University of Zambia

School of Veterinary Medicine

Microbial Assessment of Vegetables sold in selected Supermarkets in Lusaka District

By

Banda Moses

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DECLARATION

I, **Moses Banda (Comp. No: 2016145544)**, hereby declare that the dissertation hereby submitted is my own work and the work of other persons used in this dissertation has been duly acknowledged. This work has not been previously submitted for a degree, diploma or other qualification at the University of Zambia or any other university around the world.

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Moses Banda Date

CERTIFICATE OF APPROVAL

This dissertation by Moses Banda is approved as a requirement in partial fulfillment of the award of the **MSc. in One Health Food Safety (OHFS)** Degree of the University of Zambia.

Name of Examiner 1………………......... Signature ………………… Date……………………

Name of Examiner 2…………………..... Signature…………………..Date……………………

Name of Examiner 3……………………..Signature…………………..Date……………………

DEDICATION

This work is dedicated to my late aunty and mentor, Professor Drinah Banda Nyirenda, my late Father, James J. Banda, for the confidence they had in me during my early school days and my Mother who would go to the extent of selling vegetables to enable me go to school.

ABSTRACT

Nutritionally, fresh vegetables are a good source of vitamins and essential minerals, among other nutrients. This is the main reason as to why they are consumed globally. The production of vegetables faces many constraints, especially land pressure, access to safe clean water, and low soil fertility. To satisfy the increasing demand for vegetables in view of poor soils and land pressure, farmers tend to intensify production by using sewer water, cattle and poultry manure as fertilizers. This coupled with different methods of irrigation, handling, transportation and storage, further renders fresh vegetables to be vulnerable to contamination with pathogens. Here in Lusaka vegetables are sold both in the open markets as well as supermarkets.

The objective of this study was to assess whether fresh vegetables sold in selected markets in Lusaka district present a threat in terms of food borne pathogens such as *salmonella and staphylococcus* as well as bacteria belonging to the *Enterobacteriaceae* family (*Total coliforms, feacal coliforms and E.coli*).The bacterial isolates were further subjected to antibiotic resistance using 9 different drugs. chloramphenical**,** TE;Tetracycline**,**AMC;Amoxylcluvate**,**Erythromycin**,**Imepenem**,**Cefotaximine**,**NalidixAcid**,**CIP,Ciprofloxacin and Cefoxitin

In this cross – sectional study, 74 vegetables where purchased from five (5) selected supermarkets and three (3) open markets in Lusaka district.

Vegetable samples analysed by found the total number of fecal bacteria per 25 gram was recorded in 95% of the samples which is considerably high and a food safety risk. 94.6%) samples were found to be positive for *S. aureus* and (5.4%) of the samples were positive for *Salmonella* were as all samples were found to be contaminated with *E.coli(100%). Staphylococcus aureus*, 23.52% were resistant to 3 of the 6 antibiotics that were used. That is 5.88 % of the isolates showed resistance to chloramphenicol and erythromycin respectively and 11.76% showed resistance to tetracycline. Out of six (6) *E. coli* isolates, three (3) showed some levels of resistance to nine (9) of the antibiotics used. Only (16.67%) were resistant to amoxylcluvate, (16.67%) resistant to ampicilin and 83.33% were resistant to Tetracycline. Out of the 4 isolates belonging to *salmonellae*, 0nly one (1) (25.00%) showed resistance to ampicilin.The finding of resistant strains to some antibiotics was a great concern in view of food safety and public health as these vegetables have the potential of causing diarrhea diseases to consumers.

Key words: Vegetables, Pathogens, antibiotics, contamination.

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TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

ABBREVIATIONS OF TERMS USED IN THE STUDY

BP	Baird Parker
BPW	Buffered Peptone Water
CDC	Centre for Disease Control
DST	Drug Sensitivity Test
FAO	Food and Agricultural Organization
FBD	Food Bacterial Diseases
GAP	Good Agricultural Practices
GMP	Good Manufacturing Process
GRZ	Government of the Republic of Zambia
HACCP	Hazard Analysis Critical Control Points
MPV	Minimally- Processed Vegetables
MRL	Maximum Residue Limit
NCCLS	National Committee for Clinical Laboratory Standards
OHFS	One Health Food Safety
TNTC	Too Numerous to Count
WHO	World Health Organization
ZOI	Zone of Inhibition

OPERATIONAL TERMS

Antibiotics - a drug (such as penicillin or its derivatives) that inhibits the growth of or destroys microorganisms

Contamination– the introduction or occurrence of a contaminant in food or food environment.

Disinfection – the reduction, by means of chemical agents and/or physical methods, of the number of micro-organisms in the environment, to a level that does not compromise food safety or suitability.

Food-borne disease (FBD) – any symptom or syndrome resulting from a disease transmitted to human beings by contaminated foods.

Food hygiene – comprises conditions and measures necessary for the production, processing, storage and distribution of food designed to ensure a safe, sound, wholesome product fit for human consumption.

HACCP – a system which identifies, evaluates and controls hazards that are significant for food safety.

Food safety – assurance that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use.

Good Agricultural Practices (GAPs) – practices of primary production improving on conventional production and handling methods, to ensure product safety, reducing the negative impact of production systems on the environment, fauna, flora and workers' health.

Good Manufacturing Practices (GMPs) – post-harvest practices to prevent and control product safety hazards with reduced effects on the environment and on workers' health.

Hazard – a biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect.

Micro-organisms – include yeasts, fungi, bacteria and viruses.

Pathogen – any micro-organism causing human diseases.

Sensitivity - Degree of susceptibility to damage or harm from changes in the environment

Vegetables - A plant or part of a plant used as food, such as a cabbage, rape, spinach and green pepper.

CHAPTER ONE: BACKGROUND

1.0 Introduction

The study was aimed at assessing the microbial quality and safety of fresh vegetables: the case of vegetables sold in selected markets in Lusaka District. It begins with the background of the study under investigation. Thereafter, it presents the problem under investigation, purpose of the study and objectives of the study that tally with the research questions. It also provides the significance of the study, the delimitations and operational definitions of the terms used.

1.1 Background of the Study

Vegetables, both traditional and exotic, have been part of the diets of most people around the world for a long time now. To this end, agriculturalists have been planting a variety of vegetables. Nutritionally, vegetables are a good source of vitamins, dietary fibre and essential minerals, among other nutrients. In the same vein, it has been mentioned that the role of vegetables in nutrition and healthy diets is well recognized and in recent years many countries have undertaken various initiatives to encourage consumers to eat more of these products (WHO/ FAO, 2008). This, thus, implies that there is need for countries around the globe to increasingly engage in the growing of vegetables as this is what will enhance the health of many people globally and improve on their income due to high demand of vegetables necessitated by international trade and globalization. For many countries, especially developing countries, vegetables are increasingly seen as a valuable product making a substantial contribution to the economy as well as to the health of countries' population (WHO/FAO, 2008).

Cultivation of vegetables follows different approaches in terms of irrigation systems used, handling, packaging and transportation thereby making the outcome or product cultivated vulnerable to pathogens. Problems linked with pathogens in fresh produce, including the associated public health and trade implications, have been reported in a number of countries worldwide (WHO/FAO, 2008). This, subsequently, has led to various challenges associated with international trade in vegetables and impacted negatively on the income of those specialized in cultivation of vegetables for sale. Some countries have put various health guidelines to be strictly followed in the importation of vegetables. For example, it has been noted that the United States of America described the impact of the large spinach- E 0157:H7 outbreak of 2006 on the export of spinach to Canada, where spinach was sent in large volume. The outbreak led to Canadian trade restrictions of USA- grown spinach for several months, and a recall of imported spinach in Canada. In Europe there have been numerous alerts sent via the Rapid Alert System for Food and Feed that highlight the detection of pathogens on fresh produce traded among European countries or imported from outside of the European Union (WHO/FAO, 2008).

Most African countries suffer from considerable constraints in meeting quality standards, SPS measures and private sector requirements due to weak institutions, lack of infrastructure, high compliance costs or face challenges in accessing of information among other factors. This has thus affected exports of fruit and fresh vegetables to international markets such as USA and the EU owing to their stringent food safety regulations and standards imposed by governments in these countries. However with the support of the African Growth and Opportunity Act (AGOA), A United States Trade Act, enacted on 18 May 2000 as Public Law 106 of the 200th Congress, the legislation has significantly enhances market access to the US for qualifying Sub-Saharan African (SSA) products of which Zambia is one of the member countries.

