MICROBIAL PROFILING OF STREET VENDED FOODS AND THE ASSESSMENT OF FOOD HANDLER KAPs IN MASERU, LESOTHO

BY

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DECLARATION OF OWN WORK

I PONTS'O LETLOTLO JOYCE LETUKA, declare that the work hereby submitted is my own original work. Where other people's work has been used (either from a printed source, internet or any other source), this has been properly acknowledged and referenced accordingly. Furthermore, this work has not been submitted to any other institution or organization for any reason whatsoever.

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ABBREVIATIONS

BGA- Brilliant Green Agar

CAC- Codex Alimentarious Commission

CDC- Centre for Disease Control and Prevention

DDST- Double Disc Synergy Test

FAO- Food and Agriculture Organization

FDA- Food and Drug Authority

FSA- Food Safety Authority

ICMSF- International Commission on Microbiological Specifications for Food

MDR- Multidrug Resistance

PA- Pseudomonas Agar

PCA- Plate Count Agar

RTE- Ready-to-eat-food

SVF- Street Vended Foods

TNTC- Too Numerous To Count

TVC- Total Viable Count

WHO- World Health Organization

ABSTRACT

Street food vending has over the years become more popular in developing countries. This is because it requires less capital to start, making it an easy business for those with low incomes. Street food vending is described as the sale of ready-to-eat food and beverages in and around public places. This study was conducted to investigate the safety of foods sold in around the taxi and bus rank areas in Maseru, Lesotho. Three aspects were surveyed in this study. The first survey was on the knowledge, attitudes and practices of food handlers and the general perspectives of consumers about street food vending in Maseru. Secondly, the microbial quality of food and the hygiene status of food contact surfaces and food handler hands were evaluated, and lastly, the antimicrobial susceptibility profile of organisms obtained was assessed. A total of 58 food handlers and 93 consumers were involved in this study, and 30 food samples were collected for microbial analysis. For the antimicrobial susceptibility test, seven bacterial isolates were evaluated.

In this study, there were more female food handlers (n=35, 60%) than male food handlers (n23, 40%) and a majority of the food handlers had not received any food safety training (60%). On average the street food vendors who participated in this study were said to have poor food safety knowledge since they scored (49%±11). However, food handlers in this study were found to have good attitude towards food safety since none of the food handlers scored below 50%. Additionally, all food handlers in this study (100%) reported that they use sanitizers when washing service utensils and a substantial amount of the food handlers (84%) said they check the shelf life of food at the time of delivery or purchase. These are some of the good practices that help ensure

the safety and quality of food produced. In this study there were more male consumers (n=59, 63%) than female consumers (n=34, 37%), and police officer (24%) and taxi drivers constituted the majority of consumers.

The microbial analysis of food samples showed that there was more microbial contamination (TVC) in vegetables (10.91 Log_{10} cfu.ml) than in chicken samples (5.45 Log_{10} cfu/ml). With regards to food contact surfaces and food handler hands hygiene status (TVC), there was more contamination on the left hands (7.21 cfu/cm^2) than on right hands (6.82 cfu/cm^2), and tables had the lowest counts among the three sampled surfaces (6.89 cfu/cm^2). The antimicrobial susceptibility test carried out in this study showed that all isolated were multidrug resistant to at least two or more of the antibiotics used. This ability of microorganisms to develop resistance to more antibiotics daily could be due to poor infection control, inappropriate food handling, and failure to prudently use antibiotics.

In conclusion the microbial analysis results collaborated those of the surveys which showed that food handlers had poor food safety knowledge and exhibited negative attitudes towards some of the concepts of food safety hygiene. Furthermore, the results of this study should provide the government of Lesotho with enough evidence to show the need for improvement of mitigation procedures and appropriate interventions for the development of the street food trade and public health.

Keywords: street food vending, food safety knowledge, food handlers, consumers, antimicrobial susceptibility

CHAPTER 1

Introduction

1.1 BACKDROP ON STREET FOOD VENDING AND FOOD HANDLER KNOWLEDGE

Street-vended foods are defined as food and beverages prepared and/or sold by vendors in streets and other public places for immediate consumption or consumption at a later time without further processing or preparation (WHO, 1996). These include food prepared at the vendor's dwelling and brought to the street for selling or food prepared in small scale factories or those prepared and sold at the street food stalls (Gadaga *et al*., 2014).

In many countries across the world street vended foods provide populations with cheap and easily accessible diets, they are also a source of income for a vast number of people and contribute to the economy of countries. Because of industrialization, most people in the cities are leaning towards the consumption of these street-vended foods daily when out of their homes (WHO, 1996; Alimi, 2016). This is despite the fact that a concern may arise whether the food is prepared under hygienic and regulated conditions in order to avoid food poisoning. Furthermore, consumers are less interested in safety and quality of hygiene of the food but rather their convenience which also increases their risk of consuming contaminated food (Mensah *et al.,* 2002).

Apart from health and safety of street foods, there were issues in the past regarding the legality of street vended foods since this practice was considered to be illegal. However, despite all of the challenges that the South African street food market has experienced, there has been significant growth within the sector due to socio-economic changes (von Holy and Makhoane, 2006). Presently a number of organizations e.g. local authorities,

international organizations and consumer associations are fully aware of the advantages and disadvantages of street-vended foods. Their major concern is food safety but sanitation problems also form part of the risks associated with street vending (FAO 2010).

Over and above the concerns mentioned, street food vending in developing countries may present environmental and food hygiene problems which are brought about by the lack of basic water supply and proper infrastructure (Proietti *et al.,* 2014). There are a number of important sources that can cause contamination during or after food preparation e.g. serving and cooking utensils, raw materials, time and temperature abuse of cooked foods and personal hygiene of the street vendors (Rane, 2011). Some of these sources of contamination, just like the raw materials, can be made vulnerable by the use of cheap ingredients with illegal or undesirable residues and substances arising in poorly stored commodities (Proietti *et al*., 2014). These undesirable occurrences have been identified as sources of outbreaks of diseases and illnesses (Alimi, 2016).

1.2 PATHOGENIC MICROORGANISMS ASSOCIATED WITH STREET FOODS AND FOOD HANDLER ROLE IN THIS REGARD

Food poisoning is when a person gets ill from consuming food that is contaminated by harmful microorganisms or toxins they produce (Albrecht *et al.,* 1992). Food borne pathogens have been associated with bacterial infections in humans across the world, with food animals being reservoirs for such pathogens (Tanih *et al.,* 2015). Various microbes that are present in street foods to be belonging to the genus *Bacillus, Staphylococcus, Clostridium, Vibrio, Campylobacter, Listeria and Salmonella* (Rane, 2011). Some of these pathogens that are detrimental to the consumer's health may survive when raw materials are not thoroughly cooked and they are responsible for about 90% of foodborne illnesses and fatalities (Alimi, 2016; Khairuzzaman *et al.,* 2014). A study (Kubheka *et al.,* 2001) showed that the street vended fruit salads and gravies had high aerobic plate counts (APC>250) and spore count even though they had been cooked prior to consumption. These high APC counts are generally assumed to be potential health hazards.

Salmonella Enteritidis was reported to have been the cause of a food borne outbreak in Kwazulu-Natal where 216 patients presented to the local hospital (Neihaus *et al*., 2011). Estimates by the World Health Organization (WHO) for global burden of food borne diseases showed that almost 1 in 10 people fall ill annually from eating contaminated food and 420 000 died as a result. Children under the age of 5 are at high risk with 125 000 dying each year (WHO, 2015).

Food safety and security are greatly enhanced by the knowledge, attributes and practices (KAPs) of food handlers. Research has shown that the majority of food handlers knowledgeable on hygienic practices and sanitation procedures such as hand washing and proper cleaning of utensils (Akabanda, 2017). However, they may not be aware of the threat posed by microbial contamination of food due to lack of food safety education and failure to implement safe food handling practices.

There are practices that street vendors do that compromises the safety of the food they sell, for example, wearing jewelry while handling food, handling money and preparing food concurrently. Other risk factors are, uncovered heads, not using aprons, blowing air into plastic bags before putting food into them, storing food openly at the stalls and saving left over foods to be sold the next day when they were not even properly stored (Chukuezi, 2010). However, food handler practices are not the only source or attributes of contamination of the food chain. For example, if a food handler is infected with maybe Hepatitis A or Norovirus, he or she may unknowingly infect the food they are handling at the time (WHO, 2006). The basic lack of knowledge and understanding of hygiene by street food vendors could be a major barrier for the efficient and effective implementation of Hazard Analysis and Critical Control Points (HACCP) in such small scale businesses (Walker, 2003).

The WHO in 2001 came up with the Five Keys to Safer Food strategies, this in an attempt to reduce microbial load commonly responsible for foodborne illnesses. These keys are as follows:

- Keep clean
- Separate raw and cooked foods
- Cook food thoroughly
- Keep food at safe temperatures
- Use safe water and raw ingredients

Failure to observe proper food handling practices or even the five keys as provided by the WHO, compromises food safety and the food may be contaminated by common foodborne pathogens, as outlined in Table 1.1

Table 1.1 Common foodborne pathogens associated with foodborne infections

Pathogen	Sources	Incubation period	Symptoms
Campylobacter jejuni	Raw or uncooked meat and poultry, raw vegetables, raw milk	2-11 hours and symptoms can last for 7-14 days	Abdominal pain, bloody diarrhea, chills, fever, headache
Escherichia coli	Raw or uncooked beef, uncooked fruits and vegetables, raw milk	1-8 days	Diarrhea, severe cramping, nausea, vomiting, kidney damage in children
Salmonella enteritidis	Eggs, poultry, unpasteurized milk, fruits, vegetables	12hours- 3days	Fever, nausea, vomiting, severe abdominal pain, diarrhea
Listeria monocytogenes	Unwashed fruits and vegetables, water, soil	3-4days (Johnsen *et al.,* 2010)	Flu-like symptoms, encephalitis, meningitis
Staphylococcus aureus	Sandwiches, bakery products, chicken, tuna, ham	1-6 hours	Severe abdominal cramps, diarrhea, mild fever, vomiting
Bacillus cereus	Rice, leftover food, sauces, soups, food that have been kept for too long at room temperature	6-15 hours	Emetic vomiting, watery diarrhea, nausea, stomach cramps
Clostridium perfringens	Beef, poultry, gravies	6-24 hours	Diarrhea, abdominal cramps
Pseudomonas aeruginosa	Contaminated water	13 days	Septicaemia, meningitis

Source: (FDA 2018; Foodsafety.gov 2018)

There is also however, bacteria that is not just pathogenic but rather produce toxins, examples of such bacteria include *Staphylococcus aureus, Clostridium botulinum* and *Clostricium perfringens* (Waggoner, 2004). Viruses are also implicated in foodborne illnesses and the contamination is mainly from the fresh produce or processed food by virus-containing fecal material as depicted in Table 1.2.

Table 1.2 Viruses associated with foodborne illnesses

Virus	**Sources**	**Symptoms**
Norovirus	Salads, frozen fruits	Diarrhea, vomiting, body pains, headache, fever, nausea
Hepatitis A &E viruses	Contaminated water, salads	Anorexia, sudden onset of fever, malaise, abdominal discomfort, jaundice
Rotavirus	Salads, fruits, contaminated water	Watery diarrhea, abdominal pain, vomiting, fever
Astrovirus	Contaminated water	Gastroenteritis, predominant diarrhea

Source: (Atreya, 2004).

1.3 COMMON FOODS IMPLICATED IN MICROBIAL CONTAMINATION AND THE ROLE OF THE ENVIRONMENT IN STREET VENDING

Raw materials

In order to sell food at cheaper prices to consumers, vendors usually buy cheap ingredients that are sometimes detrimental to the health of consumers.

- **Meat-**mostly street vended meat is cooked at high temperatures which leads to the development of carcinogenic chemicals such as Heterocyclic Amines which are not present in uncooked meat (Statement and Cancer, 2013). Microorganisms such as *Staphylococci aureus* as well as enteric organisms can multiply and be virulent on meat and meat products prior to cooking (Oguttu, 2015), therefore to prevent cross contamination to other food substances, raw meat should always be separated from other foods (WHO, 2015). To date there has been no studies in Lesotho to evaluate or implement risk analysis methods in informal markets, neither has there been studies on the food quality of ready-to-eat meat in order to determine the risk of food borne diseases to the consumers.
- **Flour-** although low moisture foods such as flour do not offer suitable environment for microorganism growth, *Salmonella* is known to be present in wheat flour and has been traced back to outbreaks that occurred in New Zealand in 2010 and New South Wales, Australia in 1952 (Akins, 2011).

- **Spices-**spices are commonly used in almost every household and are generally used daily in cooking. Reports by the FDA have shown that spices contain contaminants that may be detrimental to health, and these range from food borne pathogens such as *Salmonella,* heavy metals, and micro toxins which carcinogenic (FDA, 2017).
- **Vegetables-** fresh vegetables provide the human body with important supplements and are therefore vital nutrients for a healthy diet (Mohamed *et al.,* 2016). There is, however, the risk of infection with *Salmonella* and *E.coli* O157 that is associated with the consumption of fruits and vegetables and recent outbreaks have been linked to spinach, lettuce and tomatoes (Heaton and Jones, 2008).
- **Eggs-** they are naturally nutritious and affordable, and in the street market they are boiled and sold as ready-to-eat foods. Food poisoning may however occur if proper care is not taken when handling and preparing them. However they are prone to contamination and this may happen in one of two ways; vertical transmission through the ovary or trans-ovarian or by horizontal transmission through the shell or trans-shell (Cox *et al*., 2012). Salmonella is usually associated with vertical transmission, while horizontal transmission usually involves contamination by faeces or environmental vectors (Wybo *et al*., 2004).

