (-) indicated by a red or pink colour (Bartholomew and Finkelstein, 1958). Grams positive (+) isolates were selected for *S. aureus* identification. Presumptive colonies of *S. aureus* from BPA were confirmed by the *S. aureus* coagulase test (Bactident Coagulase). The test was performed by transferring a colony in a

tube containing 5ml of the brain heart infusion broth and incubated at 37°C for 18 to 24 hours. A culture of 0.1ml was added to 0.3ml of the rabbit plasma and incubated at 37°C for 18 to 24 hours. Positive results were indicated by the volume of clot which occupied half the volume of the liquid. As a negative control, for each batch of plasma, 0.1 ml of sterile brain heart infusion broth was added to the rabbit plasma and incubated together with the culture containing rabbit plasma. Oxidase test was performed by inoculating a colony on the oxidase test-stripe. Color observation was done after 20-60 seconds. Yellow to red colour indicated negative results and blue to purple colour indicated positive results. Gram positive (+), Coagulase positive and Oxidase negative colonies were selected for further identification of *S. aureus*. Positive control *S. aureus* ATCC 25923 was used.

3.5.6 Confirmation of *Enterobacteriaceae*

Identification of isolates through gram staining was done to indicate a gram reaction. Slides were viewed using an optical microscope. Gram reaction as gram positive (+) was indicated by a purple or blue colour or gram negative (-) indicated by a red or pink colour (Bartholomew and Finkelstein, 1958). Gram negative (-) isolates was selected for *Enterobacteriaceae* identification. Furthermore, glucose test was done by inoculating the pure colonies from nutrient agar into test tubes containing the purple glucose agar test tubes and were incubated at 37°C for 18 to 24 hours. Oxidase test was performed by inoculating a colony and spreading it on the oxidase test-strips. Colour observation was done after 20-60 seconds. Yellow to red colour indicated negative results and blue to purple colour indicated positive results (ISO 21528-2: 2004). Gram negative, Oxidase negative and Glucose positive colonies were kept for further *Enterobacteriaceae* identification.

3.5.7 Isolation and Confirmation of enteropathogenic *E. coli* strains

Some 3-4% of *E. coli* from foods, especially biotype 1, 2 and serogroup 0157 strains are glucuronidase-negative and cannot grow at 44°C (Retnam et al.,1988). Hence, homogenized samples were also cultured based on Andrews' method (Andrews, 1992). Aseptically, a sample of 25 g was weighed out and added to 225 ml of BPW. Selective enrichment was done by inoculating 1ml of the culture into a tube containing 10 ml of Lauryl Tryptose (LT) broth and Durham tubes (Scharlau Chemie S.A, Spain) and examined for gas production after 24 hours of incubation. Samples (1 ml) producing gas, that is, displacement of the medium in the Durham tubes were transferred into EC medium (Scharlau Chemie S.A, Spain) for a further 24 hours at 37°C. Samples producing gas were then streaked on MacConkey agar (Oxoid) and were incubated at 37°C for 18 to 24 hours. For identification purposes, isolated suspicious colonies (colonies that were bright pink in colour) of *E. coli* were sub cultured on nutrient agar and incubated at 37°C for 18 to 24 hours. Identification of isolates through gram staining was done to indicate a gram reaction. Slides were viewed using an optical microscope. Gram reaction as gram positive (+) was indicated by a purple or blue colour or gram negative (-) indicated by a red or pink colour (Bartholomew and Finkelstein, 1958). Gram negative (-) isolates was selected for *E. coli* identification. Colonies were then confirmed biochemically using the IMVIC (Indole, Methyl red, Voges-Proskaur (VP) and Citrate) tests. Indole test was done by inoculating a suspected colony of *E. coli* from nutrient agar into a tube containing 5ml of the tryptone/tryptophan medium. The medium was then incubated at 37 °C for 18 to 24 hours. After incubation, 1 ml of the Kovacs reagent was added in the medium. The formation of a red ring indicated a positive reaction. A yellow-brown ring indicated a negative reaction. The VP test was performed as follows; a loop-full of the suspected colony of *E. coli* from the Nutrient agar was suspended in a sterile tube

containing 3 ml of the Voges-Proskauer (VP) medium and incubated at 37 °C ±1 for 24 ±3 hours. After incubation 2 drops of creatine solution was added followed by 3 drops of the ethanolic solution of 1-naphthol and then 2 drops of potassium hydroxide solution. The solution was shaken after each addition of the reagent. The formation of pink to bright red color within 15 minutes indicated a positive reaction. Methyl red test was performed by incubating the VP tube for an additional 48 hours at 37°C after performing the VP test. After incubation, 5 drop of methyl red solution was added to each tube. A distinct red colour indicated a positive reaction and a yellow colour indicated a negative reaction. Citrate test was done by inoculating the test tubes containing the Simmons' citrate agar with pure suspicious colonies of *E. coli* and incubated at 37°C for 18 to 24 hours. Blue colour indicated a positive reaction and green colour indicated a negative reaction.

Table 3. Summary of typical biochemical reactions (IMVIC) for Enteropathogenic *E. coli* strains

Test	Positive	Negative	<i>E. coli</i> reaction
Indole	Red ring	Yellow ring	+/-
Methyl red	Red	Yellow	+
VP	Pink-to-red	No colour change	-
Citrate	Blue	No colour change	-

IMVIC pattern of +++ - (Biotype 1) and - + - - (Biotype 2)

3.5.8 Further confirmation of the isolates with VITEK® 2 Systems

All the isolates were further confirmed with the VITEK® 2 systems (Biomerix, France) up to their species level. The gram negative bacteria were confirmed using the Gram Negative (GN) test kit VITEK® 2 cards and the gram positive bacteria were confirmed using the Gram Positive (GP) test kit VITEK® 2 cards. A fresh culture was inoculated on nutrient agar from a pure culture and incubated at 37°C for 24 hours. Aseptically 3.0 mL of sterile saline was transferred into a clear plastic (polystyrene) test tube (12 mm x 75 mm). Subsequently, a homogenous suspension was prepared by transferring sufficient (0.5-0.63 ml) morphologically similar colonies to the saline tube using a sterile swab. The suspension tube and the GN or GP cards were then placed on the cassette. Lastly, the cassette was loaded into the following the VITEK® 2 machine following the manufactures' instructions. Results were available in about six hours.

3.6 Data analysis

Measurements of colony forming units were done in triplicates and average means were calculated with standard deviation (\pm SD) for each of the locations. Kolmogorov-Smirnov test was done to check for normal distribution for each of the locations. Differences between prevalence of pathogens in different locations were analyzed using the One way ANOVA (to compare the means) and Tukey's post hoc multiple comparisons test was further used to analyse the differences. Pearson Correlation test was used to assess the relationship between the preparation methods, type of meat, time of collection as well as the serving temperatures' and the prevalence of pathogenic bacteria in the meat using Statistical package for Social Sciences (SPSS) version 24. Furthermore,

the microbiological quality guidelines for ready-to-eat (RTE) foods by (ICMSF, 2001), served as the basis for the evaluation of the microbiological quality of street vended ready-to-eat meats.