There are four main types of human pathogens associated with fresh vegetable produce: that is soil associated pathogenic bacteria (*Clostridium botulinum, Listeria monocytogenes*), feaces associated pathogenic bacteria (*Salmonella* spp, *Shigella* spp., *E. coli* 0 157:H7) and others pathogenic parasites (*Cryptosporidium, Cyclospora*) as well as pathogenic viruses (Hepatitis and enterovirus) **(**Beauchat, 1996).

Many of these pathogens are spread via a human to food to human transmission route. Handling of fruits and vegetables by infected fieldworkers or consumers, cross contamination, use of contaminated irrigation water, use of inadequately composted manure or contact with contaminated soil are just a few of the ways that transmission of human pathogens to food can occur **(Appendix 1).**

Normally well water is less likely to be contaminated with human pathogens than surface water supplies, however, all irrigation water sources are potential sources of contaminants with microbial pathogen and the overhead irrigation systems being more likely to spread contamination since water is applied directly to the edible portions of the vegetables.

On the other hand, while produce quality can be judged by outward appearance based on such criteria as color, turgidity or firmness and aroma, food safety cannot. Casual inspection of produce cannot determine whether the produce is in fact safe and wholesome to consume. Therefore, management of growing conditions is paramount and best way in preventing the contamination of fresh produce by physical hazards, harmful chemicals and human pathogens (Suslow 1997).

Most of the commercial producers have systems in place that enable them to provide good quality water for irrigation. However, for many small-scale farmers access to good quality water for irrigating their vegetables is a challenge. To overcome this challenge, some farmers resort to other sources of water for irrigation and at times the source being sewer water. For instance, a study done in Ghana revealed that wastewater irrigated vegetable production threatens public health from the perspective of microbiological and pesticide contaminations (Amoah, 2008).

Most households in Lusaka obtain their vegetables from open markets and Soweto being the most prominent market. Others particularly those in low density areas buy their vegetable from some leading super markets such as Shoprite, Food lovers, Spar, Pick and Pay as well as Food Co. in Game stores. Information concerning the main source of the vegetables and the conditions in which they are grown is not known by customers and/or consumers. What is known is that these vegetables come from the small-scale farmers who may either use sewer or contaminated water for irrigation or from established commercial farmers with proper irrigation systems in place. From a microbiological perspective, sewer water has been associated with pathogenic organisms which are causative agents for waterborne diseases.

The use of untreated Chicken manure by farmers to promote the growth of vegetables has the potential to contaminate fresh produce as chicken manure is known to harbour a lot of bacteria.

The prevalence and transmission of antibiotic resistance among bacteria associated with food from animals and raw produce have been well documented, which occurred with increased frequency worldwide (Threlfall *et al.*).

Contamination of raw produce with pathogenic and nonpathogenic microorganisms can occur at any point during production to consumption (De Roever, 1998). Antibiotic-resistant bacteria could colonize vegetables because of the direct use of antibiotics during cultivation and use of contaminated poultry manure or irrigated water to croplands (Witte, 1998). All these could result in spread of resistance to indigenous soil bacteria through horizontal transfer, which could in turn transfer resistance back to animals or humans via fresh vegetables produce (Sengeløv *et al.*, 2002).

Thus, vegetables sold in both open and supermarkets can either be a product of sewer water irrigation, contaminated with chicken manure or fresh quality produce from either small scale or commercial farmers. Hence, the interest for carrying out research on vegetables found in selected supermarket in Lusaka District.

1.2 Statement of the Problem

Fresh vegetables provide most of our daily requirements for vitamins, minerals and fibre and their role in reducing the risk of lifestyle associated illnesses such as heart disease, diabetes and cancer has resulted in a further increase in their desirability and consumption. Here in Zambia vegetables are a cheaper source of food and nutrition for most low income households especially those that cannot afford fish or meat products.

FDA and WHO have recommended vegetables to be taken daily because correct fresh produce intake alone could save 2.7 million lives a year because 31% of heart disease cases are due to an insufficient intake of fresh produce (Johnston *et al*., 2006). As a result of this recommendation, vegetable consumption increased by 29% per capita in the USA between 1980 and 2000 (Matthews, 2006). Also, in South Africa, the Department of Health has been promoting the consumption of fruits and vegetables through its '5-a-Day' eating programme, namely, consumption of least five portions of vegetables every day (Badham, 2010).

According to Coillard Hamusimbi a consultant for Musika Development Initiatives and the Indaba Agricultural Policy Research Institute (IAPRI) in Zambia (2018), Zambia's fresh produce industry is on the increase.

Interestingly, low-income households doubled expenditure on fresh fruit and vegetables (14-32%). The medium income and wealthier households have increased their fresh fruit and vegetable consumption by 11% and 5% respectively. According to the study, consumption is also expected to increase due to the rising healthy-eating momentum among higher per capita households. However, the sometimes-unhygienic trading conditions at fresh fruit and vegetable markets turn these into potential "disease hubs" and raise food safety concerns.

On the other hand, vegetables are among the food groups implicated with greater frequency of causing enteric diseases (Beuchat, 2006). All types of fresh produce have the potential to harbour pathogens and most of these bacterial pathogens have been associated with foodborne illnesses (Beuchat, 2002). The global burden of foodborne diseases was estimated to be 600 million cases and 420,000 deaths in 2010 (WHO, 2010). In Zambia the diarrhea diseases rank in the top five (5) causes of morbidity and mortality in all ages according to the Zambia demographic and health survey 2013-2014 (CSO, 2015).

As of May 12, 2018, the cholera outbreak had affected seven of the 10 provinces in Zambia, with 5,905 suspected cases and a case fatality rate (CFR) of 1.9%. Among the suspected cases, 5,414 (91.7%), including 98 deaths (CFR = 1.8%), occurred in Lusaka residents (MOH, 2018).

Consumers may tend to feel that vegetables bought from leading supermarkets have no risk associated with their consumption. Mainly because they look clean and are properly arranged in the shelves unlike in open markets where they are normally stacked on floors or dirty tables. Fruits and vegetables are unique foods since they are often consumed raw or with minimal preparation. To date, there have been no effective interventions strategies developed which can completely eliminate food safety risks associated with consumption of uncooked or fresh produce.

Therefore, preventing contamination with human pathogens, dangerous levels of chemical residues and physical contaminants is the only way to assure these foods are wholesome and safe for human consumption. Systems that assure safety and wholesomeness of fruits and vegetables during postharvest handling and fresh - cut processing fall into four prevention programs: Good Agricultural Practices (GAP's); Good Manufacturing Practices (GMP’s); Sanitation Procedures; and Hazard Analysis Critical Control Points (HACCP).

The recent increase in the number of shopping malls has worsened the problem. The supermarkets in these malls have resorted to sourcing of fresh vegetables from outside the country so as to meet supply and demand for fresh produce which local farmers at times fail to meet. On the other hand, agricultural practices and hygienic conditions vary greatly among growing regions around the world and increased global sourcing increases consumers exposure to diverse endemic microflora carried on fresh fruits and vegetables. Also, global sourcing means longer transportation and handling, giving pathogenic microorganisms’ additional time to proliferate and reach levels which can cause illness. For example, South Africa is the major and leading exporter of fresh fruits and vegetables in Africa and to Zambia inclusive. Ndiame & Jaffee (2005) reported that 73% of fruits and vegetables exported to the USA in terms of the African Growth and Opportunity Act (AGOA) were from South Africa.

Hence despite the economic importance of fresh vegetables with regard to income generation and creation of employment opportunities, it is essential to elaborate on the pathogens that may contaminate them during pre-harvest which may later predispose them to become causative agents of infectious diseases to both local and international consumers. To this effect, consumers in these supermarkets maybe at greater risk of foodborne illnesses as a consequence of exposure through consumption of contaminated fresh vegetables outsourced from neighbouring countries.

A study conducted on microbiological safety of raw-freshly consumed vegetables from open markets found in Lusaka District (Chakopo, 2017) established that these vegetables contain food-borne pathogens which may pose a health risk to consumers. Literature on the microbial safety of freshly consumed vegetables sold in supermarkets in Lusaka District is non- existing (Kinkese *et al*, 2018). This study, therefore, attempted to assess the microbial quality and safety of freshly consumed vegetables sold in Supermarkets in Lusaka District.