Equipment

It is critically important that cooking utensils must be clean at all times and should be regularly washed with clean water and soap. However, this is not always the case in street food vending (Chukuezi,2010).The author showed that most street food vendors use dirty water to wash their utensils and to make matters even worse, the water is constantly recycled and there are often complains of water shortage. Water is a critical raw material in street food vending and contaminated water can pose a great public health risk when used for washing utensils because it is a well-known vehicle for enteropathogens such as *E. coli, Salmonella* spp. and *Campylobacter* spp. (Rane, 2011). Utensils used in street vending are usually similar to those used at home, and they can be divided into two groups; traditional utensils and modern utensils (WHO 2015), as depicted in Table 1.3.

Table 1.3 Traditional and modern utensils

Traditional utensils(designed and made by local artists)	**Modern utensils (generally made from metal and plastic**
Hand grater, grinding stone, knives, wooden spoons, cloths, baskets, braai stands	Basin, bucket, pans, pots, cookers, ovens, plates, cups, spoons

1.4 LEGISLATION CONCERNED WITH SOUTH AFRICAN AND LESOTHO STREET FOOD VENDORS

In Lesotho vendors are required to rent their stalls from Maseru City Council and are required by the Public Health Order of Lesotho to be examined medically and hold valid medical certificates (Gadaga *et al*., 2014). Labour laws concerning street food vendors are vague in Lesotho, however, there is a consumer protection unit within the Ministry of Trade that protects the rights of consumers. In South Africa, The Business Act 1991 states that any person who carries on the business of selling or supplying any foodstuff in the form of meals for consumption on or off the business premises, or any perishable foodstuff is required to hold a business license. The employees of non-standard work positions enjoy limited legal protection, which means the employer disregards most or all the legal requirements. Street food vendors form a large portion of these non-standard workers and they are sometimes disregarded as employees and therefore the basic labour laws do not apply to them (WIEGO 2014). Environmental health practitioners are also authorized by the South African Food Safety Law to train food handlers (Campbell, 2011). Microbiological standards for food have been set in South Africa but none are set or even implemented in Lesotho.

1.5 RATIONALE

Street food vending is found almost everywhere and it provides a large number of people with nutritional diets and some with an income and means of survival (Alimi, 2016). However food safety is a primary concern in the street markets, hence it is important to apply sanitary measures, good hygiene measures as well as proper food handling practices essential in food safety (Ifeadike *et al*., 2014).

Improper storage, transportation, poor personal hygiene, preparation of food in bulk and in ambient temperatures especially long hours before selling are the most common contributing factors to food borne diseases. Most cases of severe food borne illnesses and fatalities are due to bacterial pathogens (Rane, 2011). Of the thousands various bacterial species, more than 90% of food borne illnesses are caused by *Staphylococcus, Salmonella, Clostridium, Listeria,Vibrio, Bacillus* and Enteropathogenic *Escherichia coli* (CDC, 2016). Food borne pathogens such as *Bacillus* spp. are the most predominant in ready-to-eat street food due to holding conditions that are favourable for these pathogens to survive and geminate (Rane, 2011). Moreover, bacterial pathogens such as *Salmonella, E.coli* and *Vibrio* may cause diarrheal diseases, including cholera, which may in some cases be fatal.

It is therefore of great importance that studies on microbial hazards of street foods are conducted. This will then assist relevant authorities on the importance of educating both the street vendors and consumers about the importance of proper food handling practices so as to prevent and reduce food borne pathogenic diseases.

In the capital town of Lesotho, Maseru, street food vending has been implicated in food poisoning due to the lack of knowledge of the vendors on proper food handling practices. This is even compounded by the fact that some cases are not reported due to inability of the consumer to relate to their exact cause of illness. This issue became evident during an informal discussion with the popular buyers of street food in Maseru, which amongst others are taxi drivers, factory workers and some high school students.

The aim of this study was therefore to establish the microbial profile of microorganisms prevalent in street-vended food in Lesotho, Maseru. The specific objectives of the study were to:

- To evaluate the knowledge, attitudes and practices of street food vendors in relation to food safety.
- To identify, characterize and enumerate microorganisms in street vended foods and preparation surfaces.
- To assess the antimicrobial susceptibility profile of organisms isolated from the street vended foods

1.5.1 Expected outcomes

The findings of this study could be able to influence the municipality to improve on the value of street food vending. Through a multidisciplinary partnership, this study is aimed at improving the knowledge of both street food vendors and consumers on the importance of food safety as well as attracting both governmental and non-governmental organizations to contribute funds towards educating street food vendors on proper food handling practices so as to combat the escalating threat of food borne illnesses. Through the improvement of street foods, it is envisaged that the market will be attractive to the consumers and this will have a positive impact on the economy.

1.5.4 Ethical considerations

- Approval letter to conduct the study will be obtained from Maseru City Council
- Consent forms will be signed by the participants
- Right to confidentiality, anonymity and freedom to withdraw shall at all times be observed
- Food samples will be paid for

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CHAPTER 2

Assessment of knowledge, attitudes and practices among street food vendors and consumer perceptions of street food vending in Maseru Lesotho

2.1 ABSTRACT

Street food vendors are those individuals that sell and prepare ready-to-eat foods and beverages in public places. This trade is popular among low income groups since it requires less capital and thus easy to start. The preparation of street vended food is normally under unsatisfactory conditions that may lead to the contamination of food, which renders them unsafe. In this study a descriptive survey was carried out amongst 151 participants (58 food handlers and 93 consumers) through self-administered questionnaire to assess the knowledge, attitudes and practices of food handlers and the experiences of consumers with street vended foods, and an observation checklist. The food handlers' questionnaire was structured into four distinctive sections to collect empirical data on; demographics, knowledge, attitudes and practices. The consumers' questionnaire comprised mostly of open ended questions that allowed them to express their views. The majority of food handlers were females (n=35, 60%) and males (n=23, 40%). About 40% the participants had undergone food safety training and 3% had no formal education background. On average the vendor population that participated in this study was considered to have poor knowledge of food safety (49%±11). Demographic characteristics had no effect on the food handlers' knowledge (gender, p=0.3281, age, p=0.3956, education level, p=0.1638), except for food safety training where the p value was 0.0040, showing that food safety training did impact on knowledge. With regard to the consumers, 63% were males and 37% were females, and only 6% reported that they never buy street vended foods.

Key words: Street food vendors, consumers, knowledge, attitudes and practices.

2.2 INTRODUCTION

Food borne illnesses have devastating effects on public health and also impact negatively on the socioeconomic status of countries globally. Currently, food poisoning is still a persisting major concern and global phenomenon that results in morbidity and significant mortality (Chukwuocha, 2009). It has been reported that people who indulge in the consumption of street foods sometimes suffer from food borne diseases such as cholera, diarrhea, typhoid fever and gastroenteritis (Rane, 2011). Contamination of food by various pathogens is facilitated by various factors including temperature, storage and relative humidity surrounding the food (Bhat *et al.,* 2012). It is in this regard that hygiene is a critical component to observe during food handling and storage.

Unhygienic practices during food preparation and storage generates conditions that facilitates the growth and transmission of food-borne pathogens and other microorganisms which may cause food poisoning (Angelilo *et al*., 2000). Moreover, the majority of food borne diseases and outbreaks have been attributed to infected food handlers coupled with unhygienic food handling practices (Akabanda *et al*., 2017). In order to counteract this problem, the government of Lesotho requires that food handlers be tested for any contagious diseases before they are allowed to work on food premises. However, food handlers can also assist in the control of food poisoning incidences through good levels of knowledge, attitudes and practices since they are directly involved in food handling (Angelilo *et al*., 2000).

Knowledge has been described by (Thanh, 2016) as “a complex process of remembering, relating, or judging an idea or abstract phenomenon”. To further elaborate, (Nee and Sani, 2011), stated that a general consensus was reached by authors that good levels of food safety knowledge among food handlers and the application of such knowledge effectively into practice is invaluable to ensure safe food production in any food establishment. Additionally it has been stated by (Glanz *et al.,* 2008), that knowledge is gained through learning practices which may either be formal or informal, individual experiences and knowledge dissemination. It has been a common assumption that behaviour emanates from knowledge (Campbell, 2011). Behaviour is also influenced by the following factors as depicted in Table 2.1, of which are partly contributed to by knowledge.

Table 2.1 Level of influence involved in human behavior

Individual factors	Knowledge, attitudes, beliefs, personality
Interpersonal factors	Social identity, support, roles
Institutional factors	Rules, regulations, informal structures
Community factors	Social networks, norms, culture
Public policy	Laws/regulations

Source: (Hind, 2015)

Numerous studies have shown that food workers' improper food handling practices are a leading cause for food borne illness outbreaks in food establishments, thus making human behavior an important component of food safety (Green, 2008). The behaviour model designed by psychologists suggests that as knowledge accumulates, attitudes change and that the change in attitudes promotes behavior changes. Therefore the hidden assumption in this model is that with increased knowledge, one also acquires skills to change their behaviour (Aunger *et al*., 2016).

According to Jain, (2014) and Campbell, (2011) attitudes involve the evaluation concepts associated with the way people think, feel and behave. Additionally it comprises of the cognitive-consistency theories, learning theories, functional theories and social judgment theories which basically imply what you know, how you feel and what you do (Simonson and Maushak, 2001). Research has shown that people who seek to have consistency in their attitudes also seek consistent correlation between their attitudes and their behaviour, and that whenever an inconsistency occurs, corrective action is taken to rectify such or some forces are initiated to return the individual to the state of equilibrium (Jain, 2014). Despite the usefulness of the KAP survey, some social scientists have argued that on its own, the KAP survey may not be enough to depict a holistic view of what is being investigated (Launiala, 2009). It is however advised that it be coupled with other in-depth information gathering tools using qualitative methods (Campbell, 2011 and Bulmer and Warwick, 1993).

On a continuous base, food handlers are provided with food safety interventions that increase their knowledge, and this is done with the hope that knowledge will automatically translate into practice (Green, 2008). This notion is however refuted in other behavioral studies, including food safety, it is believed that knowledge does not automatically prompt a certain behavior even though knowledge is a necessary component of behavior (Rennie, 1995). However, Thanh, (2015), in his study expressed that in street food vending poor knowledge and attitudes contributes to the increased chances of finding food borne pathogens in food and preparation surface thereof. In all food establishments, food handlers are encouraged to observe personal, food and environmental hygiene at all stages of food preparation in order to prevent the contamination of food contact/preparation surfaces and the food itself. This practice must occur up to the last stage wherein food reaches the hands of the consumer and these stages are portrayed in Figure 2.1.

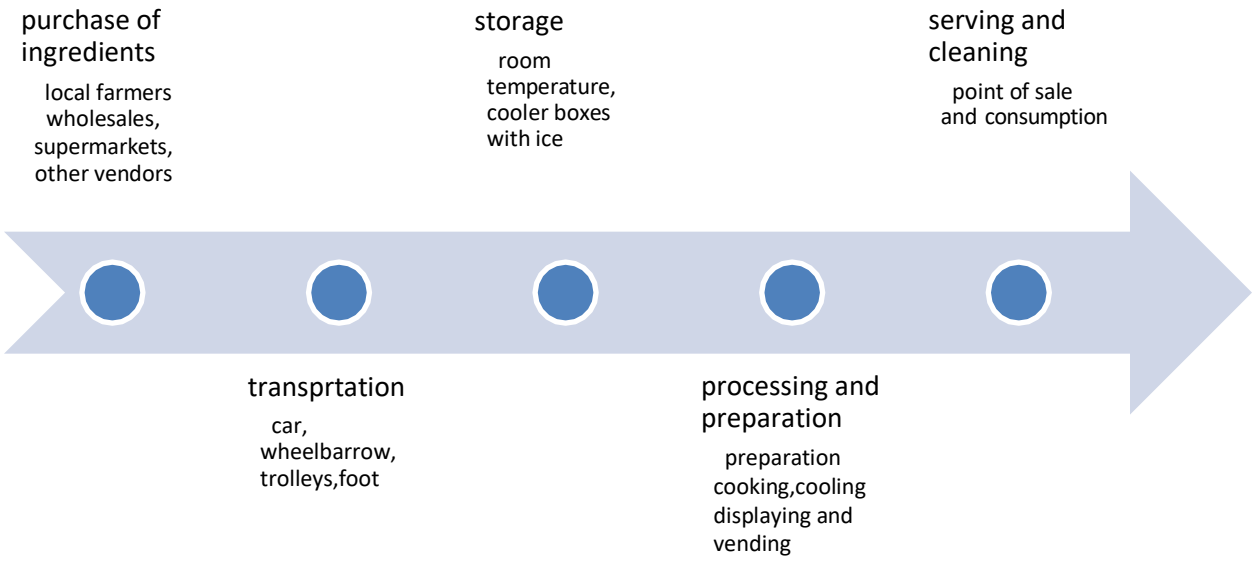

Figure 2.1 Stages of food production in street food vending (Cortese *et al*., 2016)

Even though street vended foods carry some economic and nutritional benefits, there are also detrimental effects on the health of consumers if not hygienically prepared and served (Pannu *et al*., 2016). The same author further emphasizes that consumers are sometimes unaware of the critical role they play in the prevention of food borne diseases and often overlook the incidence of food borne disease and the devastating consequences thereof.

Continuous studies have been conducted globally on food safety knowledge, attitudes and practices of food handlers, but there is not much information regarding the food safety knowledge, attitudes and practices of consumers (Samapundo *et al*., 2014).

2.2.1 Food poisoning

Foodborne illnesses are a growing public health problem globally (WHO, 2015; Conlon, 2017). They are further defined as diseases either infectious or toxic in nature, caused by agents that enter the body through ingestion of food or water (CDC, 2018). It is therefore imperative that studies and improvements are made on food safety because on a daily basis people are exposed to microbial and chemical contamination from the food they consume (WHO, 2015). A review by Whitworth, (2015), highlighted the fact that 600 million or 1 in 10 people per annum contract foodborne illnesses, and 420 000 of these people die, including 125 000 children under the age of 5. The WHO Africa and South-East Asia regions are said to have the highest burden of foodborne diseases (WHO, 2016). In low-and middle-income countries the risk of foodborne diseases is quite high, they are mostly associated with preparing food with unsafe water, poor hygiene and inadequate conditions in food productions and storage, lower levels of

literacy and education, and insufficient food safety legislation or implementation of such legislation (WHO, 2016).