3.7 Research Ethics

The UNAM research ethics was strictly adhered to in this study. Permission was sought from the Windhoek municipality for the collection of ready-to-eat meat samples from the street food vendors in Windhoek. Meat vendors were informed of the importance of the study and were assured that it is only for research purposes. To maintain privacy and confidentiality pertaining to information collected from participants, data was collected anonymously and the samples were given codes/sample identities, without disclosing participants' identities. Upon dissemination to relevant stakeholders (example, Ministry of Health and Social Services and the Municipality of Windhoek) results will not disclose any identities of participants whose meats were found to have unacceptable results.

CHAPTER 4: RESULTS

4.1 Aerobic Plate Count in ready-to-eat meat samples

Table 4 shows the prevalence of aerobic plate count in the various locations where ready-to-eat meats were purchased. For the 96 ready-to-eat samples, 32% (31 of 96) samples showed unsatisfactory high levels of aerobic plate count. Havana had the highest unsatisfactory levels (explained in Table 1) of 65% (13 of 20), followed by Okuryangava 50% (4 of 8), Prosperita 45% (9 of 20), Single Quarters 27% (4 of 15) and Dorado Park 7% (1 of 15), while aerobic plate counts were in the satisfactory range for samples purchased from Wanaheda and Greenwell. The mean

APC counts of street vended ready-to-eat meats ranged from $2.67 \pm 0.94 \log \text{cfu g}^{-1}$ (Greenwell) to $5.54 \pm 0.94 \log \text{cfu g}^{-1}$ (Havana) and the highest APC value of $7.74 \log \text{cfu g}^{-1}$ was observed in the cold, roasted beef sample purchased in the afternoon from Havana. Significant differences were recorded in aerobic plate count between the locations ($P < 0.05$). Tukey's multiple comparisons test showed that, there was a significant difference ($P < 0.05$) for aerobic plate count between Havana and all the other locations. Pearson's correlation test showed significant positive correlations for the prevalence of aerobic plate count with the time of collection and the serving temperature of ready-to-eat meats ($r = 0.436, p = 0.000$), ($r = 0.307, p = 0.002$) respectively.

Table 4. Aerobic Plate Counts in sampled locations

Locations	No. of samples examined	% Positive	% Unsatisfactory	Mean ($\log \text{cfu g}^{-1}$)
Single Quarters	15	100	27	4.13 ± 0.81
Havana	20	100	65	5.54 ± 0.94
Wanaheda	9	100	0	4.11 ± 0.71
Dorado Park	15	100	7	3.70 ± 0.72
Okuryangava	8	100	50	0.89 ± 0.99

Greenwell	9	100	0	2.67±0.94
Prosperita	20	100	45	4.51±1.19

4.2 Prevalence of *Enterobacteriaceae* in ready-to-eat meat samples

Out of the 96 samples of ready-to-eat meats, 83% (80 of 96) were found to be positive for *Enterobacteriaceae*. However, unsatisfactory levels of *Enterobacteriaceae* were found in 26% (25 of 96). Unsatisfactory levels (Table 1) of *Enterobacteriaceae* were observed in Havana 55% (11 of 20), Okuryangava 38% (3 of 8), Prosperita 25% (5 of 20), Dorado Park 27% (4 of 15) and Wanheda 1% (1 of 9). Remarkably, *Enterobacteriaceae* were in the satisfactory range for samples purchased from Greenwell and Single Quarters (Table 5). The mean *Enterobacteriaceae* counts ranged from $0.76 \pm 1.23 \log \text{ cfu g}^{-1}$ (Greenwell) to $4.10 \pm 0.77 \log \text{ cfug}^{-1}$ (Havana). The highest *Enterobacteriaceae* count of $5.76 \log \text{ cfu g}^{-1}$ was also observed in the cold, roasted beef sample purchased in the afternoon from Havana. There were significant differences in *Enterobacteriaceae* counts between the locations ($P < 0.05$). Tukey's multiple comparisons test showed that there was a significant difference for *Enterobacteriaceae* between Havana and all the other locations ($P < 0.05$). Pearson's correlation test showed significant positive correlations for the prevalence of *Enterobacteriaceae* with the time of collection ($r = 0.295$, $p = 0.003$) and with the area of collection ($r = 0.358$, $p = 0.000$). The *Enterobacteriaceae* isolates were further identified up to the species level (Table 6). The identified species include; *Enterobacter cloacae* (27%), *Enterobacter sakazakii* (13%), *Enterobacter kobei* (5%), *Enterobacter ludwigi* (8%), *Enterobacter hormaechei* (4%), *Pseudomonas luteola* (5%), *Pseudomonas aeruginosa* (4%), *Pseudomonas stutzeri* (4%), *Klebsiella pneumonia* (15%), *Klebsiella oxytoca* (14%) and *Citrobacter koseri* (2%).

Table 5. Prevalence of *Enterobacteriaceae* in sampled locations

Locations	No. of samples examined	% Positive	% Unsatisfactory	Mean (log cfu g ⁻¹)
Single Quarters	15	67	0	1.58±1.44
Havana	20	100	55	4.10±0.77
Wanaheda	9	78	1	2.17±1.48
Dorado Park	15	93	27	2.94±1.25
Okuryangava	8	100	38	3.45±0.69
Greenwell	9	33	0	0.76±1.22
Prosperita	20	90	25	2.41±1.52

Table 6. Bacteria Strains identified (using the VITEK® 2 machine) in each location

No. of identified strains/ Locations	Wanaheda	Okuryangava	Havana	Single Quarters	Prosperita	Dorado Park	Green well	Total no. of individual strains
<i>E. coli</i>	5	4	10	7	2	3	0	31
<i>S. aureus</i>	4	7	9	1	2	0	4	27
<i>L. monocytogenes</i>	0	5	5	2	2	0	0	14
<i>S. flexineri</i>	0	2	4	0	1	0	1	10
<i>S. typhimurium</i>	2	0	5	0	0	0	0	7
<i>S. enterica</i>	0	0	3	0	0	0	0	3
<i>S. enteritidis</i>	0	0	1	0	0	0	0	1
<i>E. cloacae</i>	11	8	15	6	10	6	5	61
<i>E. sakazaki</i>	8	8	6	1	5	2	2	32
<i>E. kobei</i>	4	4	2	0	3	1	0	14
<i>E. ludwigi</i>	3	2	4	0	8	3	0	20
<i>E. hormaechi</i>	4	2	3	0	3	0	0	9
<i>P. luteolo</i>	3	4	1	1	5	0	0	14