1.3 Justification of the study

In general a lot has been written on microbial contamination of fresh vegetables around the World and Zambia in particular (Amoah, 2008; Chikopo, 2017; Kinkese *et al*, 2018; WHO/FAO, 2008). Many people in Lusaka buy food that includes vegetables from the markets as it is very difficult to grow them in many households due to poor water supply and space. However, information on quality and safety of fresh vegetables sold on the Zambian markets and issues that are to do with the growing concern of antimicrobial resistance is inadequate or still lacking. Therefore, it is hoped that this study will:

i. Add to the body of knowledge on microbial quality, safety and antimicrobial resistance of fresh vegetables sold in selected open and supermarkets in Lusaka.

ii. Guide relevant stake holders (local authorities ,food producers, distributors, retailers, traders and consumers) on how to regulate and improve growing of vegetables, handling, packaging and transportation by observing safer practices in order to safeguard the health and lives of consumers

iii. Contribute to improved quality and safety of fresh vegetables for the benefit of public health.

1.4 Research Questions

The following were the research questions:

i. What is the quality and microbial safety of the fresh vegetables sold in markets of Lusaka district?

ii. Are the fresh vegetables contaminated with feacal coliforms?

iii. Are the fresh vegetables contaminated with *E. coli, staphylococcus and salmonella*?

iv. What is the antibiotic resistance of the pathogens isolated from vegetables sold in supermarkets?

1.5 General Research Objectives

The main objective of the study was to assess microbial quality and safeties of fresh vegetables sold in selected markets and establish the public health significance of these fresh vegetables.

1.6 Specific Research Objectives

Arising from the statement of the problem and general objectives of the study, the specific objectives were as follows:

i. Determination of microbial quality of fresh vegetables sold in selected markets of Lusaka District.

ii. Determination of feacal coliforms as an indication of Enterobacteriaceae

iii. Determination of microbial pathogens (*E.coli, Staphylococcus, feacal coliforms, total coliforms* and *Salmonella)*

iv. Determine antibiotic resistance of the isolated pathogens.

CHAPTER TWO: LITERATURE REVIEW

2.0 Introduction

This part focuses on the literature reviewed related to the topic under study. The literature reviewed has been categorized as international and local perspectives.

Fresh vegetables are nutritionally well-recognised as healthy components in diets. The microbiological foodborne outbreaks associated with the consumption of fresh produce have been increasing. *Salmonella* spp., *Escherichia coli*, *Staphylococcus aureus, feacal coliforms, Campylobacter* spp. and *Listeria monocytogenes* are the most common pathogens that contaminate fresh produce. This review discusses foodborne outbreaks linked to fresh produce, factors that affect microbiological contamination and measures that could be adopted to reduce the foodborne illnesses

2.1 International Perspective

Cultivation of vegetables follows different approaches in terms of irrigation systems used, application of fertilizers or poultry manure, sanitation and handling, packaging and transportation thereby making the outcome or product cultivated vulnerable to pathogens. Problems linked with pathogens in fresh produce, including the associated public health and trade implications, have been reported in a number of countries worldwide (WHO/FAO, 2008).

In recent years, increasing international trade has resulted in globalization of the food supply leading to intensification of crop production,. With increasing urbanization and scarcity of land and fresh water, fresh leafy vegetables and herbs are often grown in urban and peri-urban areas, irrigated with contaminated water or grown in areas close to other agricultural production systems, such as livestock production (Drescher, Nugent and de Zeeuw, 2000).This may lead to food safety risks arising from exposure (direct and indirect) to animal, human and industrial wastes

2.2 The use of water contaminated with feacal matter

The use of sewage water for irrigation has been observed to have distinct effects on quality and safety of crops (S.Jiang et al, 2012).It has been noted that long term exposure to polluted water and soil can have serious environmental consequences as well as health hazards such as diarrhea, abortion, hepatitis A and typhoid in humans who consume the resulted food product.

Studies done in Accra Ghana (Amoah,2008), Dar-es- salaam Tanzania (Kayombo and Mayo,2018) and Western Cape, South Africa (Lotter,2010) revealed that vegetables irrigated with polluted or sewer contaminated water contain high concentration of salts and pathogenic organisms. Also, sampled vegetables from these gardens were highly contaminated with *fecal coliforms* of up to 10^8 Cfu/kg-wet vegetables. The fresh vegetables were also contaminated with pathogenic organisms such as *Escherichia coli*, *Citrobacter ssp, Proteus ssp, Klesiella ssp,* S*almonella* spp, and *Basillus* ssp.

According to (Suslow et al.2003), the microbial quality of irrigation water is critical because water contaminated with animal or human wastes can introduce pathogens into vegetable products during preharvest and postharvest activities via direct or indirect contamination. Therefore, microbiological quality of irrigation water has a paramount importance to the safety of fresh and minimally processed vegetables. Moreover, (Ibenyassine et al 2006) reported that contaminated irrigation water and surface runoff water may be the major sources of pathogenic microorganisms that contaminate fruits and vegetables in fields. Water from the river that received both human and animal waste disposal poses a health risk due to contamination with all microorganisms of human and animal intestinal habitat such as Salmonella and Listeria spp. . Consequently, the microbiological quality and safety of fresh vegetables is a significant concern to all stakeholders all over the world.

(Tambekar and Mundhada, 2006) reported that foodborne bacterial pathogens commonly detected in fresh vegetables were E. coli, S. aureus, and Salmonella spp.

2.3 The use of untreated chicken manure

In many parts of the world organic wastes play an important role in providing nutrients to crops and improving overall soil quality. However organic manure has the potential of contaminating vegetables if not properly treated. Pathogenic enteric bacteria like E. coli O157:H7 and Salmonella have the ability to persist for extended periods in manure, with survival times ranging from several weeks to several months, and even up to nearly 2 years (Jiang, Morgan and Doyle, 2002; Franz et al., 2005; Kudva, Blanch and Hovde, 1998).

A major issue with respect to the microbiological risks associated with fresh leafy vegetables grown in soil enriched with organic waste is the ability of pathogens present in the amended soil to colonize the plant. Several experimental studies have shown associations between human pathogens and the surface of leafy vegetables grown in manure-amended soil (Islam et al., 2004; Natvig et al., 2002).

Animal feaces including poultry manure which contain large numbers of bacteria can contaminate croplands and hence agricultural products. Feacal bacteria, including, *Escherichia coli*, are responsible for serious outbreaks of diarrhea, particularly in children. Some of these microorganisms including *feacal coliforms, E. coli* and *feacal streptococci* are life threatening. Gastrointestinal diseases are ranked among the top 3 three most important health problems for most communities' in the developing world especially in high density areas. Other diseases such as typhoid, paratyphoid fever and skin diseases can also be caused by feacal contamination. H.De Bon, L.Parrot, and , F.Amponsah-Doku (2010).

Many infection outbreaks have been associated with food directly or indirectly contaminated by animal manure_by identifying *Escherichia coli* and fecal coliforms, which are indicators of fecal pollution (Ijabadeniyi et,al,2011). One such example was a major foodborne disease outbreak of *Escherichia coli* O157:H7 (ECO157) infections with bloody diarrhea and abdominal cramps which lasted from 15 December 1989 to 20 January 1990 in Missouri. (Griffin et al 1998), reported the occurrence of many outbreaks of *Escherichia coli* O157:H7 (ECO157) infections in communities, nursing homes, a day care center, and a kindergarten. They mainly took the form of gastrointestinal diseases, bloody diarrhea, hemolytic uremic syndrome, or thrombotic thrombocytopenic purpura. Contaminated manure can contact the product directly when used as a soil fertilizer or indirectly via infiltration of irrigation water or infiltration of water used to wash the product

Studies to describe the bacterial load and the occurrence of some disease-causing enteric bacteria on raw vegetables sold in both open and supermarkets have also been done. The results have revealed that the bacterial loads ranged from 3 to 8 log10 CFUg- 1 for aerobic bacteria and 1 to 4 log10 CFUg- 1 for coliforms as well as Enterobacteriaceae

Also other studies carried out on microbiological variation amongst fresh and minimally processed vegetables from retail establishers- a public health study in Pakistan. Their study acknowledges that fresh and minimally processed ready to eat vegetables are very attractive eatables amongst consumers as convenient, healthy and readily available foods, especially in the South Asian States. The work further notes that vegetables provide numerous nutrients, phytochemicals, and vitamins but also harbor extensive quantity of potentially pathogenic bacteria. Fresh cut vegetables showed the highest incidence of presumptive *E. coli* (69.9%). The results showed a poor quality of fresh vegetables in Pakistan and point to the implementation of good hygiene practices and food safety awareness amongst distributors, food handlers at retail establishments.