According to the estimates of WHO (2015), food-and-water borne outbreaks kill almost 2.2 million people per annum globally. Even though the majority of foodborne illnesses resolve themselves, complications such as severe dehydration, lactose intolerance, kidney failure, reactive arthritis, brain and neural disorders, cancer and death eventually are common (WHO, 2013). It is therefore imperative that food safety hazards are identified and controlled through the implementation of food safety management programs. Hence the current study sought to document information on knowledge, attitudes and practices of street food handlers

2.3 MATERIALS AND METHODS

2.3.1 Study design

The study design was descriptive survey and concurrent microbial analysis.

The knowledge, attitudes and practices of food handlers in all the market areas around town in Maseru were assessed, along with the food handling and hygiene practices using a questionnaire survey.

2.3.2 Sample collection

The data was collected in a space of a month, in August 2017 and a total of 151 questionnaires were administered to 93 consumers whilst 58 were given to food handlers.

2.3.3 Food safety knowledge, attitudes and practices questionnaire for food handlers

The questionnaire used in this study was derived from one that has been used in previous studies (Akabanda *et al.,* 2017; Thanh, 2015). The questionnaire was designed to analyze the three aspects namely: knowledge, attitudes and practices of the street food handlers. For that matter the questionnaire was divided into four sections of which the fourth section was about the demographic characteristics. All questionnaires were administered in Sesotho language and were completed in the presence of the researcher.

The demographic characteristics part of the questionnaire included age definition, gender, education level and food safety training. The food safety knowledge questionnaire was aimed at finding out the food handler's knowledge of food pathogens, good hygiene and food handling practices, high risk groups and food-borne illnesses. This section comprised of 18 questions from which food handlers will be scored out of, and the questions had three possible answers being "yes", "no" and "don't know". Each correct response was awarded one point while zero points were awarded for an incorrect or "don't know" response. For a person to score a 100% they should've answered all 18 questions correctly. Food handlers with any score below 50% were considered to have poor knowledge of food safety, whilst those with anything between 50% and 75% were considered to have average knowledge and those with scores above 75% were said to have good knowledge of food safety. The same strategy was used for the food safety attitudes questionnaire, which was aimed at seeing how much the food handlers understand about food safety. This part consisted of 16 questions.

For the food safety practices questionnaires, 11 questions were asked in regards to the food handlers' behavior with options of two answers being "yes" and "no" provided. In this section points were awarded on a basis of whether the food handler's actions were correct or not. Of which one point was appointed for good practice and zero points were allocated for bad practice. The score board in terms of percentages was still similar to that of the food safety knowledge and practices sections where anything <50% shows unsatisfactory behavior, 50% - 75% was averagely good behavior and >75% showed good implementation of food safety knowledge.

A different questionnaire was made for assessing the knowledge, attitudes, practices and experiences of consumers with regards to street vended foods. Consumers that were selected are high school and tertiary students, taxi drivers, people who work and live around the street food vending areas.

The vendors were randomly selected in order to avoid bias. No particular order was followed when choosing participants.

2.3.4 KAP checklist

The checklist (Appendix 1) that was used in this study has been useful for other studies (Chukuezi, 2010 and Thanh, 2015). The first part of the checklist is about demographic characteristics, which are location of the stall, age of the vendor, gender of the vendor, education level of the vendor and food safety training. Within the checklist 5 important aspects were evaluated, those being 1) the facilities, 2) environment around the stall, 3) personal hygiene 4) food storage and 5) utensils. The vendors who participated in this

part of the study are the very same vendors who were involved in answering the KAP questionnaires.

2.3.5 Data analysis

Data was captured electronically by the researcher using Microsoft Excel. Any further analysis was done by a statistician using SAS Version 9.2. Descriptive statistics namely frequencies and percentages were calculated for categorical data and means and standard deviations or medians and percentiles were calculated for numerical data. Correlation analysis was used to investigate the relationship between variables. Analytical statistics namely the Shapiro-Wilk Test was used to test for normality, the independent T-test or ANOVA was used to test for mean differences among groups, and the Mann-Whitney U-test or Kruskal-Wallis test was used to test for median differences among groups. A significance level (α) of 0.05 was used.

2.4 RESULTS AND DISCUSSIONS

A discussion document of WHO, (1996) on essential safety requirements for street-vended foods, stated that it is of great importance to develop government guidelines and regulations that are specific to the control and recognition of street foods. In addition, studies of consumer education and purchasing decision, local street food systems and food handler training are important to reduce street foods microbial hazards.

Table 2.2 depicts the demographics of 58 food handlers that participated in the study. The majority of the participants were females (*n*=35, 60%), and males constituted only (*n* = 23, 40%), therefore the majority were females. This is also a case in many other studies e.g. Thanh (2015), Lee *et al*., (2017), Mashuba (2016), Njaya (2014), Nnebue *et al*., (2014) and Campbell (2011). Njaya (2014) indicates that normally there are more women than men in the street food trade because of socio-economic indicators such as scarcity of formal employment and also the immediate need to provide for their children.

The mean age of all the respondents (55) was 36 ±10, however, the other 3 food handlers were unwilling to provide their age and the information was recorded as such. Less than half of the participants (*n*=23, 40%) had food safety training while (*n*=35, 60%) had none. However, a larger percentage (*n*=39, 67%) had high school education, fourteen (24%) had elementary education, three (5%) had higher/tertiary education and only two (3%) were illiterate.

Table 2.2 Demographic characteristics of food handlers

CHARACTERISTICS	*NUMBER*	*MEAN AND STD DEVIATION*
GENDER		
Male	23 (39.66%)	
Female	35 (60.34%)	
AGE		36±10
FOOD SAFETY TRAINING		
Yes	23 (39.66%)	
No	35 (60.34%)	
EDUCATION LEVEL		
Elementary school	14 (24.14%)	
High school	39 (67.24%)	
Higher education	3 (5.1%)	
Illiterate	2 (3.45%)	

2.4.1 Food safety knowledge

The results of food handler knowledge are shown in table 2.3. On average, the vendor population used in this study was considered to have poor food safety knowledge since they scored (49%±11). Some food handlers showed good knowledge of general sanitary practices such as regular hand washing during work (100% correct answers) and proper cleaning and sanitization of utensils (84% correct answers), although only two (3%) understood that washing utensils with detergent does not necessarily render them to be free of contamination. It is critically important that food handlers should have knowledge of important hygiene procedures. More especially in light of the fact that

street food vendor's hands have been proven to be vectors in the spread of foodborne illnesses mainly due to poor hygiene and cross contamination (Aycicek *et al*., 2014 and Lambrechts *et al*., 2014). It has been reported (Assefa *et al*, 2015) that in their study conducted at South West Ethiopia that only 57% of the vendors had good hand washing habits after using the toilet, blowing their nose or touching dirty material and thus highlighting negligence and lack of awareness on the part of the vendors with regards to food contamination sources. Moreover, Akabanda *et al*., (2017) states that washing hands should be coupled with proper use of gloves in order to minimize chances of contamination. In this study, 95% of the vendors failed to choose the correct response for the question about high risk groups in regards to foodborne diseases transmission. That is, 98% of the food handlers did not know or could not remember that *Salmonella,* Hepatitis A and *Staphylococcus*, are among foodborne pathogens. However about 64% of participants claimed to know that typhoid fever could be transmitted by food and it was also the same for those who said HIV/AIDS could also be transmitted by food. This indeed is of great concern since HIV/AIDS transmission mode is well known.

On the other hand a moderate (69%) of the respondents mentioned that bloody diarrhea could be transmitted by food. The results of this study are in agreement with other studies (Ansari-Lari *et al*., 2010, Soares *et al.,* 2012 and Akabanda *et al.,* 2017) wherein majority of the respondents did not know if *Salmonella,* Hepatitis A and *Staphylococcus* are among foodborne pathogens. The majority of the vendors (91%) were aware of the microbial risks associated with swollen cans while only (9%) didn't know of the associated microbial threat. In addition, only (41%) of the vendors knew that microbes can be found on the skin, nose and mouth of healthy looking individuals while the

majority (95%) agreed that a leave of absence is necessary during an infectious disease of the skin. Infectious diseases such as sores and boils for example may harbor microorganisms that can be transmitted to food and cause illnesses to the next person that consumes them.

Table 2.3 Food safety knowledge

QUESTION	***RESPONSE (%)***		
	CORRECT	*INCORRECT*	*DON'T KNOW*
Washing hands before work reduces the risk of food contamination	58(100%)	0(0%)	0(0%)
Using gloves while handling food reduces the risk of food contamination	28(48%)	11(19%)	19(32%)
Proper cleaning and sanitation of utensils increases the risk of contamination	49(84%)	7(12%)	2(3%)
Eating and drinking during food preparation increases the risk of food contamination	30(52%)	20(34%)	8(14%)
Preparing food in advance reduces the risk of food contamination	36(62%)	12(21%)	10(17%)
Reheating cooked food can contribute to food contamination	14(24%)	33(57%)	11(19%)
Washing utensils with detergent leaves them free of contamination	2(3%)	55(95%)	1(2%)
Children, healthy adults, pregnant women and the elderly are at equal risk for food contamination	3(5%)	50(86%)	5(9%)
Typhoid fever can be transmitted by food	37(64%)	10(17%)	11(19%)
HIV/AIDS can be transmitted by food	37(64%)	13(22%)	8(14%)
Bloody diarrhea can be transmitted by food	40(74%)	9(16%)	9(16%)
Abortion in pregnant women can be induced by foodborne diseases	43(74%)	2(3%)	13(22%)
Salmonella is among foodborne pathogens	1(2%)	0(0%)	57(98%)
Hepatitis A is among foodborne pathogens	2(3%)	1(2%)	55(95%)
Staphylococcus is among foodborne pathogens	1(2%)	0(0%)	57(98%)
Can swollen cans contain microorganisms	53(91%)	0(0%)	5(9%)
Microbes can be found on the skin, nose and mouth of healthy individuals	24(41%)	13(22%)	21(36%)
During an infectious disease of the skin, it is necessary to take leave from work	55(95%)	2(3%)	1(2%)

Figure 2.2 depicts the frequency of percentages scored by food handlers on the food safety knowledge questionnaire which comprised of 18 questions. It should be noted that no food handler scored below 22% (i.e., ≤3/18) or above 72% (i.e., ≥14/18). Only 24 of the vendors scored below 50% which indicates poor knowledge on food safety, while the rest of the food handlers scored between 50% and 75% which shows that they had average knowledge of food safety concepts. A test for normality was also done using Shapiro-Wilk test and it showed that the distribution of the knowledge percentage follows a normal distribution ($p < 0.1498$) and on average the entire group failed (49% ±11) All percentage scores were rounded off to the nearest whole number.

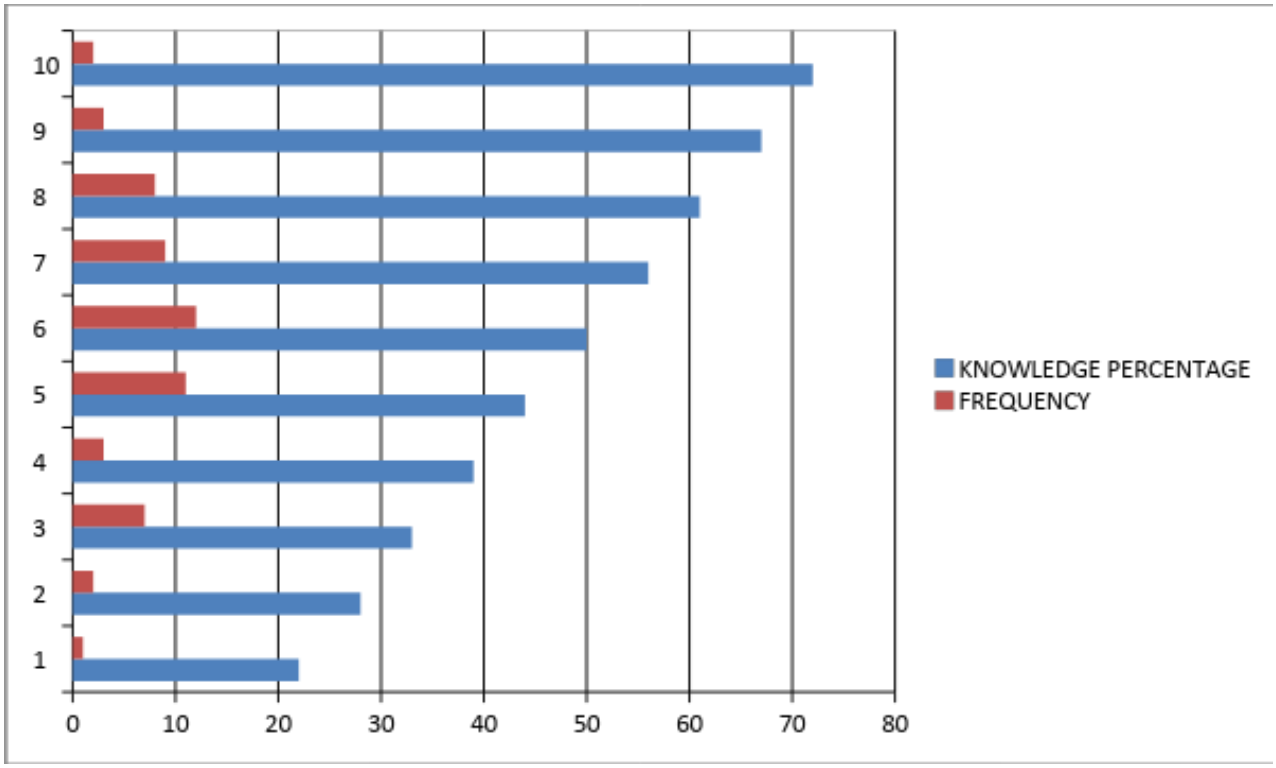

Figure 2.2 Frequency of percentage scores in the food safety questionnaire

The effects of demographic characteristics of the food handlers on their knowledge of food safety are depicted in Table 2.4. The mean values from both genders were compared, males and females reportedly scored (51±13 and 47±10 respectively), p=0.3281, therefore gender did not have any effect on the knowledge of the food handlers with regards to food safety. There was a weak positive correlation (r=0.11684) between age and knowledge, thus age did not have an effect on knowledge (p=0.3956). The mean scores of education level of the food handlers, p=0.1638, also showed that education level had no effect on knowledge of this group. However here was a significant difference with regards to knowledge between he trained and the untrained group (p=0.0040). For the comparison of food safety training means (no= 46±12, yes= 54±9) an independent T-test was employed and p=0.0040. This shows that food safety training did have an effect on the food handlers' knowledge on food safety.