<i>P. aeruginosa</i>	4	2	1	0	2	0	0	9
<i>P. stutzeri</i>	3	2	3	0	2	0	0	10
<i>K. oxycota</i>	8	5	8	2	7	4	1	35
<i>K. pneumonia</i>	10	3	3	4	7	4	2	33
<i>C. koseri</i>	2	1	0	0	1	0	0	4
Total	71	59	81	24	60	23	15	333

4.3 Prevalence of *E. coli* in ready-to-eat meat samples

Forty-two percent (40 of 96 samples) of the ready-to-eat meat samples were found to be positive for *E. coli*. However, unsatisfactory levels (explained in Table 1) of *E. coli* were 35% (34 of 96). Unsatisfactory levels of *E. coli* were observed in Havana 75% (15 of 20), Wanaheda 56% (5 of 9), Single Quarters 47% (7 of 15), Okuryangava 38% (3 of 8), Dorado Park 20% (3 of 20) and Prosperita 5% (1 of 20). *E. coli* were in the satisfactory range for Greenwell samples (Table 7). The mean *E. coli* ranged from 0 log cfu g⁻¹ (Greenwell) to 3.55±1.72 log cfu g⁻¹ (Havana). The highest *E. coli* count of 5.67 log cfu g⁻¹ was also observed in the cold, roasted beef sample purchased in the afternoon from Havana. There were significant differences in *E. coli* between the locations (P < 0.05). Turkey's multiple comparisons test showed that, *E. coli* was significantly higher in samples purchased from Havana than all other locations sampled (P < 0.05). Pearson's correlation test showed a significant positive correlation for the prevalence of *E. coli* with the time

of collection ($r= 0.449$, $p=0.000$). No enteropathogenic *E. coli* was detected from all the ready-to-eat meat samples.

Table 7. Prevalence of *E. coli* in sampled locations

Locations	No. of samples examined	% Positive	% Unsatisfactory	Mean (log cfu g ⁻¹)
Single Quarters	15	47	47	1.05±1.23
Havana	20	95	75	3.55±1.72
Wanaheda	9	55	56	1.66±1.81
Dorado Park	15	20	20	0.56±1.19
Okuryangava	8	50	38	1.45±1.77
Greenwell	9	0	0	0
Prosperita	20	10	5	1.45±1.77

4.4 Prevalence of *Salmonella* in ready-to-eat meat samples

Of the 96 samples of ready-to-eat meats, 9% (9 of 96) were found to be positive for *Salmonella*. However, *Salmonella* was only detected in the cold, roasted beef sample purchased in the afternoon from Havana, 40% (8 of 20) and in 1 cold, roasted beef sample purchased in the

afternoon from Wanaheda 11% (1 of 9) but only after media enrichment. No *Salmonella* was detected from the other locations before and after media enrichment (Table 8). The *Salmonella* isolated from the positive samples were further identified to their species level using the VITEK® 2 cards (Table 6) as *Salmonella typhimurium* (64%), *Salmonella enterica* (27%) and *Salmonella enteritidis* (9%). There was a significant difference ($p < 0.05$) in the prevalence of *Salmonella* between the Wanaheda and Havana samples. Pearson's correlation test showed significant positive correlations for the prevalence of *Salmonella* with the meat preparation method ($r= 0.549$, $p=0.000$), serving temperature ($r= 0.380$, $p=0.005$) and the type of meat ($r=0.290$, $p=0.005$).

Table 8. Prevalence of *Salmonella* in sampled locations

Locations	No. of samples examined	% Positive	% Unsatisfactory	Mean (log cfu g ⁻¹)
Single Quarters	15	0	0	No growth
Havana	20	40	40	Detected
Wanaheda	9	11	11	Detected
Dorado Park	15	0	0	Not detected
Okuryangava	8	0	0	Not detected
Greenwell	9	0	0	Not detected

Prosperita	20	0	0	Not detected
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4.5 Prevalence of *L. monocytogenes* in ready-to-eat meat samples

The prevalence of *L. monocytogenes* was 15% (14 of 96 total ready-to-eat meats). However, 11% (11 of 96) showed unsatisfactory high levels of *L. monocytogenes*. Unsatisfactory levels (explained in table 1) of *L. monocytogenes* were 50% in Okuryangava (4 of 8 samples), 20% in Havana (4 of 20 samples), 13% in Single Quarters (2 of 15 samples) and 5% in Prosperita (1 of 20 samples). No *L. monocytogenes* was detected in samples from Greenwell, Dorado Park and Wanaheda (Table 9). The mean counts of *L. monocytogenes* in the street vended ready-to-eat meats ranged from no growth (Dorado Park, Greenwell and Wanaheda) to 1.94 ± 1.73 log cfu g⁻¹ (Okuryangava). The highest counts *L. monocytogenes* value of 4.56 log cfu g⁻¹ was observed in the cold, roasted beef sample purchased in the afternoon from Havana. There were significant differences in *L. monocytogenes* between the locations ($P < 0.05$). Tukey's multiple comparisons test showed that, *L. monocytogenes* was significantly higher in samples purchased from Okuryangava than all other locations sampled ($P < 0.05$). Pearson's correlation test showed significant positive correlations for the prevalence of *L. monocytogenes* with the time of collection ($r = 0.283$, $p = 0.005$) and the serving temperature ($r = 0.221$, $p = 0.030$).

Table 9. Prevalence of *L. monocytogenes* in sampled locations

Locations	No. of samples examined	% Positive	% Unsatisfactory	Mean (log cfu g ⁻¹)
Single Quarters	15	13	13	0.43±1.16
Havana	20	25	20	0.804±1.53
Wanaheda	9	0	0	No growth
Dorado Park	15	0	0	No growth
Okuryangava	8	63	50	1.95±1.72
Greenwell	9	0	0	No growth
Prosperita	20	10	5	0.19±0.58

4.6 Prevalence of *S. aureus* in ready-to-eat meat samples

The prevalence of *S. aureus* (Coagulase positive) in ready-to-eat meat samples were 52% (50 samples). However, unsatisfactory levels of *S. aureus* were 7% (7 of 96). Unsatisfactory levels (explained in table 1) were only detected in Okuryangava and Havana with prevalence of 50% and 15% respectively. *S. aureus* counts were in the satisfactory range for samples purchased from Single Quarters, Wanaheda, Dorado Park, Greenwell and Prosperita. The mean *S. aureus*

counts of street vended ready-to-eat meats ranged from no growth (Dorado Park) to 3.46 ± 1.81 log cfu g⁻¹ (Okuryangava) (Table 9). The highest *S. aureus* value of 5.12 log cfu g⁻¹ was observed in the cold, roasted beef sample purchased in the afternoon from Havana. Significant differences were recorded in *S. aureus* between all the locations ($P < 0.05$). Tukey's multiple comparisons test showed that, there were significant differences for *S. aureus* between Dorado Park and Okuryangava, between Single Quarters and Havana, between Prosperita and Okuryangava, as well as between Dorado Park and Havana, ($P < 0.05$). Pearson's correlation test showed a significant positive correlations for the prevalence of *S. aureus* with the serving temperature ($r = 0.293$, $p = 0.040$).