An assessment on microbial quality of fruits and vegetables sold in Sango Ota in Nigeria acknowledges that fresh fruits and vegetables promote good health but harbor a wide range of microbial contaminants. In this study, nine bacteria belonging to eight genera were found in fruits and vegetables. *Staphylococcus aureus* (29.2%) was the most frequently isolated followed by *Staphylococcus* spp. (12.5%), *Klebsiella* spp. (12.5%) and *Salmonella spp*. (12.5%). The effect of acetic acid (Vinegar) concentration (0.5- 2.5%) and exposure time (0-10 min) on the microbial load of five vegetables were also assessed. Increasing vinegar concentration from 0.5- 2.5 % reduced microbial load by 15 – 82%. The study recommended that consumers' awareness on the dangers of consuming pathogen contaminated foods and the need to insist on properly processed or stored sliced produce needs to be re-awakened. Jeddi et al (2014) carried out a microbial evaluation of fresh, minimally- processed vegetables and bagged sprouts from Chain Supermarkets in Tehran, Iran. The aim was to evaluate the bacterial and fungal quality of minimally-processed vegetables (MPV) and sprouts. The findings of the study established that the load of aerobic mesophilic bacteria was minimum and maximum in the fresh- cut vegetables and fresh mung bean sprouts respectively. According to the results from the present study, effective control measures should be implemented to minimize the microbiological contamination of fresh produce sold in Tehran, Iran. Chaturvedi et al (2013) Eni *et al* (2010), Jeddi et al (2014) , Sair et al (2017), Sabry et al (2011).

There are other studies that were carried out to describe the bacterial load and the occurrence of some disease-causing enteric bacteria on raw vegetables sold in Saudi markets and open markets in Lusaka district. The studies further aimed at analyzing antibiotic resistance among bacterial population of raw vegetables in Lusaka districts and Saudi markets and the results revealed that none of them contained *Bacillus cereus, Salmonella*, and *Escherichia coli* O157:H7.

However, *Staphylococcus aureus* and *Shigella* were detected in 11.8% and 4.4% of the samples, respectively. The bacterial loads ranged from 3 to 8 log10 CFUg- 1 for aerobic bacteria and 1 to 4 log10 CFUg- 1 for coliforms as well as Enterobacteriaceae. The isolates exhibited resistance in decreasing order for ampicillin (76.5%), cephalothin (69.5%), trimethoprime-sulfamethoxazole (36.7%), aminoglycosides (21.9%), tetracycline (17.2%), fluoroquinolones (17.2%), amoxycillin-clavulanic acid (13.3%), and Chlorempenicol (7.8%). These investigations indicate the occurrence of antibiotic resistance among bacterial isolates populating raw vegetables which is a public health concern. Sabry et al (2011) and Chakopo (2017)

2.4 Relationship between Chicken manure and antibiotic resistance in amended soils

Chicken manure chicken manure has the potential to contribute to the spread of antibiotic resistance from animal intestines to the soil environment. The fact that some human pathogens with multiple antibiotic resistances were detected in harvested vegetables after growing in manure-amended soil demonstrates a potential threat to human health. (Yang et al, 2014).

Soil and organic fertilizer (manure) can be sources of ARB in microorganisms isolated from fresh vegetables. Antibiotic residues in poultry manure or soil may find their way to vegetables thereby contaminating fresh vegetables.

Vegetables produced in or close to soil such as carrots and leaf vegetables are at special risk for contamination with soil-borne bacteria, either belonging to natural soil microbiota or introduced into soil by manure fertilization. While the application of manure in ready-to-eat crops is discouraged, for example, by the U.K. Food Standards Agency (www.food.gov.uk); it is not generally prohibited by European law or in other parts of the world.

Several studies have been carried out to focus on the prevalence of ARB or AMR genes in vegetables at harvest and the soil from which these vegetables were harvested (Wang *et al.*, 2015; He *et al.*, 2016; Lau *et al.*, 2017; Tien *et al.*, 2017); these studies found that there is a negative impact that manuring have with regard to the presence of antimicrobial resistance of microorganisms in fresh vegetables.

China is the largest producer and user of antibiotics in the world, based on market sales data (Hvistendahl .M, 2012). In 2013, the total antibiotic usage in China was approximately 162,000 tons, with animal consumption accounting for about 52% of the total antibiotics, and the rest used by humans. It is noteworthy that a high proportion of VAs are mainly excreted via urine and feces, and 30%−90% of them in the feces are parent compounds or metabolites and subsequently, these residual antibiotics can enter the soil environment following the land application of animal or Chicken manure at a level of 15,000−150,000 $kg \cdot ha^{-1}$ per year in vegetable farming in China (Fang H,2015).

2.5 Local Perspective

The study by Chakopo (2017) identified the bacterial food-borne pathogens found in freshly consumed vegetables sold in open markets in Lusaka. The work acknowledges that fresh vegetables normally carry natural non- pathogenic epiphytic microorganisms, but during growth, harvest, transportation and further handling of the produce can make the vegetables to be contaminated with pathogens from animal and human sources. To this effect, the microbial content may represent a risk factor for consumers' health and therefore a food safety problem. Based on the findings of the study, bacterial species identified mainly included *Klebsiella, Enterobactor, Escherichia coli, Proteus, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and streptococcus*. Susceptibility of the isolated bacterial to the common chemotherapeutic agent showed that some bacteria were susceptible and others were not susceptible to the drugs. The study concluded that microbial contamination of fresh vegetables with opportunistic pathogens can be considered as a food safety concern as consumption of these produce may represent a potential risk for the consumer's health. Kinkese *et al* (2018) studied contamination of complementary weaning foods for children with *Escherichia coli* and *Salmonella* species in Lusaka District in Zambia. The study samples of complementary weaning foods included pumpkin leaves and beans which are fresh vegetables. The study examined *Escherichia and Salmonella* levels of contamination. The magnitude of foodborne diseases with most harmful potential pathogens that can cause diseases of human and animal origin in Zambia is not fully known. The results of the study established that out of 244 complementary food samples contaminated with bacteria, 91 had E. coli and 38 had Salmonella. The rest, 115 food samples, were contaminated with other bacteria such as *Staphylococcus, Bacillus* and *Streptococcus*. Furthermore, isolated *E. coli* and *Salmonella* exhibited resistance to metronidazole and Ceftazidime thereby raising some serious concerns as the drugs are used for first choice for enteric infections.

CHAPTER THREE: MATERIALS AND METHODS

3.0 Introduction

The section focuses on materials used in the collection of samples, analysis of the sample, data entry and data analysis.

3.1 Study design

The study design was cross sectional.

3.2 Sampling area/population

The study was conducted in Lusaka district. The district has a population of over 2,526,102 million people (CSO, 2018) and has the highest number of open markets and shopping mall that houses some big supermarkets. Most households in Lusaka obtain their vegetables from open markets and Soweto being the most prominent as various vegetable farmers bring their vegetable produce here for sale at very cheaper prices. And as a result most people and traders from other smaller markets often flock to Soweto market to buy these vegetables and then resell in other markets such as Chawama, Mandevu and Kalundu markets. Also a good number of households particularly those in low density areas buy their vegetables from super markets such as Shoprite, Food lovers, Cheers, Spar, Pick and Pay as well as Food Co in Game stores among many others often located in big shopping malls. In super markets vegetables are sourced from farmers who directly supply the supermarkets and are often sold under what appears to be a clean environment. In some cases, the supermarkets also outsource vegetables from neighbouring countries especially exotic vegetables.

3.3 Sampling

Both purposive and random sampling techniques were applied in sample collection. Purposive Sampling was used in selecting samples which are normally consumed mainly on a larger scale by buyers such as rape ,cabbage green beans and as such could exposes people to greater risk if contaminated.