Table 2.4 Effects of education level, gender, food safety training and age on food safety knowledge

CONSTRUCT	*EDUCATION LEVEL*				*GENDER*		*FOOD SAFETY TRAINING*		*AGE*
	Illiterate	***Elementary school***	***high school***	***Higher education***	***Male***	***Female***	***Yes***	***No***	**18-54**
	36±20	48±12	49±11	59±6	51±13	48±10	54±9	46±12	
PEARSON CORRELATION COEFFICIENTS									0.11684
INDEPENDENT T-TEST						0.3281		0.0040	
ANOVA			0.1638						
On the age demographic, the Pearson Correlation Coefficient was reported on									

2.4.2 Food safety attitudes questionnaire

Table 2.5 below shows the results of the street vendor food safety attitudes. About 84% of the food handlers agreed that thoroughly cooked food is free from contamination and 95% agreed that foodborne diseases can be prevented by proper hand hygiene. Proper hand hygiene is important in food safety since hands of food workers are capable of spreading germs that can cause foodborne diseases. With regards to personal hygiene, more than half of the respondents agreed that wearing masks (76%), gloves (67%) and caps (98%) is an important practice that can reduce contamination of food. A study (Akabanda *et al.,* 2017) also showed that 60% of the food handlers indicated that the use of caps, masks and gloves can minimize the risk of contamination. However, only about 55% of the respondents had a positive attitude towards proper storage of cleaning products/chemicals and food products. The results of the study are lower than those obtained by (Kunadu *et al.,* 2016) whereby 93.9% of the respondents had a good attitude towards proper chemical storage. With regard to separating cooked and raw foods, the majority (98%) of the participants agree and the results of this study are higher than those obtained by Kunadu *et al.,* (2016) where it was reported that only 73.4% agreed that raw and cooked foods should be kept separate. The World Health Organization (1996) recommends that stalls should be designed and constructed such in a way that raw and cooked food are always kept separate in order to avoid cross contamination. On the aspect of temperature control, almost all the respondents (95%) stated that it is important to regularly check the temperature of refrigerators/ freezers. Although none of the vendors used refrigerators or even ice boxes, their knowledge on

this important issue remains satisfactory. The United States Department of Agriculture (USDA) advices that food be refrozen only if it was defrosted in the refrigerator or if it was thoroughly cooked first. The street food vendors defrost their meat using hot water, some cold water and some let it thaw at room temperature, therefore refreezing of such meat is unadvised. With regards to the above mentioned issue the majority of food handlers (95%) had an idea on the effect of temperature on food since they agreed that it is necessary to check the temperature of refrigerators/freezers periodically to reduce the risk of food contamination. In Lesotho all food handlers are required by the Department of Health to have their health status evaluated before they are issued with food handler's certificate. This is also the case for countries such as South Africa and Ghana (WIEGO 2014 and FAO 2016). In the current study almost all the respondents (98%) agreed that it is important to have one's health status evaluated prior to employment. Respondents also agreed that individuals with abrasions or cuts on their hands should not touch food without gloves (91%). All the respondents (100%) were mindful of the fact that failure to properly sanitize knives and cutting boards could lead to cross contamination, similarly (97%) showed positive attitude towards cleanliness of dish towel and the role played by dish towels in food contamination. Thus the general attitudes of food handlers towards food safety were satisfactory.

Table 2.5 Food safety attitudes

QUESTION	***RESPONSE(%)***		
	CORRECT	*INCORRECT*	*DON'T KNOW*
Thoroughly cooked food are free from contamination	49(84%)	4(7%)	5(9%)
Proper hand hygiene can prevent foodborne diseases	55(95%)	2(3%)	1(2%)
Can a closed can/jar of cleaning product be stored together with closed cans/jars of food products	32(55%)	22(38%)	4(7%)
Raw and cooked food should be kept separately to reduce the risk for food contamination	57(98%)	1(2%)	0(0%)
It is necessary to check the temperature of refrigerators/freezers periodically to reduce the reduce the risk for food contamination	55(95%)	1(2%)	2(3%)
Defrosted foods can be refrozen	42(72%)	9(16%)	7(12%)
The health status of food handlers should be evaluated before employment	57(98%)	1(2%)	0(0%)
The best way to thaw meat is in a bowl of cold water	42(72%)	14(24%)	2(3%)
Wearing masks is an important practice to reduce the risk for food contamination	44(76%)	9(16%)	5(9%)
Wearing gloves is an important practice to reduce the risk for food contamination	39(67%)	11(19%)	8(14%)
Wearing caps is an important practice to reduce the risk for food contamination	57(98%)	1(2%)	0(0%)
The ideal place to store raw meat in refrigerator is in the bottom shelf	49(84%)	2(3%)	7(12%)
Eggs must be washed after purchase	12(21%)	35(60%)	11(19%)
Dish towels can be a source of contamination	56(97%)	1(2%)	1(2%)
Knives and cutting boards should be properly sanitized to prevent cross contamination	58(100%)	0(0%)	0(0%)
Food handlers who have abrasions or cuts on their hands should not touch food without gloves	53(91%)	3(5%)	2(3%)

The frequency of percentages scored by food handlers on food safety attitudes questionnaire are depicted in Figure 2.3. The results show that none of the participants scored below 50% and two of the participants answered all questions correctly. Forty nine of the respondents (84%) had scores ≥75% which indicates that the respondents had good attitudes towards food safety. Only nine (16%) of the respondents had scores ranging from 50% to 69%.

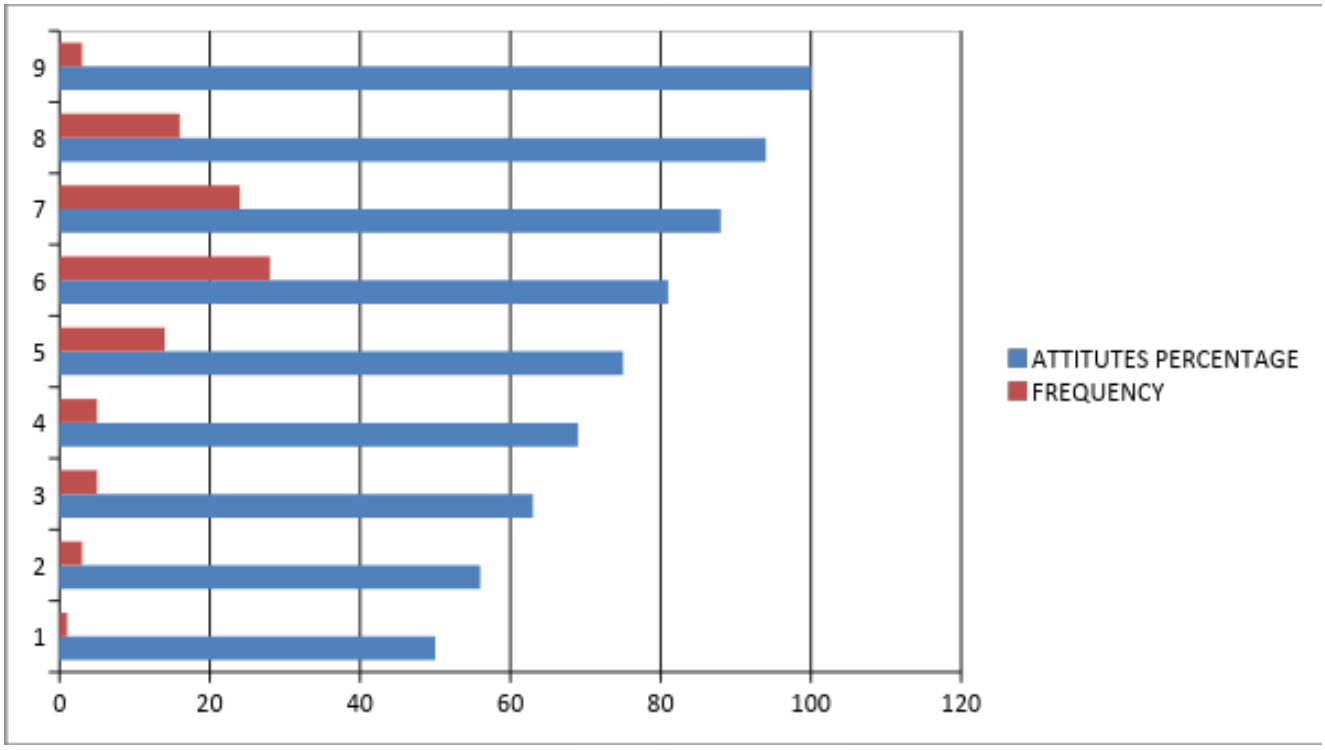

Figure 2.3 Frequency of percentage scores in the food safety attitudes questionnaire

The table below demonstrates the relationship between the demographics of food handlers and their attitudes towards food safety. The results show that there was no relationship between the demographic characteristics and attitudes since the p value of education level, gender, food safety training and age were all above 0.05 (0.5209, 0.3675, 0.5373 and 0.6332 respectively).

Table 2.6 Effects of food safety training, education level, gender and age on food safety attitudes

CONSTRUCT	*FOOD SAFETY TRAINING*		*EDUCATION LEVEL*				*GENDER*		*AGE*
	yes	*no*	*e. school*	*h. school*	*h. education*	*illiterate*	*Female*	*Male*	
MEDIAN	81.25	81.25	81.25	87.5	81.25	68.75	81.25	81.25	
MEAN AND STD DEVIATION									81(±11)
P VALUE	0.5373			0.5209			0.3675		0.6332
There was a weak negative correlation between age and attitudes, r= -0.06579									

2.4.3 Food safety practices

The food safety practices among street food handlers are depicted in Table 2.7. In the assessment of food safety practices, all street vendors (100%) reported that they did not use gloves when distributing unwrapped food. A substantial number of the vendors (64%) stated that they use aprons while working while only (9%) use masks only when necessary. About 50%of the vendors reported that they eat and drink during working hours. Additionally (25%) of the vendors admitted to wearing nail polish while handling food, and 58 (100%) reported that they use sanitizer for cleaning utensils while none of the respondents reported the use of sanitizer for washing fruits. Almost all the vendors (98%) prepared their food in advance and 79% adequately cleaned storage areas for the safe keeping of new products. About 84% of the vendors stated that they check expiry dates of perishable foods after delivery or before purchase. However it has been reported by Avanza *et al.,* (2000) that good knowledge does not necessarily influence good practice since food vendors usually compromise food safety due to financial burdens. Furthermore, a study (Clayton *et al.,* 2002) mentioned that 63% of the food handlers who had knowledge in food safety did not necessarily show corresponding positive behaviour towards food safety practices.

Table 2.7 Food handler practices

QUESTION	***RESPONSE (%)***	
	YES	*NO*
Do you use gloves during the distribution of unpackaged foods? if not, go to question 3	0(0%)	58(100%)
Do you wash your hands properly before or after using gloves?	0(0%)	0(0%)
Do you wear an apron while working?	37(64%)	21(36%)
Do you wear a mask when you distribute unwrapped foods?	5(9%)	53(91%)
Do you eat or drink during work hours?	29(50%)	29(50%)
Do you wear nail polish when handling food?	14(25%)	43(75%)
Do you prepare meals in advance (i.e., from one shit to another)	57(98%)	1(2%)
Do you properly clean the storage area before storing new products?	46(79%)	12(21%)
Do you use a sanitizer when washing service utensils?	58(100%)	0(0%)
Do you use a sanitizer when washing fruits	0(0%)	58(100%)
Do you check the shelf life of food at the time or delivery/purchase?	49(84%)	9(16%)

2.4.4 Observation checklist

The results of the observation checklist are presented in appendices. The predominant food preparation surface in the stalls of vendors who participated in this study was wooden table (79%), while plastic and zinc/iron tables constituted 2% and 19% respectively. Almost all the vendors (98%) prepared their food at the stalls and only one street vendor (2%) who sells porridge (motoho) and home-made bread (maqebekoane) prepared food from home. This seems to be a common practice and studies (Samapundo *et al*. 2015; Alimi, 2016) also had few vendors who usually prepared their food at home. More than half of the stalls (64%) were not properly constructed, and it could be speculated that this might be due to a combination of lack of funds and lack of proper knowledge. Only twenty one (36%) of the stalls were constructed in a way that afford protection from the sun, dust and winds thus a possible reduction in microbial transmission through dust. About thirty six stalls (62%) harboured rodents and flies, while no animals or pests were observed around the other 22 (38%) stalls. A study, Mjoka and Selepe (2017) also found common presence of animals, insects and rodents and this posed an environmental health risk. About 60% of the vending stalls had access to portable water supply, all the stalls had no adequate hand washing facilities, vendors used buckets or have someone pour water over their hands to wash. With regards to access to adequate waste water and food disposal facilities only 43% of the vendors had access o such facilities. A study (Thanh, 2015) reported that 57% had access to portable water supply.