Table 10. Prevalence of *S. aureus* in sampled locations

Locations	No. of samples examined	% Positive	% Unsatisfactory	Mean (log cfu g ⁻¹)
Single Quarters	15	7	0	0.57 ± 0.61
Havana	20	75	15	2.21 ± 1.56
Wanaheda	9	44	0	1.22 ± 1.60
Dorado Park	15	0	0	No growth
Okuryangava	8	88	50	3.46 ± 1.81

Greenwell	9	44	0	1.22±1.49
Prosperita	20	50	0	1.12±1.32

4.7 Prevalence of *Shigella* in ready-to-eat meat samples

Of the 96 samples of ready-to-eat meats examined for the presence of *Shigella*, 6% (6 of 96 samples) were found to be positive for *Shigella*. Okuryangava, Havana and Prosperita had unsatisfactory levels (explained in Table 1) of 13% (1 of 8), 20% (4 of 20) and 5% (1 of 20) respectively. No *Shigella* was detected in ready-to-eat meat samples from Wanaheda, Single Quarters, Dorado Park and Greenwell. The mean *Shigella* counts of street vended ready-to-eat meats ranged from no growth (Dorado Park, Wanaheda & Single Quarters) to 0.51 ± 1.08 log cfu g^{-1} (Havana) (Table 11). The highest *Shigella* value of 3.3 log cfu g^{-1} was observed in the cold, roasted beef sample purchased in the afternoon from Havana. The *Shigella* isolates from the positive samples were further identified to their species level (Table 6) as *S. flexineri*. There were no significant differences ($P > 0.05$) in comparing means for *Shigella* between all the locations. Pearson's correlation test showed no significant correlations for the prevalence of *Shigella* with the serving temperature, meat preparation method, area of collection, time of collection, meat type ($P > 0.05$).

Table 11. Prevalence of *Shigella* in sampled locations

Locations	No. of samples examined	% Positive	% Unsatisfactory	Mean (log cfu g ⁻¹)
Single Quarters	15	0	0	No growth
Havana	20	20	20	0.51±1.08
Wanaheda	9	0	0	No growth
Dorado Park	15	0	0	No growth
Okuryangava	8	13	13	0.66±1.41
Greenwell	9	0	0	No growth
Prosperita	20	5	5	0.06±0.25

CHAPTER 5: DISCUSSION

5.1 Aerobic Plate Count

In this study, 32% of the ready-to-eat meat samples showed unsatisfactory high levels of aerobic plate count. The mean APC counts of street vended ready-to-eat meats in this study ranged from 2.67±0.94 to 5.54±0.94 log cfug⁻¹ and the highest APC value of 7.74 log cfu g⁻¹ was observed in the cold, roasted beef sample purchased in the afternoon from Havana. As compared to results of

other authors, the highest mean APC value obtained in this study was higher than that was reported by Cho et al., (2011) for various street foods, including hot ready-to-eat meats in Korea with the value of $4.71 \pm 1.53 \log \text{ cfu g}^{-1}$. In the study reported by Ologhobo et al. (2010), the highest APC value of $6 \log \text{ cfu g}^{-1}$ was obtained for Nigerian roasted chicken '*suya*' (Olanyinka, Temitope and Innocent, 2008). In a different study, unsatisfactory levels of APC were (33% of 24) for Laguna hot grilled chicken samples and (20% of 25) for hot grilled pork (Manguiat and Fang, 2013). In the same study, Taichung hot grilled chicken samples had unsatisfactory levels of (11% of 18) while hot grilled pork samples had unsatisfactory levels of (17% of 12) samples (Manguiat and Fang, 2013). High aerobic colony counts alone do not make foods unsafe, but they indicate poor handling, storage or inadequate general hygiene but samples are of unsatisfactory microbiological quality because of high aerobic colony counts ($\geq 5 \log \text{ cfu g}^{-1}$) (Olanyinka et al., 2008). The 65% unsatisfactory levels obtained for the samples from Havana are a cause for concern. It was observed that there is no open market in Havana and vendors usually prepare meat on the streets (alongside the road), no toilets are present at the vending site and consumers as well as vendors urinate in the same street where vending takes place. Moreover, waste water and garbage are discarded in the streets and foods are not effectively protected from dust and flies. Some street vended ready-to-eat meat is prepared in the morning, held at ambient temperatures during street selling and sold without reheating. Furthermore, it was observed that some cooked meats were left uncovered and exposed to microbial contaminants during the entire selling period. These factors are likely to be linked to the high aerobic plate counts recorded in Havana and the positive correlations of specific locations, time of collection and serving temperature with the APC prevalence.

5.2 Prevalence of *Enterobacteriaceae* in ready-to-eat meat samples

Unsatisfactory levels of *Enterobacteriaceae* were 26%. Unsatisfactory levels of *Enterobacteriaceae* likewise were highest in Havana (55%). The mean *Enterobacteriaceae* count in this study ranged from 0.76 ± 1.23 log cfu g⁻¹ to 4.10 ± 0.77 log cfu g⁻¹ with the highest *Enterobacteriaceae* count being 5.76 log cfu g⁻¹ was also observed in the cold, roasted beef sample purchased in the afternoon from Havana. These findings are comparable with other studies done worldwide. In a study that was done in Greece, the highest mean *Enterobacteriaceae* count was 7 log cfu g⁻¹ and (38.5% of 52) of ready-to-eat pastries were unsatisfactory (Kotzekidou, 2013). In another study that was done in Nigeria, (21.4% of 18) street vended ready-to-eat fish had *Enterobacteriaceae*, specifically the *Klebsiella* species (Odu and Ameweiye, 2013). The identified species in this study include *Enterobacter* species, *Pseudomonas* species, *Klebsiella* species and *Citrobacter* species. These microbes are responsible for cross-contaminating meats because they are members of the intestinal flora of humans and animals and can survive in a variety of environments (WHO, 2014). The *Pseudomonas* and *Enterobacter* species are of particular concern as they proliferate in unhygienic, moist conditions which in this case of street food vending is likely to happen as waste water and garbage are discarded in the streets (Djoulde et al., 2015). On the other hand, domestic animals such as cattle, goats and chickens are principal hosts for *Klebsiella* species (Siri, Sithebe and Atebe, 2011). Improper farm management techniques or improper hygiene practices during meat processing may facilitate contamination of raw meat and water sources with *Klebsiella* species (Siri et al., 2011). Thus, most infections caused by *Klebsiella* species result from consumption of contaminated foods such as rotten meats, undercooked meats or contaminated water (Haryani et al., 2011). The presence of *Klebsiella* species in ready-to-eat street foods such as ready-to-eat meats indicate poor food preparation and handling practices, cross