Due to limited time and inadequate resources only 74 vegetable samples weighing about 200g-300g each, were purchased from selected open and supermarkets in Lusaka district. All the bought samples where then packed in sterile Ziplock bags and were transported in cooler boxes containing ice packs until they reached the laboratory at the Food Science laboratory of University of Zambia where the samples were analysed for *total coliforms, E. coli, feacal coliforms*, *Staphylococcus* and *Salmonella.*

On the basis of and manner of consumption, samples were trimmed of spoiled parts, the outer leaves were removed in the case of cabbage. Leafy vegetables were cut aseptically and cucumbers and green peppers were sliced into smaller pieces. Analyses were carried out within 2 to 4 hours of purchase.

3.4 Sampling frame

Only fresh vegetables sold in the selected markets were included in this study. That is five (5) supermarkets and three (3) open markets

3.5 Experimental procedure

Pre-enrichment of all samples collected was done in the laboratory before analysis on detection of *Salmonella* and *Staphylococcus* and *E. coli* and the enumeration of total viable count, feacal coliforms and *E. coli*.

A mixture of 25 grams of sample and 225 ml of Buffered Peptone Water (BPW, Oxoid CM0509) was made and left at room temperature for 1 hour. Ringer's solution was then made using Ringers tablets (Merck, 115525) and subsequently used for serial sample dilutions. Aerobic plate counts of viable organisms was done by the pour plating method on plate count agar (PCA, Oxoid), followed by incubation at 21 °C for 48 h; colonies were recorded as CFU/g.

$$\textbf{CFU/g} = \frac{\textbf{No\# of colonies}}{\textbf{Dilution factor x no\# of plates plated}}$$

Enterobacteriaceae and coliforms were cultured and enumerated on violet red bile glucose (VRBG, Oxoid CM1082) agar by the pour plate method with an over-lay, followed by incubation at 35 °C for 24-48 hrs.

The detection and enumeration of *S. aureus* was done by plating the serial dilutions with the drop technique onto Baird Parker Agar (BP, Oxoid CM0275) containing egg yolk tellurite emulsion (Oxoid, SR0054).

Presence of *fecal coliforms* and *Escherichia coli* (thermotolerant) was determined using eosin methylene blue agar (Scharlau) after incubation at 44.5_C for 24 h.

Salmonella was detected in a six successive steps process. Pre-enrichment in BPW at 35 °C for 24 h, followed by enrichment of 100 μL in Rappaport–Vassiliadis (RV, Oxoid CM0669) broth incubated at 42 °C for 48 h. The isolation was done on two selective media, brilliant green agar (BGA, Oxoid CM0263) and xylose lysine deoxycholate (XLD, Oxoid CM0469) agar at 35 °C for 24-48 h. Confirmation of suspicious colonies of *Salmonella spp.* was done using Triple Sugar Iron (TSI, Oxoid CM0277) slants.

3.6 Antibiotic Resistance test (ART)

The antibiotic resistance of the isolated strains was determined on cation-adjusted Mueller-Hinton agar (Hi-Media) using Kirby-Bauer disk diffusion method according to the standards and interpretive criteria described by NCCLS (2006).

Antibiotics used included erythromycin, amoxycillin-clavulanic acid (AMC 20/10), nalidixic acid (NAL 30), ciprofloxacin (CIP 10), Chloramphenicol (C30), Cefoxitin (FOX 30), Cefotaximine (CTX 30), Erythromycin (E), Impenem (IPM) and Tetracycline (TET 30).

Procedure

Using Kirby-Bauer-CLSI modified disc agar diffusion technique (DAD) (NCCLS, 2006). The 24-hour broth culture of the isolates was diluted to McFarland standard (10^5 – 10^6cfu/ml). One millilitre (1.0 ml) of standardized culture of each isolate was used to flood the surface of Mueller Hinton agar (MHA) plates and then allowed to dry while the Petri dish lid was in place. The standard antibiotic discs were then aseptically placed at reasonable equidistance on the inoculated MHA plates and allowed to stand for 30 minutes to allow the antibiotics to dif-fuse in the agar medium. The plates (prepared in duplicates for each isolate) were then incubated at 37°C for 16 hr to 24hr (Ehinmidu, 2003). The diameter of the zones of inhibition produced by each antibiotic disc was measured using the vernier caliper and recorded. The average mean diameter zones of inhibition were taken.

The ZOI readings were then compared with the standard ZOI Charts for antimicrobials. **Appendix 3.0**

3.7 Limitations of the Study

The study had its own limitation that included:

- Available funds limited the purchase of more vegetable samples
- Due to limited resources it was not possible to have a larger sample size and also limited the inclusion of other pathogenic bacteria of public health significance.

CHAPTER FOUR: RESULTS

This section presents the findings of the study whose main objective was to assess quality and microbial safety of fresh vegetables sold in selected markets and establish the public health significance of these fresh vegetables.

4.1 The fresh vegetables:

Fresh vegetables were found to be contaminated with higher levels of Enterobacteriaceae and pathogenic microorganisms such as *salmonella* and *staphylococcus* as shown in **Tables 1.0. and 2.0**

With the exception of two samples that were minimally processed and were wrapped in clean plastic wrap all the other 74 samples that were analysed tested positive for *Staphylococcu*s (100%), *feacal coliforms* (94.6%)as well as *E. coli* (100%). Only four (4) samples of the 74 tested positive for *Salmonella* (5.4%).

Table 1.0 *Salmonella* and *Feacal coliforms*

N=74 (Fresh vegetables)	**Detected in 25g sample**	
	Salmonella	**Feacal coliforms**
	4 (5.4%)	70 (94.6%)

Table 2.*0 E.coli* **and *Staphylococcus***

N=74 (fresh vegetables)	**MICROBIOLOGICAL QUALITY (CFU PER GRAM)**		
	Satisfactory	**Acceptable**	**Unsatisfactory**
Microbiological criteria	**≥20**	**20≥100**	**≥100**
E.coli	0(0%)	0(0%)	74(100%)
Staphylococcus	0(0%)	2**(2.7%)**	72**(97.3%)**
Total coliforms	0(%)	0(%)	74(100%)

4.2 Results of Antibiotic Resistance Testing (ART)

Nine antibiotics were used in this study and these are a representative of both the broad spectrum antibiotic compounds and also those that are commonly used in clinical settings.

Potential antibiotic resistant strains were identified by their ability to grow in the presence of different concentrations of antibiotics.

Of the 17 isolates belonging to the *Staphylococcus aureus*, 23.52% of the isolates showed some levels of resistance to 3 of the 6 antibiotics that were used. That is 5.88 % of the isolates showed resistance to chloramphenicol and erythromycin respectively and 11.76% showed resistance to tetracycline. The detailed information is presented in **Figure 1.0**