Almost all the stalls used in this study are in the vicinity taxi and bus ranks, with the exception of one vendor that is situated near the traffic department. The environment around the stalls in the taxi and bus ranks is full of litter and some ranks do not have drainages for waste water disposal and some vendors still prefer to litter even though skip bins are provided. The number of food handlers observed to be washing hands in clean water each time before handling, preparing or serving food was unsatisfactory (21%), however all participants (100%) confirmed that they wash hands each time after using the toilet. The CDC (2016) recommends that hands be washed thoroughly before, during and after food preparation, after using the toilet and also after touching garbage just to mention a few. This was mainly to reduce and prevent disease transmission. Further observations were made on vendor personal hygiene, 41% worked without using an apron and 100% they all handled food with bare hands without ever using gloves, while six individuals (10%) were found to have either long nails or nail polish. This is of concern since studies (Abdalla *et a.,l* 2009), revealed that organisms such as *Salmonella typhi,* non-*typhi salmonella Campylobacter* spp. and *E. coli* can survive on fingertips and hands surfaces, sometimes even after hand washing.

Mensah (2002), states that hygienic aspects of food vendors are cause for concern for food safety officers. Failure to wash hands before, during and after food preparation might lead to food borne illnesses or even further complications (Redmond *et al.,* 2004). There were more vendors who covered their hair (79%) than those who did not. It is deemed necessary that vendors should constantly wash their hands since they handle money and food concurrently and money has the potential to transmit pathogens. The findings of this study show that almost all the vendors (98%) handled money while

serving or preparing food and never washed hands prior to touching food again. Some consumers buy food then have them packed in former packs and polythene bags. It was observed that almost all the vendors (91%) blow air into the polythene bags before packing the food inside. This habit/practice releases microbe into the polythene bag and this may contaminate the food therefore causing illness to the consumer. It was further noticed that 24% of the vendors utilized dirty cloths repeatedly to clean the tables and sometimes chairs and while 23 (87%) did not adequately cover their jewelry. It was sometimes observed during this study that vendors had bad habits of blowing their noses, wiping away sweat with hands or even coughing into hands and then continuing to cook without pausing to wash hands. This act facilitates the possible transmission of microorganisms to the food through the hands. Only two vendors (3%) were observed to be practicing these unhygienic acts, while none were seen to be smoking during food preparation and serving. Furthermore, it has been observed (Mosupye and von Holy, 2001) that vendors used the same knife for chopping vegetables raw meat and poultry and salads on the same surface without cleaning. In this study about 25(43%) of the participants were observed to also use use the same utensils for both raw and cooked foods. Practices such as these enhance cross contamination and should be emphasized when teaching food handlers about food safety. However, (83%) of the participant kept (83%) kept raw food and cooked food separately and 67% kept their food in sealed containers while 33% kept the food openly in the stalls. It has been further observed by Chukuezi (2010) that 71.43% of the vendors stored their food in covered warmers or utensils, and the same was observed in this study. Leaving food uncovered exposes them to dust that possibly contains bacterial cells or spores that

may be detrimental to the consumers' health. All participants in this study did not keep leftover food or previously cooked food in ice boxes, although there has been studies (Odonkor, 2011) that reported that this practice of refrigerating leftover food. This is of great concern since food preparation conditions such as storage potentially contribute to the hazard of street food (CDC, 2017; Odonkor, 2011). A substantial majority (79%) of the vendors kept their utensils uncovered and 59% washed their utensils with clean warm soapy water, while 29% used clean cold soapy water and very few (12%) used dirty water that has been used previously for dishwashing due to lack of water supply. Only 40% of the food handlers cleaned their utensils after serving, while 60% normally left them inside the pots and used them for the next customer serve.

2.4.5 Consumers demographics

Table 11 below shows the demographic characteristics of 93 consumers that participated in the study. In this study there were more males consumer (n=59, 63%) than female consumers (n=34, 37%). The mean age of the consumers was 32±9 and the ages ranged from 18-54. Regarding the occupation of the consumers, there was a wide range of occupations mentioned, with the exception of 17% that was unemployed, 18% that was self-employed, while 8% were not willing to disclose their form of employment. Police officers (24%) and taxi drivers (13%) comprised the majority of consumers.

Table 2.8 Demographic characteristics of street food consumers in Maseru

CHARACTERISTICS	*FREQUENCY (%)*	*MEAN AND STD DEVIATION*
GENDER		
Male	59(63%)	
Female	34(37%)	
AGE		32±9
OCCUPATION		
Civil servant	1(1%)	
Factory worker	2(2%)	
Farmer	1(1%)	
Government employee	1(1%)	
Health officer	3(3%)	
House keeper	4(4%)	
Inspector at taxi rank	1(1%)	
Musician	1(1%)	
Not specified	7(8%)	
Nurse	1(1%)	
Nurse assistant	1(1%)	
Pharmacist	1(1%)	
Police officer	22(24%)	
Self employed	7(8%)	
Stock manager	1(1%)	
Students	4(4%)	
Taxi driver	12(13%)	
Teller	2(2%)	
Travel consultant	1(1%)	
Unemployment	16(17%)	
Vendor	2(2%)	
Volunteer	1(1%)	

2.4.6 Consumers' general questionnaire

Consumers that participated in this study were asked whether they consume or do not consume street vended food and their reasons thereof. Subsequently there was a follow up question for those who consume street vended foods with the goal of understanding their perceptions and experiences with street vended foods. Only a small portion(6%) of the consumers mentioned that they do not eat street vended food mainly due to the hygiene and food safety concerns, and also including not wanting to waste money.

Out of the 87 respondents who mentioned that they do eat street vended food, (74%) highlighted that this is once in a while and 26% said they consume the food daily. When asked to state reasons for preference of street vended foods (54%) mentioned that they are cheap and affordable as compared to restaurants, while (15%) said it is because they are readily available and 13% said it is because they are sold closer to their work place. Failure to observe good hygiene and proper food handling practices could lead to contamination of food and consequently illness of the consumers. In this study 11% of the respondents confirmed that they have been previously ill from contaminated street foods, and suffered symptoms such as abdominal pains (50%), diarrhea (40%), allergic reactions (20%) and vomiting (10%). No incidences of food poisoning had been reported to the relevant authorities despite 25% of the consumers having confirmed that they knew where such reports could be made. Of the ten (11%) of the respondents that became ill from eating street foods, 80% said they did not report these incidences because they did not know where to report and 20% mentioned that they did not report because of lack of action from the relevant authorities. Consumers were also asked to state if the street food vendors are seen to be regularly using personal protective equipment/clothing such as masks, hair nets/caps and plastic gloves. About 13% said yes for use of masks, 50% said yes to the use of hair nets/caps and 14% said yes to the use of plastic gloves. With regard to the question on worker's personal hygiene practices, 53% confirmed that they have seen the food handlers wash their hands before and after handling food. All the respondents (100%) agreed that personal hygiene in food establishments is important for the prevention of food contamination,

and they all agreed that there are improvements that need to be done in street food vending in order to help the sector grow further and these are outlined in Figure2.4.

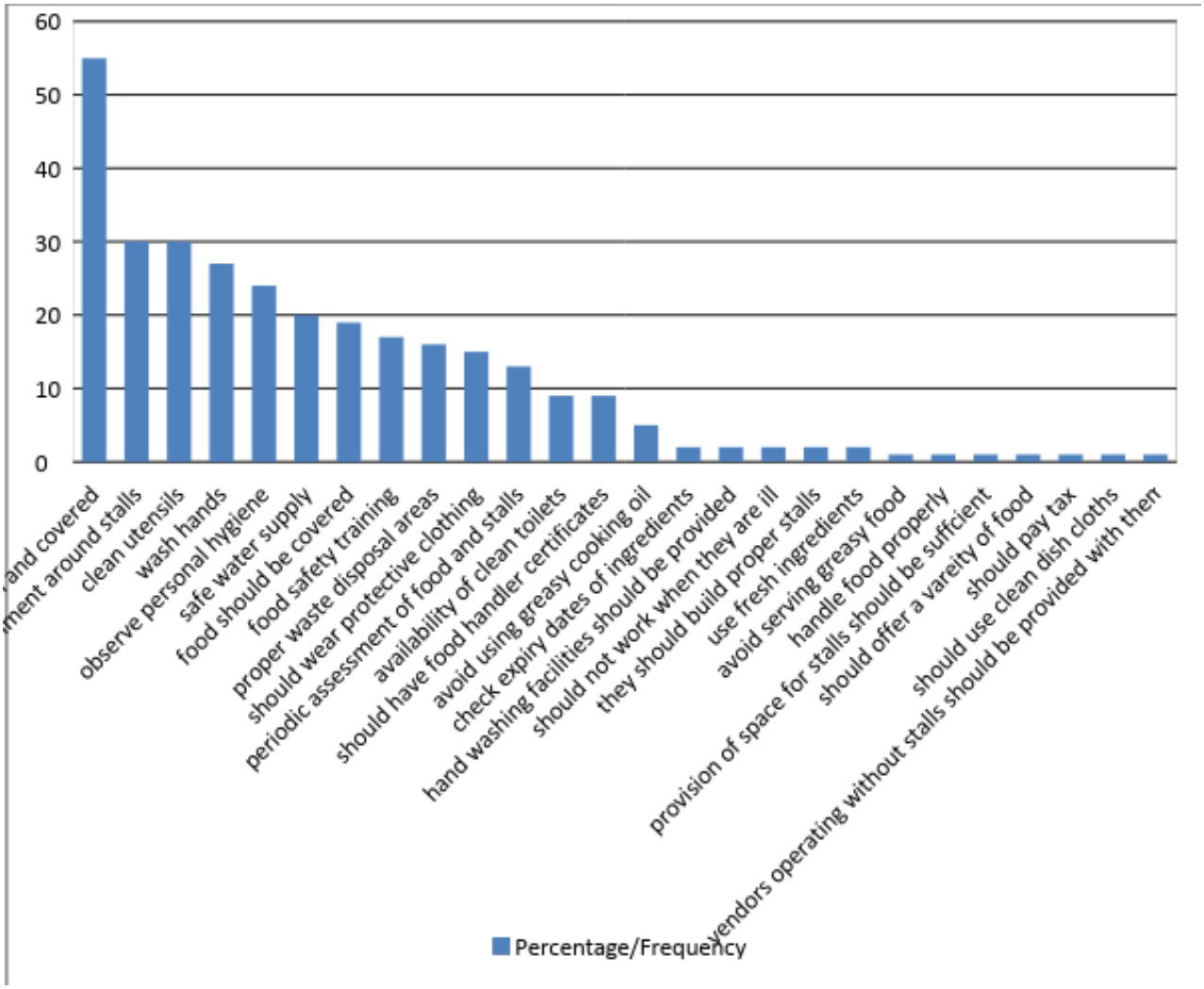

Figure 2.4 Consumer suggestions toward street food vending improvements

NOTE: It should be noted that the sum of the frequency percentages is higher than 100%, this is due the fact that one person could give more than one suggestion

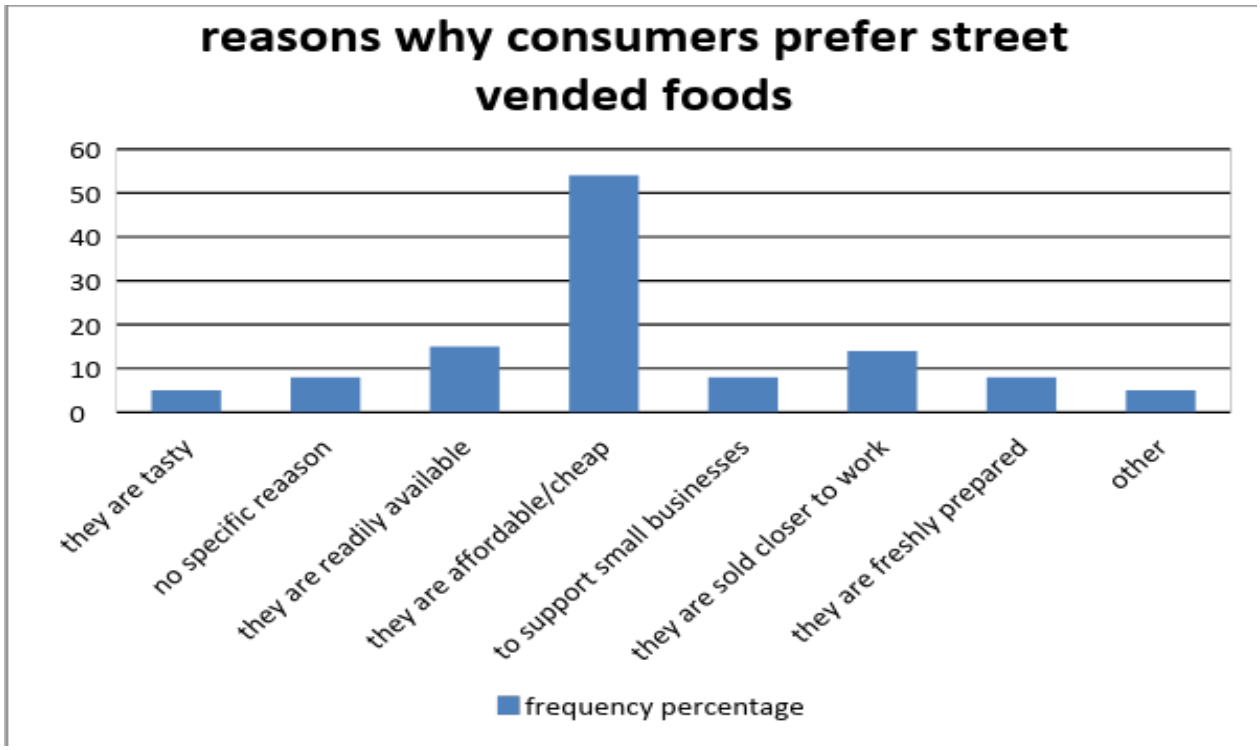

Figure 2.5 reasons given by participants for why they prefer street foods over restaurant food

2.5 CONCLUSIONS

In this study, the following recommendations were made for the improvement of hygiene, prevention of foodborne illnesses and the enhancement of food vendor effectiveness: a) local government entities such as the municipality should offer support in terms of infrastructure (construction and allocation of proper stalls, construction of sewage pipelines for disposal of waste water, construction of toilets with adequate hand washing facilities), collection of waste and provision of waste bins/skips and safe and clean water supply; b) street vendor should be provided with food safety training and should be encouraged to avoid preparing food from home, the reason for this is that food prepare long before it is served runs the risk of bacterial manifestation if not adequately stored, which is often the case for street vendors in Maseru since the lack refrigeration facilities; c) food handler certificates, which are a requisition in Lesotho for any persons intending to work in any food establishment should be acquired by all street food handlers before they are allocated a stall or allowed to operate since the tests conducted before the issuance of this certificate help identify those persons with illnesses such as Hepatitis A that maybe transmitted to food and treatment is undertaken before working at any such food establishment; d) street vendors should be advised against working individually in stalls, but rather in pairs in order to be more efficient . That is, if one of them is focused on the cleaning of the stall and utensils/equipment, the other may be focused on food preparation and serving hence minimizing the chances of cross contamination; e) from the results obtained, all vendors reported that they did not use gloves when handling food. The use of protective clothing (clean clothes, aprons and gloves) should be immensely emphasized to the vendors. .