contamination, unclean hands of the vendors and contact with sewage or contaminated water (Akusu, Kiin-Kabari and Wenedo, 2016). *Enterobacter cloacae* were the most prevalent among the isolated *Enterobacteriaceae*. These findings concur with the work of Flamir, Gozalbo and Rico (2010) who found *Enterobacter cloacae* and *Klebsiella oxytoca*, to be the most prevalent in ready-to-eat-salads served in the dining halls of a pre-school and a primary school in Spain. *Enterobacter* spp. are the sixth most common cause of nosocomial infection in particular, *Enterobacter cloacae* have been implicated in a broad range of clinical syndromes (WHO, 2014).

5.3 Prevalence of *E. coli* in ready-to-eat meat sample

Forty-two percent of ready-to-eat meats were positive for *E. coli* and 35% had unsatisfactory levels of *E. coli*. Unsatisfactory levels of *E. coli* were highest in Havana (75%). The mean *E. coli* ranged from no growth to 3.55 ± 1.72 log cfu g⁻¹. These results were higher than those obtained in Korean street food samples including hot ready-to-eat meats, whereby 9% of 20 street-vended foods had *E. coli* (Cho et al., 2011). In another study, *E. coli* was detected in 5 of 43 (11.6%) of grilled chicken samples from street vendors in Mexico, (Diaz-Lopez et al., 2011). In a different study, *E. coli* was detected in 10 (72%) of the Taiwan cold cooked chicken samples, of which 29% were found to be unsatisfactory because they had counts that were >2 log cfu g⁻¹ (Manguiat and Fang, 2013). The unsatisfactory counts of *E. coli* may be attributed to inappropriate handling, where street vended ready-to-eat meats like the cold beef roasted sample purchased in the afternoon from Havana which had the highest *E. coli* counts is displayed and sold in the open air and handled by vendors with unwashed bare hands. This is consistent with results obtained by Niyonzima et al. (2015) who found severe microbial contaminations of displayed foods through handling. The

contaminations could also be attributed to substandard cutting and preparation practices, particularly poor hygienic conditions of the premises that may result from rubbish, sewage and other noxious substances present in the vicinity (WHO, 2011). Niyonzima et al. (2015); Mosupye and Von Holy (2000) reported that bacteria from dirty washing water and other sources of utensil surfaces may constitute a risk for microbial contamination during food vending. The *E. coli* and *Enterobacteriaceae* are indicators of sanitation and could signify unhygienic conditions during food handling and preparation (ICMSF, 2001). Additionally, *E. coli* and *Enterobacteriaceae* counts are standard methods for determining the microbial contamination of street vended foods, especially raw meats and ready-to-eat meats (Gorman, Bloomfield and Adley, 2002). Cross contaminations of raw meats and ready-to-eat meats may occur at the vending sites during cutting and chopping (Mosupye and von Holy, 2000). In this study, it was observed that raw beef and poultry as well as ingredients such as tomatoes, onions and spices were prepared using the same materials without in-between cleaning, which may result in cross-contaminations between different raw materials and products. Nonetheless, overall results showed that 67% of the hot stewed, roasted and fried ready-to-eat meats were microbiological satisfactory and safe for consumption. There could be several reasons for the unsatisfactory levels, including cross contaminations from unclean raw ingredients, contact with contaminated surfaces, improper handling of foods, vendors' inadequate knowledge of food hygiene and inadequate or unavailable cold storage (Manguiat and Fang, 2013; Mamun et al., 2013). Zige, Ohimain and Mynepali (2013) showed that several factors such as the use of contaminated water during food processing, serving foods without wearing hand gloves and head coverings, use of unclean towels, use of dirty water for washing utensils and cross contaminations between raw and processed foods during transportation and storage could be the probable causes of bacterial contamination in the street

vended ready-to-eat foods. In this study, enteropathogenic *E. coli* were not detected in any of the ready-to-eat meat samples from any sampling sites, signifying the adequacy of the cooking process. These findings agree with those reported by Diaz-Lopez et al. (2011), whereby *E. coli* (shiga toxin and enterotoxin producer) were not detected in any of the 43 samples of grilled chicken and with those reported by Manguiat and Fang (2013) whereby no *E. coli* 0157 was detected in any of the grilled pork and chicken.

5.4 Prevalence of *Salmonella* in ready-to-eat meat samples

Salmonella was only detected in 1 and 9 cold, roasted beef samples that were purchased from Wanaheda and Havana respectively (9% of 96), but only after enrichment of the culture media. The detection of *Salmonella* spp. in a 25-g sample is considered potentially hazardous or unacceptable according to the microbiological guide that was used (ICMSF, 2001). The fact that *Salmonella* species were only detected following enrichment might be an indication that they were present in the ready-to-eat meats at low levels. In a study that was done in Taiwan, the prevalence of *Salmonella* in ready-to-eat meat samples was reported to be 41% of 79 (Manguait and Fang, 2013). More recently, Simasiku (2016) reported a prevalence of 0% and 14% for *Salmonella* in raw chicken and beef samples respectively, from retail and wholesale markets in Namibia. In a different study, Shilangale et al. (2015) reported a prevalence of 0.85 % for *Salmonella* in raw beef samples from abattoirs in Namibia. In another study that was done on red meats and meat products in Algeria, the prevalence rate of *Salmonella* was reported to be 26.61% (Mezali and Hamdi, 2012). A study in Cameroon on ready-to-eat meats (beef, chicken and pork) reported the prevalence of *Salmonella* was reported to be 16% of 200 ready-to-eat meats (Djoulde et al., 2015). Diaz-Lopez et al. (2011) reported a higher prevalence of *Salmonella* in grilled chicken from street