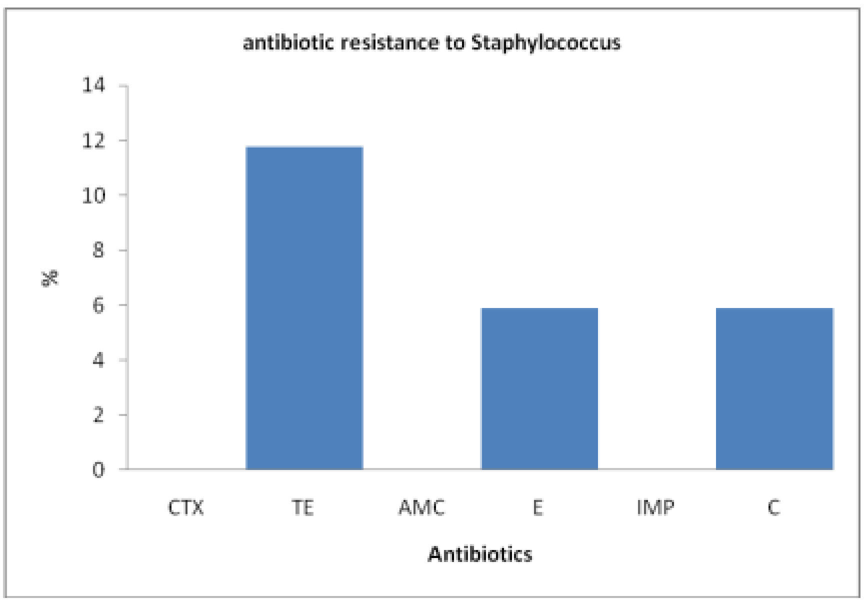
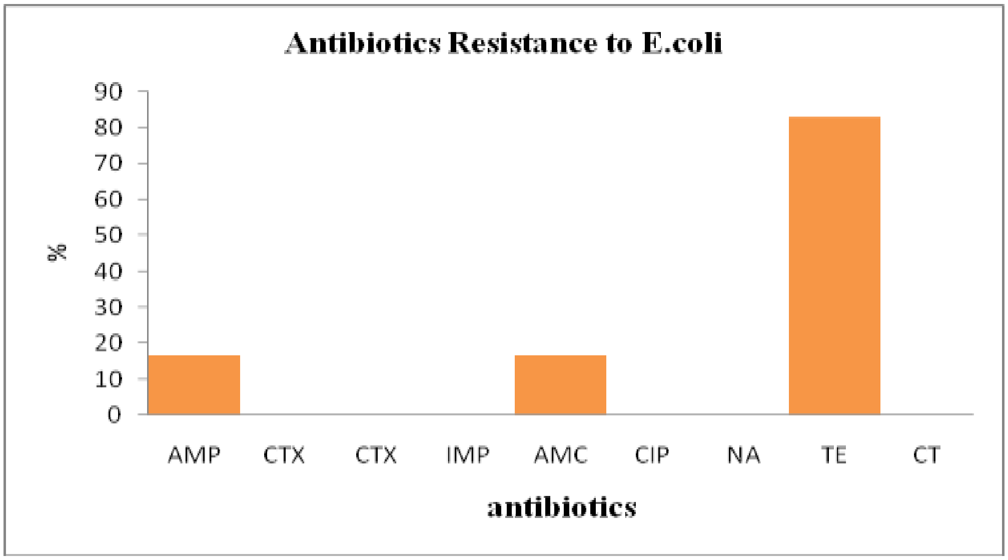


Figure 1.0 - antibiotic resistance of the isolated staphylococcus pathogens to 6 different drugs

Out of six (6) *E. coli* isolates, three (3) showed some levels of resistance to nine (9) of the antibiotics used as shown in **Figure 2.0**. Only (16.67%) were resistant to amoxylcluvate, (16.67%) resistant to ampicilin and 83.33% were resistant to Tetracycline**.**

Figure 2.0 - Antibiotic resistance of isolated E. coli pathogens to 9 different drugs



Out of the 4 isolates belonging to *salmonellae* family that were detected, 0nly one (1) (25.00%) showed resistance to ampicilin as indicated in **Figure 3.0**.

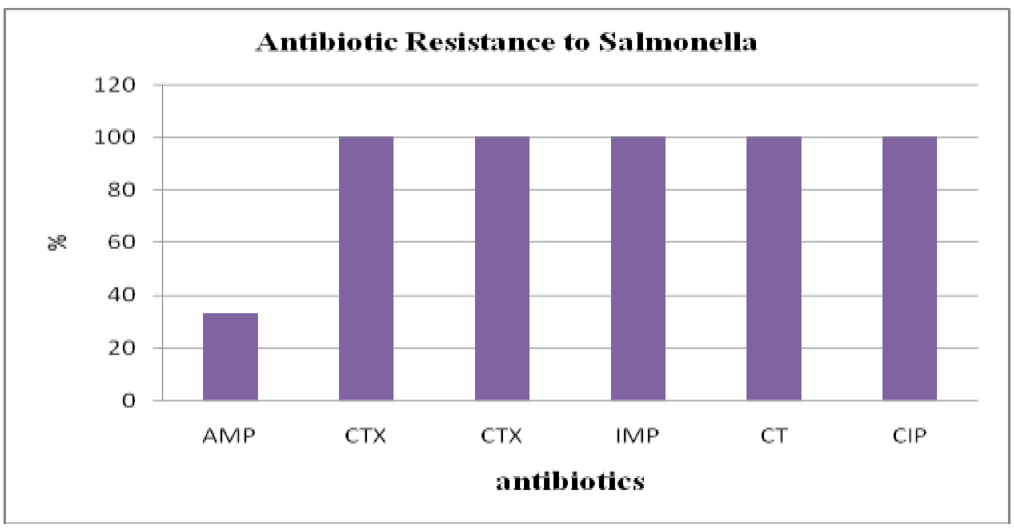


Figure 3.0 - Antibiotic resistance of the 4 isolated Salmonella pathogens to 6 different drugs

KEY:C;Chloramphenical**,**TE;Tetracycline**,**AMC;Amoxyl-cluvate**,E;**Erythromycin**,**IMP ;Imepenem**,**CTX ;Cefotaximine **,**NA;Nalidix Acid**,**CIP ;Ciprofloxacin **,**CT ;Cefoxitin

4.3 Interpretation of Results

Although there is some correlation between the size of the zone of inhibition and susceptibility of the organisms to the antibiotic, the former is a function of many variables e.g. density of the inoculum, depth of the medium, diffusibility of antibiotic etc.

The size of the inhibition zone at which organisms is considered Resistant; intermediate or sensitive is given in the zone size interpretative chart. **See Appendix 3.**

CHAPTER FIVE: DISCUSSION

5.0 Discussion

For the purpose of this study a total number of 74 vegetable samples were purchased from different supermarkets as well as open markets. These were analysed for hygiene indicator organisms that included *Feacal coliforms* and *Total Coliforms* and the presence of bacterial pathogens such as *Salmonella, Staphylococcus, E. coli*. .

5.1 Bacteriological quality

The bacteriological quality of the fresh vegetables with regard to microbial safety and quality are presented in Table 1.0 and table 2.0. According to the data recorded in the table, most of the vegetables sold in selected markets of Lusaka districts are highly contaminated with bacteria. Almost all the vegetables were contaminated with *E. coli* and about 97.3% staphylococcus. These therefore suggest that the quality of fresh vegetables sold in Lusaka district contains high levels of microbes which can pose a serious health risk to unsuspecting consumers. These findings are similar to those conducted by (Chakopo 2017) and (Kinkense et.al 2018) that conclude that fresh vegetables sold in Lusaka Markets are a safety concern due to contamination with microbes. This was as expected since most of the fresh vegetable produce is grown in outdoors and as such can be prone to contamination with microbial pathogens via a wide variety of mechanisms see **Appendix** 1

Some of these microbes are generally associated with some acute to chronic foodborne diseases such as dysentery, diarrhea, cholera and typhoid fever **(Appendix 2)**. For example, *Staphylococcus* is generally related to gastrointestinal infections because of its ability to produce very strong toxins in the body which brings about fever and vomiting. The group members of Enterobacteriaceae are widely known for intestinal infection and diarrhea diseases when ingested in foods (FBD).Therefore fresh raw vegetables normally purchased from the markets can be associated with pathogenic microbial contamination; they thus require thorough washing with fresh clean water and were possible boiling to cook so as to reduce the number of microbial communities. Moreover, refrigeration can also help in increasing shelf life of vegetables by inhibiting or slowing down the rate of microbial growth.

In the present study, the overall mean count of *staphylococci* from vegetable samples ranged from 2.1x 10^5 to 2.5x10^6(**Appendix 6)**. This was higher than the microbiological studies made on similar samples lettuce and green pepper from super market in Addis Ababa, Ethiopia, (Guchi and Ashenafi, 2010), who reported 4.55 and 4.97 x10^2, respectively. Higher counts of *staphylococci* from super market vegetables may be due to skin contact and environmental contamination probably coupled with contamination of food stuffs during distribution and handling which may allow bacterial growth and subsequent production of toxins, (Erkan et al, 2008).

In the vegetable samples analysed by this work, the total number of fecal bacteria per 25 gram was recorded in 94.6% of the samples which is considerably high and a food safety risk.

Although the original source of contamination of produce was not known the fact that almost all samples were contaminated with E.coli(100%) and 94.6% with feacal coliforms point to the use of manure from farm animals which has long been suspected of being a leading vehicle of pathogen transmission. Concerning the different results and the fact that the major source of contamination of fresh products is microbial pathogens, we agree with (Ijabadeniyi et al,2011) who reported that, at the preharvest stage, the sources include feces, irrigation water, inadequately composted manure, soil, air, animals, and human handling.