Akabanda *et al*., (2014) instigated that the proper use of gloves coupled with hand washing when handling food positively contributes towards the prevention of cross contamination. Food handlers should further be advised to always wash their gloves adequately and replace them whenever necessary for them to actually serve their intended purpose. The implementation of all these recommendations is imperative to ensure proper food, personal and environmental hygiene practices which will in turn prevent food borne illnesses

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CHAPTER 3

Assessment of bioburden of food samples, food preparation surfaces and food handler hands

3.1 ABSTRACT

The intake of food contaminated with considerable amounts of pathogenic microorganisms is one of the causes of death around the world, and people acquire foodborne diseases annually. When food is prepared in a large scale or by more than one person, which is mostly the case in the street food vending trade, chances of microbial contamination are increased. The aim of this study was to assess the general hygiene status of food preparation surfaces and food handler hands and the prevailing microbiota associated with street foods of Maseru Lesotho. In this study Rodac contact plates with PCA were used for collecting samples from food handlers' hands and tables. Food samples were aseptically collected using sealable stomacher bags and subsequently transported back to the laboratory for further analysis. The average TVC (Total Viable Count) enumerated for hands and tables were as follows: left hand- 10.64cfu/cm^2, right hand- 10.40cfu/cm^2 and tables- 12.13cfu/cm^2. In food samples, the average values for moroho were as follows: TVC- 8.06×10^9, presumptive *E.coli*- 3.33×10^6, presumptive Salmonella sp.- 2.76×10^7, presumptive pseudomonas sp.- 1.36×10^7, while those of chicken were : TVC- 2.86×10^5, presumptive *E.coli*- 3.43×10^4, presumptive Salmonella sp.- 2.05×10^6 and presumptive pseudomonas sp.- 5.88×10^5. The following organisms were found to be present in both moroho and chicken samples using RapId kits: *E.coli, E.cloacae, C.testosteroni* and *P.flourescens.* These organisms, with the exception of *E.coli*, have rarely been implicated in foodborne outbreaks. The presence of these organisms could likely be influenced by the food handler KAPs, the poor infrastructure of the stalls, the environment around the stalls or the poor sanitation conditions surrounding the whole street food trade in the city. Since some of the

organisms found forms part of the human microbiome, it is recommended that food handlers are medically examined before being given permits to work so as to minimize sources of food contamination.

Keywords: Hygiene, food preparation surfaces, food samples

3.2 INTRODUCTION

Proper food handling and observation of hygiene in street food vending is paramount as it ensures the safety and quality of food produced. The unpremeditated contamination of food might pose a risk on the consumers and economies globally (Ali *et al*., 2017). This is a concern since it has been highlighted by Assefa *et al*., (2014) that hands of food handlers play a major role in the contamination of food. The same study it was revealed that 49.6% of the food handlers had at least one or more potential bacterial pathogens and 31.7% tested positive for enteric pathogens.

3. 2 PREVALANCE OF FOODBORNE MICROBIOTA ASSOCIATED STREET FOODS

Street vended foods are described as ready-to-eat food and beverages prepared and sold on the streets or other similar public places (Rane, 2011). Street food vending is a common practice globally even though it is unregulated in third world countries, it provides solutions for socio-economic shortcomings while also providing affordable ready-to-eat meals (Imathiu, 2017). While street food vending may have certain advantages to the retailer such as increased profits, flexibility, low capital and costs including job creation, it has however been associated with microbial contamination especially in developing countries (Islam *et al*., 2015). The Mountain Kingdom of Lesotho is a country land locked by South Africa and is just 30 000 square kilometers in space with a population estimation of 2.26 million. Population density is low in the highlands, however, Maseru which is the capital city has the highest population density (World Population Reviews, 2018). The increase in population densities in the capital city of Lesotho has led to an increased number of street vendors and also an increase in food sales. This situation is similar in South Africa where an increase in population and migration to cities has resulted in street vendor and food sale increase (Mpeli, 2014).

Street food vending in Lesotho has increased significantly over the years and this trade is now an integral part of the food supply chain. Moreover, the trade has contributed to economies globally, especially in Africa and the development of people's livelihoods (Okojie *et al.,* 2014). A survey conducted by The Lesotho Times, 2017, indicated that vendors have been working in the industry since 1985 but there has been little improvement with regard to their working conditions. Some of the challenges vendors

face include; harassment by national police and city council officials including lack of infrastructure (Leduka *et al.,* 2015). For many years researchers have carried out the assessment of microbiological aspects of food safety, however foodborne diseases continue to over burden even industrialized countries such as the Netherlands with about 700 000 cases of illnesses and 80 deaths being recorded annually (Havelaar *et al.,* 2010). Furthermore, a study by (Al Mamun *et al*., 2013), highlighted that foodborne illnesses that are of microbial origin are a major problem associated with street vended food. A global approach to ensure food safety and security whereby individuals or associations of different discipline work together is therefore important due to the nature of microbes and transmission mode.

The intake of food contaminated with considerable quantities of pathogenic microorganisms represents one of the causes of death around the world, and millions of people encounter foodborne diseases annually (Baghapour *et al*., 2014). Another mode of transmission is cross contamination through food preparation surfaces, this is due to the ability of microorganisms to form biofilms on food contact surfaces (Di Ciccio *et al*., 2015). Food contact surfaces including food handlers' hands have been shown to be vectors of foodborne pathogens in ready-to-eat (R-T-E) food establishment and have led to 97% of foodborne diseases (Lambrechts *et al*., 2014). The former being regarded as potential vehicles of pathogens and mostly found to contribute significantly towards cross-contamination (Niemira *et al*., 2014). Sanitation has been described as the hygienic means of promoting health through prevention of human contact with the hazards of waste including proper disposal of sewage and water. Environmental sanitation entails the actions undertaken for the maintenance of basic environmental

conditions that may be detrimental to the wellbeing of people (Hassan 2015). It has also been emphasized (Okareh 2015), that sanitation and good personal hygiene are important in all food establishments especially in the prevention of foodborne outbreaks which mainly derive from failure to observe satisfactory standards in the preparation, processing, cooking, storing or retailing of foods. Therefore it is imperative that the assessment of sanitation of food contact surfaces in street food vending is properly conducted and this should be on regular basis.

Additionally (Cosby *et al.*, 2008), further highlights that in many instances risk factors for foodborne illnesses have been identified as inadequate cooking, temperature abuse, improper food storage and cross contamination between raw and cooked foods, including food contact surfaces. Therefore it is encouraged that every food establishment, regardless of the size, should provide safe food by adhering to food safety principles. It has been stated by Cusatu *et al*., (2012), that in order to achieve the food safety concept, food safety management plans and HACCP (Hazard Analysis and Critical Control Point) principles should be correctly implemented. The principle of HACCP is that it is a preventive system to guarantee the safety of food for the consumer. It focuses on the prevention of physical, biological and chemical hazards and it is based on seven established principles (FAO, 1997) as outlined in Figure 3.1.

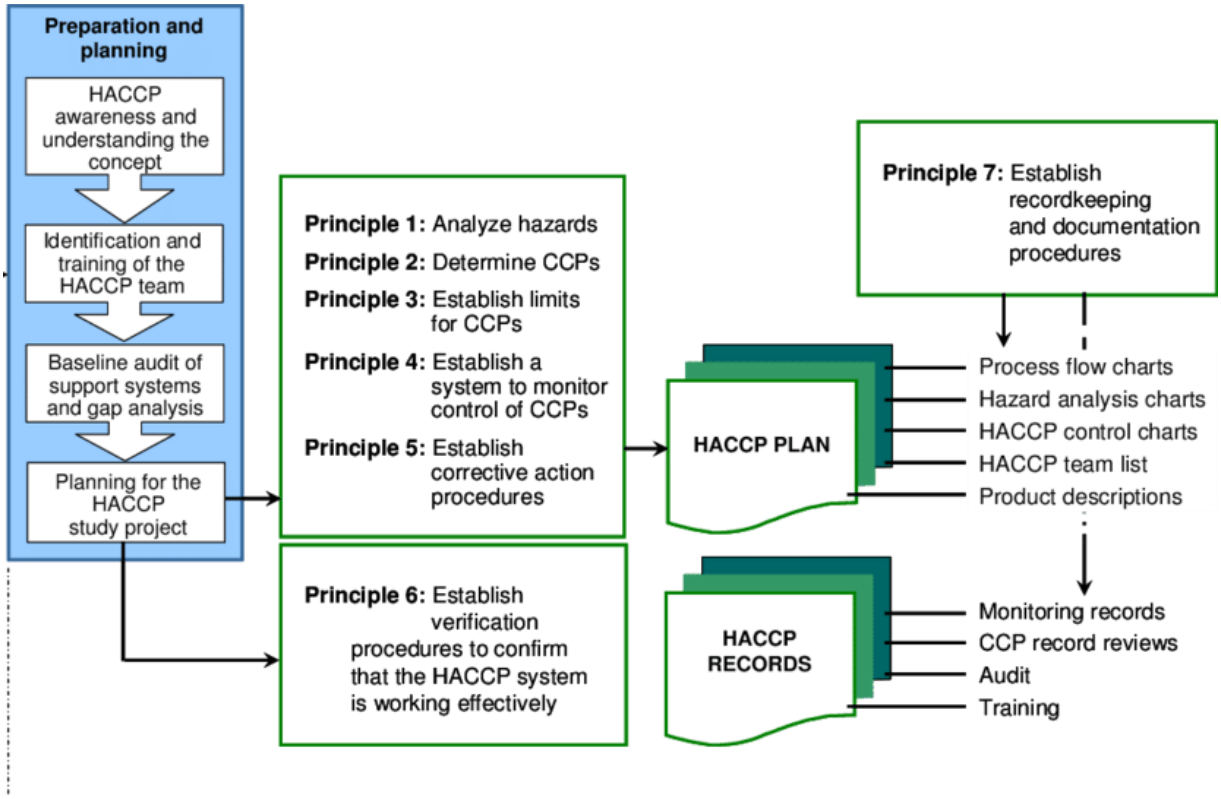

Figure 3.1 The seven principles of HACCP (Mendis *et al.,* 2009)

It is in this light that (CDC, 2006), further recommends that when observing good cleaning practices, disinfection should also be incorporated since cleaning and disinfection are two distinct steps. Disinfection is described by (William 2008) as “a process that eliminates many or all pathogenic microorganisms, except bacterial spores, on inanimate surfaces”. In a food preparation area, both food contact surfaces e.g. knives, cutting boards, utensils, tables, gloves, aprons and non food contact surface areas e.g. floors, walls should be cleaned and disinfected in order to deprive microorganisms and pests of breeding opportunities (Gaulin *et al*., 2011). Effective disinfection of clean contact surfaces is necessary to reduce bacteria to an acceptable

level. Since disease causing bacteria can survive on various food contact surfaces, e.g. cutting boards, utensils, food handler hands and plates, it is advised that the surfaces be adequately sanitized and cleaned with regimen of hot water, detergent and sanitizer (Nhlapho *et al*., 2014).

Cleaning is the first and necessary step before disinfection and the process of cleaning encompasses the removal of all foreign bodies from surfaces, these being organic matter, salts and visible soils (CDC, 2016). The cleaning and sanitization regimen for food contact surfaces is important because these surfaces can be exposed to contamination due to the infrastructure and location of stalls. Additionally, it has been stated by (Kok and Balkaran, 2014) that South African environmental conditions such dusty roads provide ideal conditions that favour bacterial growth. Food handlers have also been implicated in the contribution of conducive environment for microbial growth through unhygienic practices such as failing to properly maintain and keep utensils and equipment in good repair (Abdalla *et al*., 2009). The most common food poisoning microorganisms in street vended food have been identified as *Salmonella*, *Staphylococcus aureus*, *Clostridium perfringens*, *Escherichia coli* and *Bacillus cereus* (Rane 2011).

Temperatures at which street foods are cooked and fried are sometimes insufficient to eliminate all pathogenic microorganisms in food, and even in instances where they are enough, the handling and vending of food by the vendors re-exposes them to contamination (Alimi, 2011). Furthermore it has been highlighted by (Bryan, 1988; Muinde, 2005) that undesirable vending practices such as cutting raw and cooked foods with the same knife and cutting board or even using surfaces that have debris of

previously prepared food without any cleaning in between contributes towards cross-contamination. In another study (Kubheka *et al.,* 2001), it was observed that vendors were cutting meat and vegetables with the same utensils and bare hands without any cleaning being done in between.

Food handlers can contaminate food with their hand through cross contamination and also during or after gastrointestinal infections. Risk factors for hand contamination include toilet use, sneezing into hands, blowing nose or even touching dirty and contaminated surfaces: Hence the need for frequent hand wash in order to prevent the contamination of food. It has been suggested (CDC, 2017), that hands be washed for at least 20 seconds with running water and soap to significantly reduce the total viable count of bacteria on hands before, during and after food preparation. However hand washing has been found to be a challenge for street food vendors due to lack of clean water supply or proper hand washing facilities (Mpeli, 2004). Staphylococci are common bacterial colonizers of the human skin and mucous, with *Staphylococcus epidermidis* and *Staphylococcus aureus* being the most virulent (Otto, 2010). In addition there are enteric pathogens that are part of the transient group of microorganisms, however these can be easily removed by proper hand washing (Assefa, 2015). However in developing countries where running water is scarce, any available water may be used with soap, or alternatively alcohol based sanitizers with at least 60% alcohol (CDC 2016).