vendors than in retail outlets. Yan et al. (2010) reported that 81 *Salmonella* isolates were recovered in 20.9% of retail foods, including chicken and pork meats. The presence of this pathogen has been associated with inadequate cooking, cross-contamination from an unhygienic environment and food handlers as well as poor handling practices (Center for Food Safety, 2014). In this study, the ready-to-eat samples had undergone through different preparation methods such as stewing, frying and roasting as heat treatments that may help to destroy the microorganisms in the food. According to Odu and Ameweiye (2013), poor food preparation and handling practices as well as inadequate cooking can result in unsatisfactory microbial quality. The authors also stated that, under-cooking is sometimes done intentionally to minimize food shrinkage. The high bacterial counts and presence of some pathogens like *Salmonella* suggest that cooking was sometimes inadequate to destroy microorganisms in some ready-to-eat meat samples. No analyses were done for the raw meats in this study, but raw chicken, beef and their entrails are known to have high microbial loads due to their compositions, which favor the growth of microorganisms (ICMSF, 2001; Yan et al., 2010). In this study, all the highest counts of all the pathogenic bacteria were obtained from beef samples and no *Salmonella* was detected from chicken. This could be a reflection of good hygiene practice during processing and handling of ready-to-eat chicken. On the other hand, it could be because of the intervention strategies that are put in place from the feed mill and subsequent controls put in place during the initial breeding, hatching, growing and transportation phases before the processing and preparation of the final product (Simasiku, 2016). Although, the source of the meat samples was not investigated in this study, the high counts of pathogenic bacteria obtained from beef samples could be due to the fact that raw beefs were obtained from non-commercial abattoirs or other sources where the HACCP (Hazard Analysis Critical Control Point) system is not implemented fully. In simple words, HACCP is a safety tool that is used in food production to

prevent or reduce the risk of contamination. The *Salmonella* isolated from the positive samples were further identified to their species levels as *Salmonella typhimurium*, *Salmonella enterica* and *Salmonella enteritidis*. *S. typhimurium* is the serovar that is often being reported to be among the most frequently isolated elsewhere as compared to the *S. enterica* and *S. enteritidis*. In a study that was done in Ethiopia on the prevalence of *Salmonella* in street vended ready-to-eat foods, *S. typhimurium* was found to be the most frequently isolated serotype (Muleta and Ashenafi, 2001). In another study that was done on the prevalence of *Salmonella* in meat and meat products, *S. typhimurium* was among the top five most frequently isolated in Algeria (Mezali and Hamdi, 2012). In Taiwan, the *Salmonella* isolated from the positive samples of street vended ready-to-eat chicken and pork was serotyped as *S. typhimurium* (Manguiat and Fang, 2013). These findings suggest that *S. typhimurium* could be an important bacterium of public health concern in Namibia as it is in other parts of the world. In a more recent study that was done by Simasiku (2016), the results showed that *S. enterica* was the most prevalent in chicken and beef samples from retail and wholesale markets in Namibia and could be among the top species that cause salmonellosis in Windhoek, Namibia. According to Hendriksen et al. (2011), the majority of salmonellosis cases in humans are caused by a limited number of *Salmonella* serovars which may vary over time from one country to another. In the US, 34% of all *Salmonella* isolates from human sources were from *S. enteritidis*, *S. heidelberg*, *S. newport*, *S. javia* and *S. typhimurium* which later contributed to 16% of all *Salmonella* outbreaks (National Outbreaks Annual Reporting System (NORS), 2013).

5.5 Prevalence of *L. monocytogenes* in ready-to-eat meat samples

In this study, 11% of the 96 ready-to-eat meat samples showed unsatisfactory levels of *L. monocytogenes*. The mean *L. monocytogenes* counts of street vended ready-to-eat meats ranged from 0 to 1.94 ± 1.73 log cfu g⁻¹. The highest *L. monocytogenes* count of 4.56 log cfu g⁻¹ was observed in the cold, roasted beef sample purchased in the afternoon from Havana. Unsatisfactory levels *L. monocytogenes* were relatively low in this study compared to this study in Namibia, investigating the prevalence of *L. monocytogenes* in ready to eat foods sourced from retail outlets and university cafeterias in Namibia, *L. monocytogenes* loads were higher in hotdogs (10.87 ± 0.79 cfu g⁻¹) and polony (22.20 ± 1.16 log cfu g⁻¹) than in other foods such as apples (13.60 ± 0.81 log cfu g⁻¹), meat salads (10.40 ± 0.81 log cfu g⁻¹), and egg salads (13.80 ± 0.58 log cfu g⁻¹), however, loads were low to warrant fears of possible outbreaks of human listeriosis (Mogomotsi and Chinsebu, 2012). Diaz-Lopez et al. (2011) reported that *L. monocytogenes* were not detected in any of the 43 grilled chickens. In another study the prevalence of *L. monocytogenes* observed in 20 ready-to-eat foods such as sandwiches and sliced meats was 7% and 3.7% respectively in the United Kingdom (Little et al., 2009). A study in Greece on ready-to-eat foods reported the prevalence of *L. monocytogenes* to be 20% of 30 sandwiches (Kotzekidou, 2013). *L. monocytogenes* is widespread in the environment; animals can also carry the bacteria without seeming ill and can cause contamination of foods such as meats and dairy products during meat processing, for example, during slaughtering (Gilbert et al., 2000). Moreover, meat may have been cross-contaminated by *L. monocytogenes* through contact with animal feces (Gilbert et al., 2000). High prevalence of foodborne pathogens including *L. monocytogenes* can be attributed to cross contaminations from environmental sources and to poor handling of the foods by the vendors during preparation (Fenlon, Wilson and Donachie, 1996; Gorman, Bloomfields and Adeley, 2002).

The environmental conditions under which street vendors worked, as well as their food handling practices, is not different from those observed in other countries (Bryan et al.,1997; Bryan et al.,1998; Kusumaningrum et al., 2003). *L. monocytogenes* is the most common causative agent of human listeriosis. Therefore, the isolation of *L. monocytogenes* in the present study might reflect a health risk to the consumers, particularly pregnant women (Enyenyé et al., 2012). The differences in reported isolation rates from different countries of *L. monocytogenes* and other pathogenic bacteria in foods might be due to variations in sampling methods, handling, hygiene practices and isolation techniques.

5.6 Prevalence of *S. aureus* in ready-to-eat meat samples

Overall, *S. aureus* unsatisfactory levels were 7%. The mean *S. aureus* counts of street vended ready-to-eat meats ranged from no growth to $3.46 \pm 1.81 \log \text{ cfu g}^{-1}$. The highest *S. aureus* value of $5.12 \log \text{ cfu g}^{-1}$ was observed in the cold, roasted beef sample purchased in the afternoon from Havana. As compared to the results of other authors, unsatisfactory levels of *S. aureus* were relatively low in this study. In a study, from 200 samples of street vended ready-to-eat meats sold in Cameroon, 20 (10%) were contaminated with *S. aureus* (Djoulde et al., 2015). In another study *S. aureus* were detected in Taiwan street vended ready-to-eat chicken and pork with unsatisfactory levels of 17% of 24 hot grilled chicken (Manguiat and Fang, 2013). *S. aureus* can be isolated from humans (respiratory passage, skins and superficial wounds) and associated environments (Food Standards Australia New Zealand, 2009). *Staphylococcus* spp. are common environmental bacteria and could have been introduced into the food after cooking through cross-contamination, for instance from utensils, vendors hands when touching foods, dishcloths, or the water during dish washing or hand washing (Djoulde et al., 2015). It was observed that most vendors start preparing

meat at 06:00 a.m. Some vendors prepare meat at home, especially those that sell at construction sites at Prosperita and only start selling between 11:00-13:00 h. Some vendors heat the meat before serving to the consumer, while some serve it without heating, which could be a factor linked to the positive correlation of unsatisfactory levels of *S. aureus* and the serving temperature.