For this reason, this calls for good agriculture practices to be encouraged so that farmers and others sectors involved in fresh produce remain mindful of the environment and of public health concerns. This may require systems be developed through multidisciplinary research that includes more agriculturists, medical, biological, environmental, and socioeconomic components coming together in awareness programs that will make fresh vegetables safe for human consumption. But, in the immediate future, the use of properly composted poultry manure should help reduce the number and range of pathogens and hence avoid the application of contaminated manure.

Fresh vegetables are good sources of nutrients; however, they could pose a health risk if consumed raw (USFDA, 2001). The results of this study indicated that *Salmonella, E. coli* were present in a number of fresh vegetables which make them unsafe for human consumption.

(94.6%) samples were found to be positive for *S. aureus* and (5.4%) of the samples were positive for *Salmonella*. The results agreed with previous studies that detected *S. aureus* (Viswanathan and Kaur, 2001) in fresh vegetable samples. The possible source of raw vegetable contamination by *S. aureus* and *Salmonella* could be the hand of sellers, washing water, or insects at retails. The results are similar to the findings of (Mukherjee *et al.* 2006) who detected *E. coli* contamination in fresh vegetable samples and was mostly of leafy greens, lettuces, cabbage. This may be owed to the large surface area of these leafy vegetables, which could encounter contaminated water (irrigation and sprinkling) or other possible contaminants, including feces, flies, and dust. Also higher prevalence of fecal *E. coli* has been demonstrated by others (Viswanathan and Kaur, 2001) just as was the case with this study.

Although most of the vegetables consumed in Zambia are subjected to heat (cooking) before consuming, the growing trend of shops selling ready to eat fresh vegetables is another source of worry. Some of the samples analysed during this study were those used in a ready-to-eat products (Cabbage & Cucumber), implying that they could be consumed, by all groups of population, directly, without any further processing like washing or heat treatment. Normally ready-to-eat foods especially salads are often considered as a healthy option for many meals and most of the consumers may not be aware that it could contain high numbers of microorganisms, including pathogenic bacteria and pose adverse health risk.

The scientific world, however, started raising concerns in regards to the microbiological safety of Food of non Animal origin (FoNAO) and green leafy vegetables, in particular (European Food Safety Authority 2013). The fact that opportunistic pathogens in form of *Staphylococcus* was detected in almost all the samples analysed raises a lot of concern as these bacteria might pose a danger if the vegetables particularly a salad will be consumed by vulnerable person i.e. young, old, pregnant and immunocompromised (YOPI).

Enterobacteriaceae are a public health concern of great importance around the world affecting both humans and animals with considerable economic impact. Although illness associated with fresh produce is not common, a wide variety of fresh fruits and vegetables have been associated with diseases caused by some of these microbial pathogens and actually, when an outbreak does occur, the impact can be widespread and devastating. This is mainly because greater volumes of fresh vegetables are being produced and sold in the markets which are then distributed over much greater geographical areas and to many more people. This coupled with increased global trade potentially increases exposure to foodborne pathogens.

5.2 Antibiotic resistance

All the isolated bacteria were further tested for antibiotic resistance testing (ART) against variable concentrations ranging from (5**µg** to 30**µg**) of Nine antibiotics, Tetracycline, Chlorempenicol, Imepenem, Nalidix acid, Cefotaxime, erythromycin, Amoxyl-cluvateomycin. The results of ART showed that some of them were found to be resistant to these antibiotics.

The bacterial isolates from various fresh vegetables were analysed for their antibiotic resistance (figure 1.0, 2.0 and 3.0). Overall, 23.58% (*n*=74) of the 17 staphylococcus isolates showed antibiotic resistance to at least three (3) of the nine (9) antimicrobial agents used.

The broad use of poultry manure in agriculture is the most likely cause for resistant bacteria to antibiotics, which may enter the food chain and potentially harm humans (Khachatourians, 1998). Among the most common antimicrobial agents used in poultry production are drugs that are either identical or related to those administered to humans. In this study, 76.5% of bacterial population from vegetables at retail level showed resistance to at least one or three (3) antibiotics. This is in agreement with previously published data that found high resistance rates in bacterial populations from vegetables (Hamilton-Miller and Shah, 2001; Chakopo,2017).

CHAPTER SIX: CONCLUSION AND RECOMMENDATION(S)

6.1 Conclusion

Overall, this study revealed the presence of a high load of microorganisms in the commonly consumed vegetables items in both open market and selected supermarkets found in Lusaka district. Both *E. coli* and *feacal coliforms* were detected in almost all the samples except two samples that tested negative for feacal coliforms. This entails that the hygienic quality of the fresh vegetable samples was poor since higher bacterial counts were recorded beyond the standard safe limits. There is need for safety measures to be put in place which should be combined with Good Agricultural Practices during production of fresh vegetables so as to safe guard the health of consumers. The detection of pathogens (*Salmonella* and *staphylococcus*) shows that consumption of fresh vegetables has the potential to cause negative impacts on human health which is a public health concern.

The slowly increasing resistance patterns for most of the commonly used antibiotic by these pathogens pose another public health concern which can likely complicate the treatment options and increase the cost of treatment and patient management in general. To this end, all those involved in fresh vegetable production and related associations and individual companies should invest in implementing good manufacturing practices and hazardous analysis critical control points (HACCP) programs from production through processing and retail distribution so as to prevent the disease outbreaks related to consumption of fresh vegetables.

6.2 Recommendations

- In future a larger sample size will be needed and also the inclusion of other pathogenic bacteria of public health importance.
- Only nine different antibiotics were used in this study and it may be important to include many more antibiotics in future.
- It may also be important in future to look at microbial quality of poultry manure and irrigation water used by many vegetable farmers.
- In view of the results obtained in this study were most of the fresh produce were contaminated with bacterial pathogens, there is need for regular monitoring of microbial contamination of vegetables sold in markets especially supermarkets by local authorities if foodborne diseases are to be avoided.

- Contamination of fresh produce can occur at any point in the chain of production (from farm to fork) and can be exacerbated by improper handling and storage of the product prior to consumption. There is thus need to train all the people involved in food chain of fresh vegetables in hygiene and proper handling and storage of fresh vegetables to minimize the levels of contamination. Also farmers should be trained on how to use good agriculture practices in the growing and handling of vegetables to reduce the risk of contamination with pathogenic bacteria.
- It will be important if local authorities can come up with a policy especially on how to regulate the use of untreated manure in vegetable growing.

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APPENDICES

Appendix 1: Mechanisms by which produce can become contaminated

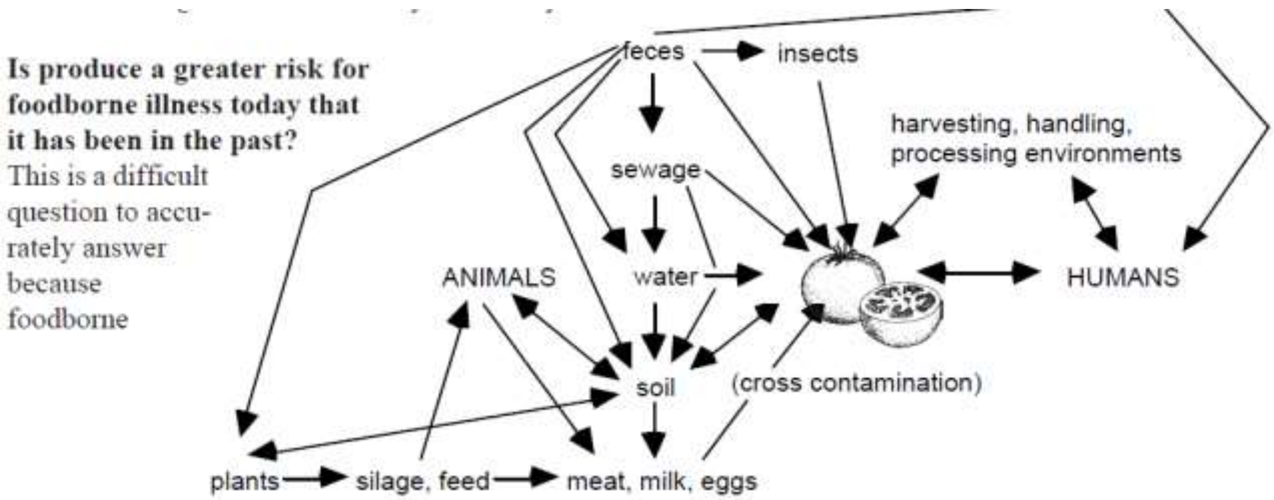

Figure 1. Mechanisms by which produce can become contaminated with pathogenic microorganisms (adapted from Beuchat, 1996)