3.4 MATERIALS AND METHODS

3.4.1 Study area

The Mountain Kingdom of Lesotho is a country land locked by South Africa and is just 30 000 square kilometers in space with a population estimation of 2.26 million. Population density is however low in the highlands and Maseru which is the capital city has the highest population density (World Population Reviews, 2018).

Figure 3.2 The map of Lesotho depicting all 10 districts including the capital city, Maseru (Lonely Planet 2018).

3.4.2 Study design

The research design consisted of mixed method research comprising both qualitative and quantitative strands.

3.4.3 Sample collection

3.4.3.1 Recovery of pathogens from contact surfaces

Samples were collected from food contact surfaces (tables, n=30) and hands of the food handlers (n=60) using Rodac contact plates.

Contact plates (PCA) were used for the recovery of viable counts from tables and food handler hands. The plates were pressed on to the appropriate surfaces for approximately 10 seconds (Kusumaningrum *et al*., 2003). Samples were transported back to the laboratory on ice pack and subsequently incubated at 37^{o}C for 24hrs. Enumeration of the contact plates was carried out and TVCs were recorded as cfu/cm^{2} for the purpose of recording the bioburden.

3.4.3.2 Recovery of food samples

A total of 30 food samples (moroho=15, chicken=15) were collected from 30 street food vendors. The food samples were collected using the same serving utensils used by the vendors and were separately packaged in sterile bags and transported to the laboratory using cooler boxes and ice packs. For analysis, 10g of each food sample was weighed and homogenized in 90ml of sterile peptone water. Ten fold serial dilutions were conducted using sterile peptone water and aliquots of 0.1ml were cultured on PCA and different selective media using the easy spiral automated plater (Interscience) and incubated at the appropriate temperature. Selective media were as follows: VRB MUG, BGA, PA supplemented with glycerolkk. The isolated colonies were enumerated using a

Scan 1200® Automated Colony Counter (Interscience). RapId and API tests were conducted for further identification of isolates.

3.5 RESULTS AND DISCUSSIONS

3.5.1 Total Viable Count (food handler hands and tables)

Total viable count is an important index used in the quality and safety assessment of contact surfaces (Huang *et al.,* 2013). It is not a count for a specific microorganism but rather a test that gives an estimate of the total living microorganism such as bacteria, moulds and yeast species on the sample surface. According to the results of this study, 37% of all sampled surfaces were TNTC (counted as 300 colonies), meaning the sampled surfaces were not clean hence the high values of viable organisms. A study (Nhlapho, 2014) obtained much higher counts whereby 80% of the sampled surfaces were TNTC. The highest count obtained in this study was 18.25 cfu/cm^2 while other surfaces had no detectable viable microorganisms using the current method. The average TVC recorded for tables and food handler hands were as follows; left hand- 7.21cfu/cm^2, right hand- 6.82cfu/cm^2 and tables- 6.89cfu/cm^2. The high contamaination of food handler hands and surfaces that food come into contact with risks the transmission of potentially harmful microorganisms to food and consequent foodborne illnesses if such food is consumed. Furthermore, Hassan *et al.,* (2018) mentioned that with the persisting problem of food handler hands and food contact surface contamination and failure to observe good hygiene, messages of health education on food safety and hygiene need to be intensively and properly communicated to food

handlers. According to legislation, the limit for TVC on food contact surfaces is 100cfu. In this study 61% of the surfaces had counts exceeding the set limits.

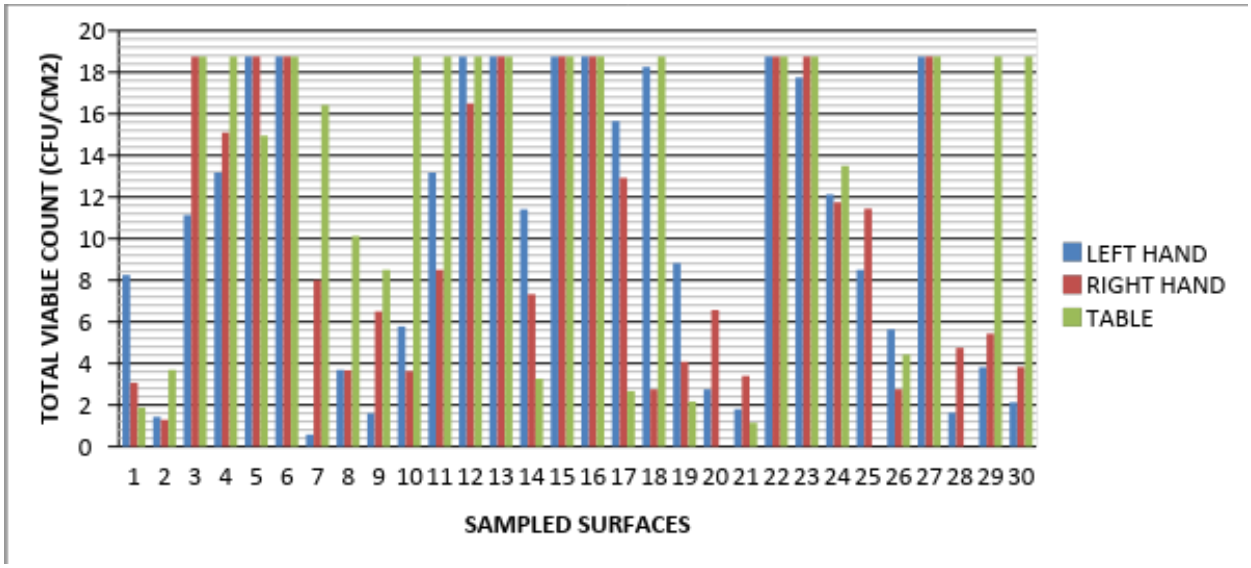

Figure 3.3 Comparison of three sampled food contact surfaces (TVC)

3.5.2 Microbiological analysis of street vended foods

As previously mentioned, in this study a total of 30 food samples (moroho=15, chicken=15) were analyzed to determine the microbiota present.

Total Viable Count

The rationale behind the measure of TVC is to assess the prevalence of microorganisms in a specified sample, in order to determine the general hygiene status thereof**.** Figure 3.4 (A & B) represents the TVC of both chicken and vegetables (moroho). The results show high microbial counts for vegetables (10.91 Log_{10} cfu/ml) than chicken (5.45 Log_{10} cfu/ml). This is higher than the results of a study by Badrie *et al.,* (2004) in Trinidad, West Indies where a mean of 4.1 Log_{10} cfu/g was found in burger

patties. These results were expected as the lack of adequate and clean water supply could be a contributing factor to whether the vegetables are adequately washed before preparation or not.

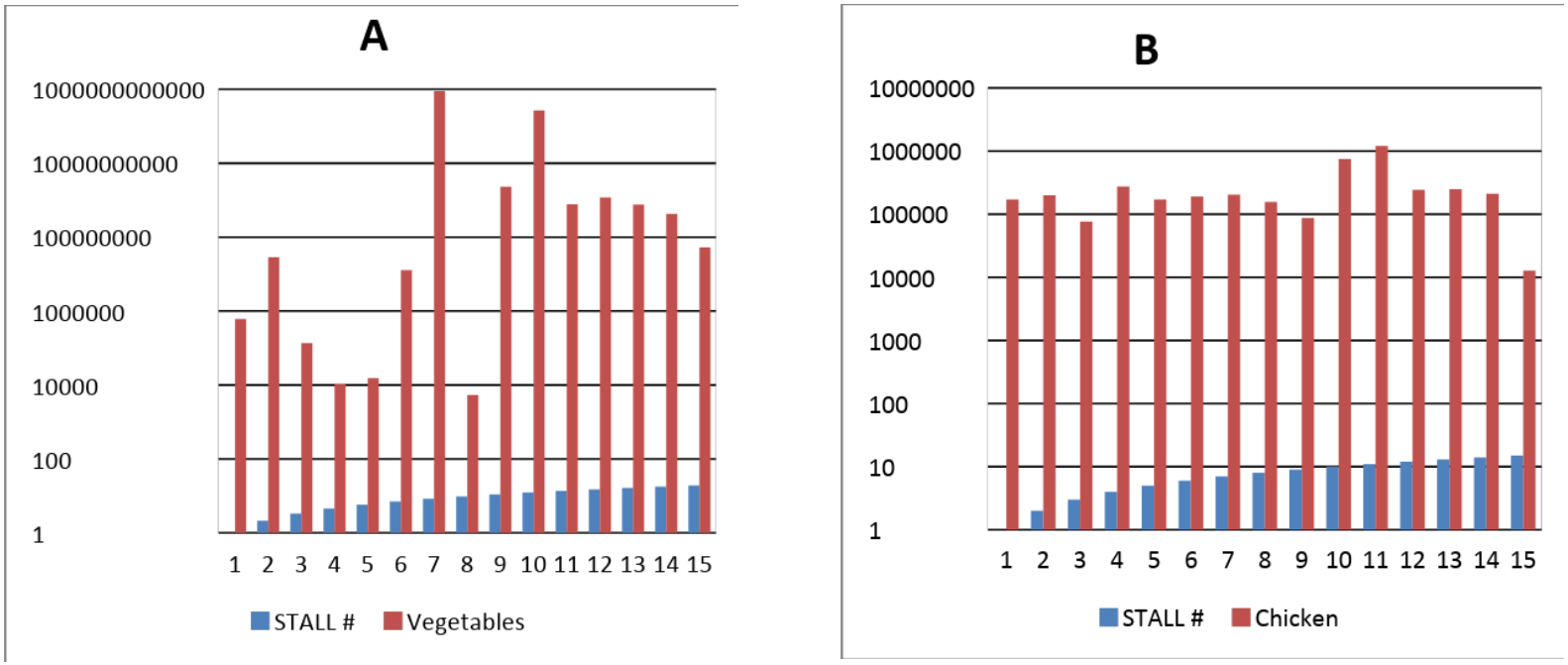

Figure 3.4 Total Viable Count obtained from vegetables (moroho) and chicken samples

Presumptive *Escherichia coli*

E.coli is one of the species belonging to the Coliform group of bacteria and is usually found in the lower intestines of warm blooded animals (Merck 2018). This organism is normally used as an indicator organism for faecal contamination in food and water (Odonkor *et al*., 2013). According to (Gerba 2009; Nhlapho 2014) pathogens may be found in faeces at concentrations of 10^4 to 10^{11} per gram, which leads to the conclusion that the contamination of samples with even just a tenth of a milligram of faeces may encompass a million of infectious bacterial cells. Figure 3.5 (A & B) depicts the microbial counts obtained from both vegetable and chicken samples. Results obtained were in a range of not detectable to 4.90×10^7 Log_{10} cfu/ml for vegetables and undetectable to 2.30×10^7 Log_{10} cfu/ml for chicken. Some studies by (Eromo *et al*., 2016; Bereda *et al*., 2016), showed *E. coli* to be the most predominant microorganisms isolated from food samples in a range of 29.6% -51.5% respectively. The infectious dose of this organism is relatively low (10^1-10^2/g) and these low doses usually cause illness in children, the elderly and the immunocompromised persons (Republic of South Africa 2001).

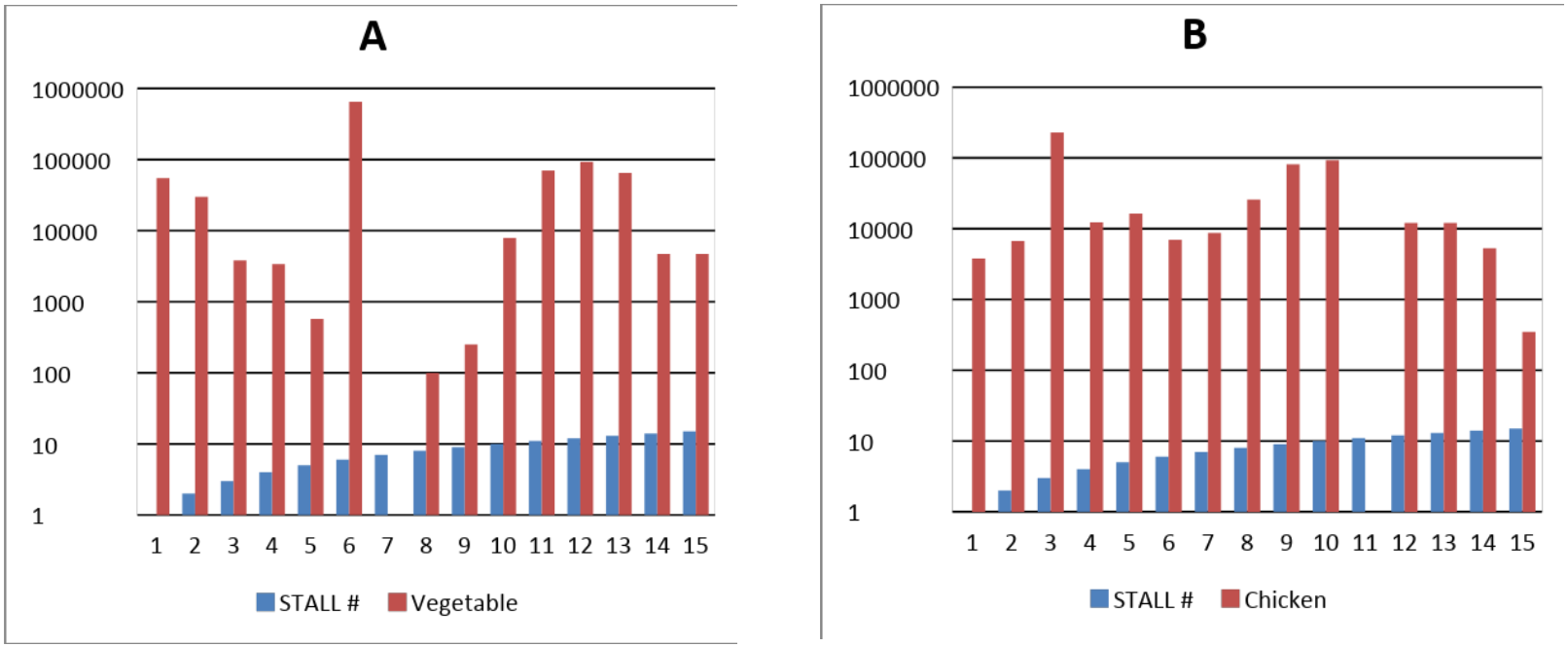

Figure 3.5 Counts of presumptive *Escherichia coli* obtained from moroho and chicken samples

Presumptive *Salmonella* spp.