5.7 Prevalence of *Shigella* in ready-to-eat meat samples

Shigella was detected in 6% of the samples. Havana, Okuryangava and Prosperita samples had unsatisfactory levels of 20%, 13% and 5% respectively. The detection of *Shigella* spp. in a 25-g sample is considered potentially hazardous or unacceptable according to the microbiological guide that was used (ICMSF, 2001). The mean *Shigella* counts of street vended ready-to-eat meats ranged from 0 log cfu g⁻¹ to 0.51±1.08 log cfu g⁻¹. The highest *Shigella* value of 3.3 log cfu g⁻¹ was observed in the cold, roasted beef sample purchased from Havana. The *Shigella* isolates were further identified to their species as *S. flexneri*. Unsatisfactory levels were relatively high in this study. As compared to other studies done elsewhere, *Shigella* spp. were detected in 1.7% of the 1600 street-vended meats and dairy products samples analysed in Egypt. The incidence of *Shigella* spp. was higher in meat products (2.0%) than in dairy products (1.4%). *S. flexneri* was the most common spp. (1.2%), followed by *S. sonnei* (0.4%) and finally, *S. dysenteriae* (0.1%) (Ahmed & Shimamoto, 2014). In another study, *Shigella* spp. was detected in 0.6% of the 10 street vended ready-to-eat meat products in Ethiopia (Garedew et al., 2015). A study in Turkey showed that *Shigella* was not isolated from any street vended meat or dairy products (Centinkaya et al., 2008). Contamination of foods by these bacteria is usually through the fecal-oral route and is most commonly due to fecal contaminated water and unsanitary handling by food handlers (Niyogi, 2005). This could be the reason why *Shigella* spp. is detected in street vended ready-to-eat meats because in most cases, running water is not available at the vending sites and hand and dishwashing

are usually done in one or more buckets or pans of water, sometimes without soap. The water is not only used for dish washing, but also for cleaning of the meat preparation areas and for hand washing by vendors or their customers before and after eating. Vendors also wash their hands in the dish water when returning from toilets. Animals are not considered as common reservoirs for *Shigella* species, hence, it might be that during meat processing, *Shigella* spp. present on the surface of the animal tissue may have been transferred to meat surfaces through workers' hands and knives (Jackson et al., 2013). Nygren et al. (2012) analysed 120 reported foodborne shigellosis outbreaks in the United States (US) between 1998–2008. The contributing factors identified in these outbreaks included infected food handlers (58%), bare-handed contact of the food handler with ready-to-eat food (38%), inadequate cold-holding temperatures (15%) and inadequate cleaning of food preparation equipment (15%) and using faecal contaminated water. Shigellosis is endemic in most developing countries and is estimated to cause at least 80 million cases of bloody diarrhea and 700,000 deaths each year (WHO, 2005). Ninety-nine percent of infections caused by *Shigella* spp. occur in developing countries; Egypt was listed as the most often reported destination for travel-associated *Shigella* spp. In England, Wales and Northern Ireland between 2007 and 2009 significant numbers of shigellosis outbreaks resulted from the consumption of contaminated foods (Anonymous, 2011). A review of 816 foodborne outbreaks associated with infected food workers showed that 4% involved *Shigella* (WHO, 2005). The most severe form of shigellosis is caused by *S. dysenteriae* serotype 1. *S. sonnei* causes the mildest form of diseases, while *S. flexneri* and *S. boydii* can cause either severe or mild illness (FDA, 2012). In all the sampling locations, the presence of *Shigella* and other pathogenic bacteria differed from location to location. This variation can be explained by differences in cleaning regimes, meat sources, handling, processing and storage protocols. However, it is worth noting that because bacteria occur everywhere in the

environment, their presence in the locations does not automatically translate into foodborne disease risks.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

In conclusion, this study has shown that pathogenic bacteria of public health concern are prevalent in street vended ready-to-eat meats in Windhoek, Namibia and the prevalence of pathogens in ready-to-eat meats is dependent on the sampling location, type of meat, preparation methods, serving temperature and the time of purchase. The general prevalence and unsatisfactory rates of foodborne pathogens in street vended ready-to-eat meats in the Havana area of Katutura, Windhoek were found to be relatively higher when compared to most findings of other researchers, warranting fears of possible health risks among the consumers. This could be due to poor hygienic practices during processing and handling in that location. On the other hand, it might be due to the fact that the raw meats were obtained from non-commercial abattoirs or other sources where the HACCP (Hazard Analysis Critical Control Point) system is not implemented fully. Improved sanitary conditions are necessary for consumer protection. Most of the high bacteria counts were only observed in beef samples as compared to chicken and no *Salmonella* was detected from chicken which could be because of the intervention strategies that are put in place from the feed mill and subsequent controls put in place during the initial breeding, hatching, growing and transportation phases before the processing and preparation of the final product. The fact that potentially hazardous *Salmonella* and *Shigella* as well as unsatisfactory levels of *L. monocytogenes*, *S. aureus*, *E. coli* and *Enterobacteriaceae* were isolated in ready-to-eat meats suggests that consumers are exposed to health risks due to foodborne pathogen infections. These

results suggest that *Salmonella*, *Shigella*, *L. monocytogenes*, *S. aureus*, *E. coli* and *Enterobacteriaceae* spp. could be among the top foodborne pathogens that cause foodborne diseases in Windhoek, Namibia. Havana location had the highest bacteria counts and had samples that were contaminated with *Salmonella* and *Shigella*.

6.2 Recommendations

In order to establish if the bacteria strains that were isolated from ready-to-eat meats are the same with the strains that cause infections in humans, it is recommended that different studies to be done. For example, assessment of prevalence of foodborne poisoning pathogen among consumers of ready-to-eat meats in order to establish the similarities between the bacteria strains that cause infections in humans and those that are found in street vended ready-to-eat meats in Windhoek, Namibia.

The prevalence and unsatisfactory bacterial pathogen prevalence rates, especially the one for Havana, were slightly higher than those reported in other findings elsewhere; this could be due to poor hygiene practice during processing and handling in such locations. Therefore, it is recommended to the Ministry of Health and Social Services, the Municipality of Windhoek and all relevant stakeholders in the food industry to provide training and continuously educate street-food vendors on food hygiene and sanitation for the street vendors as it might result in the improvement of the microbiological quality of street vended foods. The results of this study can also provide valuable information for the design of monitoring and surveillance programs that would detect pathogenic bacteria in the entire food chain for the food microbiological control.