Appendix 2: Table of selected bacteria and associated symptoms

Microganism	**Incubation period**	**Symptom**	**Infectious dose (number of cell)**	**Source**	**Examples of produce associated with Outbreaks**
Bacteria					
E.coli	2 to 5 days	Watery diarrhea often containing blood, abdominal pain and can lead to hemolytic uremic syndrome and kidney failure especially in children.	10 to 1000	Animal feces, especially cattle, deer and human	Sproughts,lettuce,apple Cider
Salmonella spp	18 to 36 hours	Abdominal pain diarrhea,fever,vomiting	10 to 100,000	Animal and human feces	Alfalfa sprout,apple cider,melon,tomatoes
staphylococcus	1 to 6 hours	Nausea, vomitting, diarrhea			Milk n milk products, ham, poultry, salads, custards

Appendix 3: Table Zone diameters Interpretive Chart (ATLAS Medical)

Serial No.	Antimicrobial Agent	Code Disc	Content Test (µg)	Organisms	Zone Diameter Nearest Whole mm		
					Resistance ≤	Intermediate	Sensitive ≥
	Amoxy-cluvate	AC	20±10	*Entrobacteriaceae*	13	14-17	18
				Staphylococcus spp.	19	----	20
	Cefotaximine	CX	30	*Entrobacteriaceae Staphylococcus* spp.	14	15-22	23
	Chloramphenicol	CK	30	*Entrobacteriaceae Staphylococcus* spp.	12	13-17	18
	Ciprofloxacin	CIP	5	*Entrobacteriaceae Staphylococcus* spp.	15	16-20	21
	Erythromycin	ER	15	*Entrobacteriaceae Staphylococcus* spp.	13	14-22	23
	Imipenem	IMP	20	*Entrobacteriaceae Staphylococcus* spp.	12	13-14	15
	Nalidix acid	NA	30	*Entrobacteriaceae*	13	14-18	19
	Tetracycline	TE	30	*Entrobacteriaceae*	11	12-14	15
				Staphylococcus spp	14	15-18	19

Appendix 4: Budget

ITEM	QUANTITY	UNIT COST	TOTAL COST (ZMK)
PURCHASE OF VEGETABLES	74	10.00	740.00
STERILE PLASTIC BAGS	80	50.00 for 10	400.00
BENCH FEES,MEDIA,ANTIBIOTICS and DISPOSABLE PETRI DISHES	5 PARAMETERS		5610.00
TRANSPORT	15 ROUND TRIPS		500.00
		TOTAL KWACHA (ZMK)	**7,250.00**

Appendix 5: Time frame of the study

The expected time of completion for this research is estimated at three months beginning the first week of November, 2018**.**

ACTIVITY	PERIOD 2018				PERIOD 2019			
	September	October	November	December	January	February	March	April
Sample collection and Laboratory work								
Data Collection								
Data Analysis								
Report writing								
Thesis Submission								

	MICROBIOLOGICALQUALITY (CFU PER GRAM)

Appendix 6 : Microbial quality and Contaminants of the analysed fresh vegetables

		MICROBIOLOGICAL QUALITY (CFU PER GRAM)							
		Total coliforms	Salmonella	Feacal coliforms			E.coli	Staphylococcus	
	Sample	Satisfactory	Unsatisfactory	Satisfactory		Unsatisfactory	Unsatisfactory	Acceptable	Unsatisfactory
1	**Chinese cabbage**	$4.1x10^{6}$				Detected	≥1,100		$7.6x10^{5}$
2	**Chomolia**	$3.3x10^{6}$				Detected	≥1,100		$1.8x10^{6}$
3	**Chibwabwa**	$3.7x10^{6}$				Detected	≥1,100		$1.0x10^{6}$
4	**Rape**	$4.3x10^{5}$				Detected	≥1,100		$1.6x10^{6}$
5	**sweetpotato leaves**	$1.3x10^{6}$				Detected	≥1,100		$4.6x10^{6}$
6	**Mupilu**	$4.1x10^{6}$				Detected	≥1,100		$4.0x10^{6}$
7	**Chinese cabbage**	$2.8x10^{6}$				Detected	≥1,100		$7.0x10^{5}$
8	**Bondwe**	$4.1x10^{6}$				Detected	≥1,100		$1.3x10^{6}$
9	**Cucumbers**	$2.7x10^{7}$				Detected	≥1,100		$2.5x10^{5}$
10	**green beans**	TNTC	detected in 25g sample			Detected	≥1,100		$1.4x10^{5}$
11	**Lettuce**	$4.1x10^{6}$				Detected	≥1,100		$1.1x10^{6}$
12	**Rape**	$2.8x10^{6}$				Detected	≥1100		$2.5x10^{6}$

Sample		Microbial quality (CFU/gram)						
		Total coliforms	Salmonella	Feacal coliforms		E.coli	Staphylococcus	
		Satisfactory	Unsatisfactory	Satisfactory	Unsatisfactory	Unsatisfactory	Acceptable	Unsatisfactory
13	**Chinese cabbage**	3.2×10^{6}	detected in 25g sample		Detected	$\geq$1,100		2.4×10^{6}
14	**sliced cabbage**	4.0×10^{1}		Not Detected		$\geq$1,100	$\leq$100	
15	**half cabbage**	4.1×10^{6}	detected in 25g sample	Not Detected		$\geq$1,100		2.0×10^{2}
16	**Spinarch**	4.1×10^{6}			Detected	$\geq$1,100		6.0×10^{5}
17	**shredded cabbage**	2.8×10^{7}			Detected	$\geq$1,100		4.0×10^{2}
18	**shredded Carrots**	1.76×10^{6}			Detected	$\geq$1,100		2.0×10^{2}
19	**Rape**	8.2×10^{5}			Detected	$\geq$1,100		1.1×10^{5}
20	**sweetpotato leaves**	2.2×10^{6}			Detected	$\geq$1,100		3.3×10^{6}
21	**Carrots**	9.0×10^{5}			Detected	$\geq$1,100		2.1×10^{6}
22	**Rape**	8.4×10^{6}			Detected	$\geq$1,100		5.6×10^{5}
23	**green beans**	3.0×10^{6}		Not Detected		$\geq$1,100		7.0×10^{5}
24	**Crisp Lettuce**	5.0×10^{6}			Detected	$\geq$1,100		2.1×10^{5}
25	**Chibwabwa**	3.0×10^{6}			Detected	$\geq$1,100		1.2×10^{6}
26	**wrapped cabbage**	3.0×10^{6}			Detected	$\geq$1,100	5.0×10^{1}	
27	**Rape**	7.0×10^{6}			Detected	$\geq$1,100		1.1×10^{6}
28	**Spinarch**	4.9×10^{6}			Detected	$\geq$1,100		1.9×10^{6}
29	**Rape**	1.4×10^{4}			Detected	$\geq$1,100		1.0×10^{6}
30	**Chinese cabbage**	2.0×10^{6}			Detected	$\geq$1,100		7.0×10^{5}
31	**green pepper**	5.0×10^{6}			Detected	$\geq$1,100		2.3×10^{6}
32	**Lettuce**	3.0×10^{2}			Detected	$\geq$1,100		1.7×10^{6}
33	**Cucumbers**	1.0×10^{7}			Detected	$\geq$1,100		1.9×10^{6}
34	**Rape**	3.1×10^{4}			Detected	$\geq$1,100		5.1×10^{5}
35	**Rape**	6.0×10^{6}			Detected	$\geq$1,100		4.3×10^{6}
36	**Chibwabwa**	4.3×10^{6}	detected in 25g sample	Not Detected		$\geq$1,100		2.7×10^{6}
37	**Bondwe**	3.1×10^{4}			Detected	$\geq$1,100		2.0×10^{6}
38	**Chinese cabbage**	1.0×10^{5}			Detected	$\geq$1,100		7.9×10^{6}
39	**sweetpotato leaves**	6.5×10^{6}			Detected	$\geq$1,100		1.1×10^{7}
40	**Rape**	5.2×10^{4}			Detected	$\geq$1,100		1.8×10^{6}