The source of the meat samples was not investigated in this study. The raw beef and chicken meats may have been obtained from non-commercial abattoirs or other sources where the Hazard Analysis Critical Control Point (HACCP) system is not implemented fully. HACCP system may be the useful tool to minimize the risks. Therefore, it is recommended to the Veterinary Services, Ministry of Health and Social Services, the Municipality of Windhoek, and all relevant stakeholders in the food industry that the HACCP system should be compulsory in all food production establishments in Namibia as a tool to reduce the health risks to consumers. As of now, HACCP system is only compulsory to the EU export food establishments. The HACCP system should be made compulsory to all local food processing establishments, including abattoirs and retail markets in the country.

Further studies should be done to determine the prevalence of pathogenic microorganisms in other street vended ready-to-eat foods in Namibia such as fruits, fat cakes and salads, as well as to determine the microbiological quality of some meat ingredients like spices, onions and tomatoes to find out if they have any effect on the microbiological quality of ready-to-eat meats. The microbial quality of street vended foods should be assessed on a regular basis to acquire sufficient data for use in conducting microbial risk assessments.

Failure to control foodborne pathogens in a country presents a potential public health problem for other countries. To minimize the burden of these pathogens, it is helpful to monitor foodborne pathogens, especially *Salmonella* and *Shigella* species distribution in many countries, implement foodborne pathogens control measures throughout the food production chain and monitor the effectiveness of the control measures.

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Appendices

Appendix 1

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
<i>Salmonella</i>	96	0	0	.00	.000
<i>E. coli</i>	96	.00	5.67	1.3156	1.79661
<i>Listeria</i>	96	.00	4.56	.4353	1.11853
<i>Shigella</i>	96	.00	3.96	.2386	.73527
<i>S. aureus</i>	96	.00	5.90	1.2168	1.60041
PCA	96	1.45	7.74	4.3623	1.22321
<i>Enterobacteriaceae</i>	96	.00	5.76	2.6272	1.59156
Valid N (listwise)	96				

Statistics

		<i>Salmonella</i>	<i>E. coli</i>	<i>Listeria</i>	<i>Shigella</i>	<i>S. aureus</i>	PCA	<i>Enterobacteriaceae</i>
N	Valid	96	96	96	96	96	96	96
	Missing	0	0	0	0	0	0	0
Mean		.00	1.3156	.4353	.2386	1.2168	4.3623	2.6272
Std. Error of Mean		.000	.18337	.11416	.07504	.16334	.12484	.16244
Median		.00	.0000	.0000	.0000	.0000	4.2400	3.0550
Std. Deviation		.000	1.79661	1.11853	.73527	1.60041	1.22321	1.59156
Variance		.000	3.228	1.251	.541	2.561	1.496	2.533
Minimum		0	.00	.00	.00	.00	1.45	.00
Maximum		0	5.67	4.56	3.96	5.90	7.74	5.76

Appendix 2

ANOVA and Pearson Correlations tables

		ANOVA	
		Sum of Squares	Df
<i>Salmonella</i>	Between Groups	.000	6
	Within Groups	.000	89
	Total	.000	95
<i>E. coli</i>	Between Groups	150.050	6
	Within Groups	156.591	89
	Total	306.641	95
<i>Listeria</i>	Between Groups	28.489	6
	Within Groups	90.367	89
	Total	118.856	95
<i>Shigella</i>	Between Groups	6.463	6
	Within Groups	44.896	89
	Total	51.359	95

<i>S. aureus</i>	Between Groups	96.039	6	16.007	9.672	.000
	Within Groups	147.284	89	1.655		
	Total	243.323	95			
PCA	Between Groups	63.915	6	10.653	12.119	.000
	Within Groups	78.228	89	.879		
	Total	142.143	95			
<i>Enterobacteriaceae</i>	Between Groups	101.143	6	16.857	10.755	.000
	Within Groups	139.499	89	1.567		
	Total	240.642	95			

Correlations

		<i>E. coli</i>	Area of collection
<i>E. coli</i>	Pearson Correlation	1	-.344**
	Sig. (2-tailed)		.001
	N	96	96
Area of collection	Pearson Correlation	-.344**	1
	Sig. (2-tailed)	.001	
	N	96	96

** . Correlation is significant at the 0.01 level (2-tailed).

Correlations

		Time of collection	<i>E. coli</i>
Time of collection	Pearson Correlation	1	.449**
	Sig. (2-tailed)		.000
	N	96	96
<i>E. coli</i>	Pearson Correlation	.449**	1

Sig. (2-tailed)	.000	
N	96	96

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations

		Meat preparation method	<i>E. coli</i>
Meat preparation method	Pearson Correlation	1	.280**
	Sig. (2-tailed)		.006
	N	96	96
<i>E. coli</i>	Pearson Correlation	.280**	1
	Sig. (2-tailed)	.006	
	N	96	96

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations

		Serving temperature	<i>E. coli</i>
Serving temperature	Pearson	1	.195
	Correlation		
	Sig. (2-tailed)		.057
	N	96	96
<i>E. coli</i>	Pearson	.195	1
	Correlation		
	Sig. (2-tailed)	.057	
	N	96	96

Correlations

		Area of collection	<i>Listeria</i>
Area of collection	Pearson	1	-.226*
	Correlation		
	Sig. (2-tailed)		.027
	N	96	96
<i>Listeria</i>	Pearson	-.226*	1
	Correlation		
	Sig. (2-tailed)	.027	
	N	96	96

N	96	96
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*. Correlation is significant at the 0.05 level (2-tailed).

Correlations

		Time of collection	of <i>Listeria</i>
Time of collection	Pearson Correlation	1	.283**
	Sig. (2-tailed)		.005
	N	96	96
<i>Listeria</i>	Pearson Correlation	.283**	1
	Sig. (2-tailed)	.005	
	N	96	96

** . Correlation is significant at the 0.01 level (2-tailed).

Correlations

		Area of collection	of <i>S. aureus</i>
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Area of collection	Pearson Correlation	1	-.336**
	Sig. (2-tailed)		.001
	N	96	96
<i>S. aureus</i>	Pearson Correlation	-.336**	1
	Sig. (2-tailed)	.001	
	N	96	96

** . Correlation is significant at the 0.01 level (2-tailed).

Correlations

		Time of collection	of <i>Enterobacteriaceae</i>
Time of collection	Pearson Correlation	1	.295**
	Sig. (2-tailed)		.003
	N	96	96
<i>Enterobacteriaceae</i>	Pearson Correlation	.295**	1
	Sig. (2-tailed)	.003	
	N	96	96

** . Correlation is significant at the 0.01 level (2-tailed).