Salmonellosis, a result of infection with *Salmonella* spp from food and water, is a common cause of mortality and morbidity globally (Siala *et al*., 2017 and Malorny *et al*., 2008). *Salmonella* is categorized into two, non-typhoidal and typhoidal, and they are both found in humans hence they can be easily transferred to food during preparation (MarlerClerk 2018). Figure 3.6 (A & B) depicts presumptive *Salmonella* spp. counts. These were found to be in a range of 3.60×10^{3} Log_{10} cfu/ml -1.30×10^{8} Log_{10} cfu/ml and 2.18×10^{0} Log_{10} cfu/ml -2.22×10^{7} Log_{10} cfu/ml for both vegetables and chicken respectively and they are above the levels that are normally necessary to cause salmonellosis (10^{7}-10^{9} cells/g) (Republic of South Africa 2001).

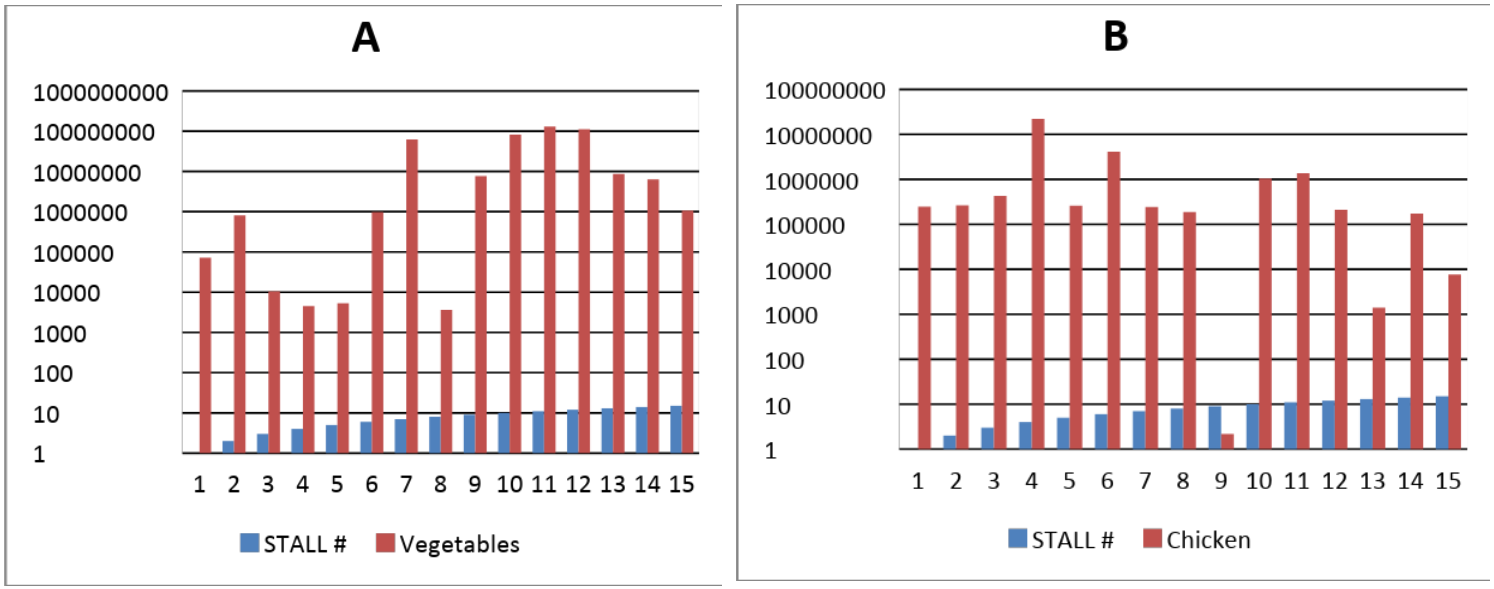

Figure 3.6 Counts of presumptive *Salmonella* sp. recovered from moroho and chicken samples

Presumptive *Pseudomonas* spp.

Pseudomonas spp. can cause spoilage in food and cause infection in various risk groups such as children, the elderly and cystic fibrosis patients (Hargreaves 2014). *Pseudomonas* spp. also has the ability to grow and cause spoilage even in temperatures as low as 4ºC (Dogan *et al.,* 2003). *Pseudomonas aeruginosa* specifically has been implicated in acute ventilator-associated pneumonia and chronic lung infections (Goldberg 2010). Figure 3.7 (A & B) represents the results of presumptive *Pseudomonas* spp. and results range from 1.10×10^{4} Log_{10} cfu/ml -7.50×10^{6} Log_{10} cfu/ml and 9.60×10^{3} Log_{10} cfu/ml -4.90×10^{6} Log_{10} cfu/ml for both vegetables and chicken respectively.

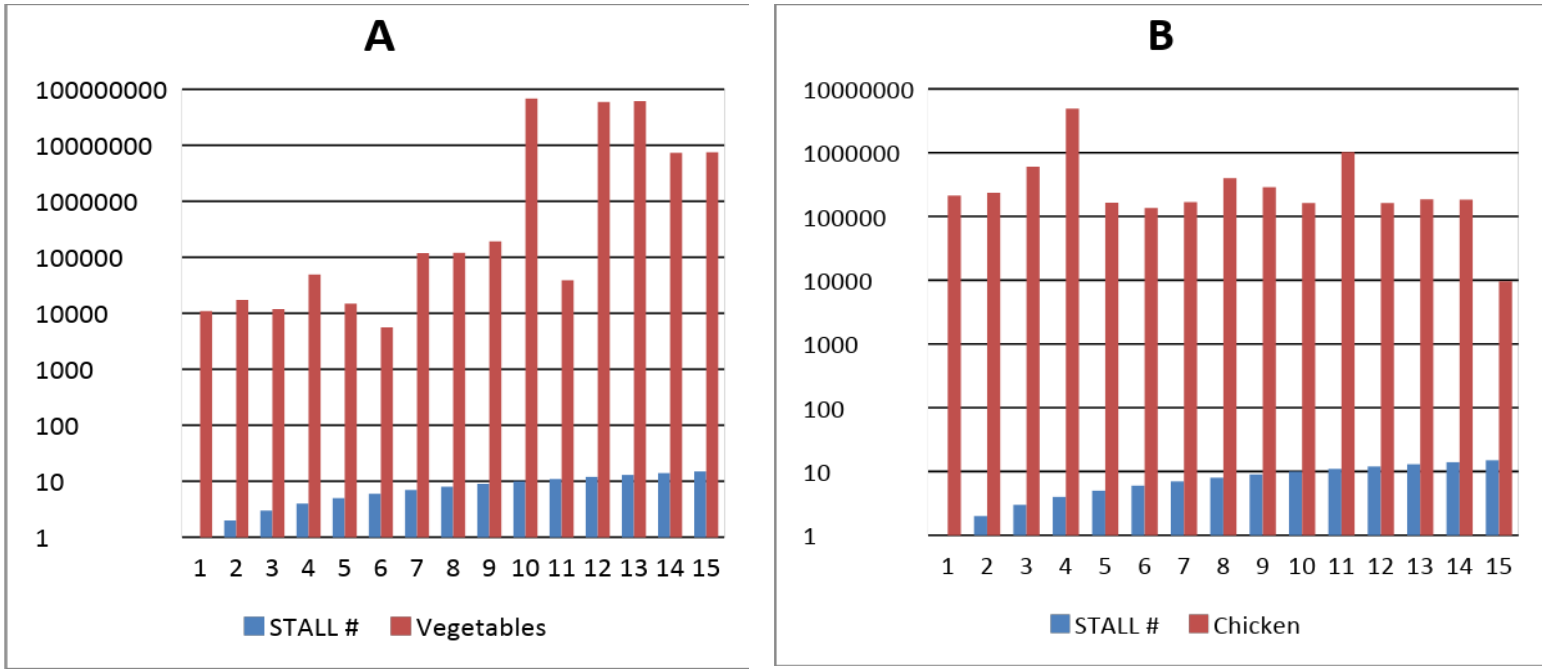

Figure 3.7 Counts of presumptive *Pseudomonas* sp. obtained from moroho and chicken samples

Isolated microorganisms

In food establishments, contamination and recontamination of food before and after preparation could occur from various sources such as the preparation surfaces, hands of the food handlers, utensils or even the air if food is stored openly without being covered. According to the International Commission on Microbiological Specifications for Foods (ICMSF, 1995), the marginal limits set for Aerobic Plate Count is $\geq 10^4 < 10^5$. The mean TVC count for vegetables (moroho) was 8.06×10^9 Log_{10} cfu/ml and 2.80×10^5 Log_{10} cfu/ml for chicken. The implication in this regard could be hygienic in nature perhaps during preparation of the food or their handling thereafter. Okareh *et al*., (2015) reported that in their study that all food samples fell within the marginal limits, however that did not imply the absence of hygiene problems in the handling of such food.

The counts in this study could further be attributed to the absence of hand washing facilities, poor cleaning and sanitizations practices and the design/construction of facilities. For example, most of the vendors that sell braid meat leave it uncovered on the braai stands until it is sold thereby exposing it to all sorts of contaminants brought by either dust or flies. Furthermore lack of clean and adequate water supply discourages the practice of good and frequent hand wash. Besides microbial contamination, street vended food may have other characteristics that negatively impact on human health and these include; the type of food product, the overuse of food additives, the nature and extend of chemical contamination (Kok *et al*., 2014).

As mentioned previously in this chapter, microflora from chicken and vegetables were further characterized using RapId kits and the species identified are shown in Table 3.1. the majority of microorganisms were *Escherichia coli* (16%), *Enterobacter cloacae* (11%), *Enterobacter asburiae* (9%), *Brevundimonas vesicularis* (9%), *Enterobacter cancerogenus* (7%), *Citrobacter freundii* (7%) and *Sphingomonas paucimobilis* (7%).

Table 3.1 Identified organisms from food samples

FOOD SAMPLE	NUMBER OF AREAS COLLECTED	BACTERIAL ISOLATES
Vegetables (moroho)	15	*Burkholderia cepacia, Stenotrophomonas maltophia, Enterobacter cloacae, Citrobacter freundii, Escherichia coli, Comamonas testoteroni, Brevundimonas vesicularis, Pseudomonas putida, Pseudomonas flourescens, Aeromonas hydrophila,*
Chicken	15	*Acinetobacter calcoaceticus, Enterobacter cloacae, Enterobacter cancerogenus, Enterobacter asburiae,Escherichia coli, Comamonas testosteroni, Sphingomonas paucimobilis, Pseudomonas oryhabitans, Pseudomonas flourescens,Pseudomonas stutzeri*

3.6 CONCLUSIONS

The results of the previous chapter on food handler KAPs and consumer perceptions showed that street food consumption is common amongst Maseru population, and consequently street food vending contributes to dietary intake. However, street foods are prone to microbial contamination and the aspect of good hygiene practices plays a major role in this matter. Soussa (2008), mentioned that failure to adhere to safety and hygiene practices in most instances causes food to become a breeding place for microorganism. Contact surfaces and food handler hands also play a role in the cross contamination of food. The results obtained in this study indicate that 61% of all the sampled surfaces had a TVC above 100cfu/cm^2 which is the acceptable limit for contact surfaces (South African R962). Therefore the counts obtained in this study are of unacceptable practices by the vendors and this can have harmful effects on the health of consumers. In this study some of the microorganisms isolated from food samples such as *Escherichia coli,* are potential enteric pathogens and have been known to cause gastroenteritis, while most of them are of soil and water origin. It has been reported (Muinde and Kuria, 2005) that water used for preparation in street food vending is often from contaminated or untreated sources, hence resulting in high bacterial load on food. This is also the case for the street food vendors in Maseru in which access to safe and clean water supply is a challenge. Since the vegetables prepared come in contact with soil, failure to adequately wash them due to the lack of water or using contaminated water for washing purposes could pose a high risk for consumers. It has been further recommended (Feglo *et al*., 2012) that vegetables be washed with previously boiled water in order to reduce contamination. The average

TVC found in vegetable (moroho) and chicken samples in this study were higher than that which is allowed by the South African Microbiological standards which indicates that cooked food should contain less than $\log_{10}$ 6.00 cfu/g. It was therefore concluded that it is critical to prioritize microbial testing of food and food safety interventions to remedy such situations. During the collection of samples it was observed that vendors do not cover chicken after braaiing, rather it is left on the braai stands until served. This practice exposes the meat to contamination either by dust or vectors such as flies. It is therefore recommended that vendors store food appropriately regardless of whether it is before or after preparation. Furthermore, in light of protecting consumer health, the WHO (1996) recommends that vendors be advised to display notices in their stalls relating to the safety of their food. For example during the listeriosis outbreak in 2018, one could put up a sign showing the implicated food type and the associated risks of consumption. In Lesotho this could be achieved with participation of organizations such the Ministry of Health, Maseru City Council and other stakeholders.

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CHAPTER 4

Antimicrobial susceptibility profile of microflora isolated from street vended foods in Maseru, Lesotho

4.1 ABSTRACT

An antimicrobial is defined as an agent that kills or inhibits the growth of all microorganisms including bacteria, viruses and fungi to prevent or treat infections in both animals and humans. Despite the great contribution of antimicrobials to health systems, the development of resistance by microbial pathogens currently presents a global public health challenge, leaving a void for the development of new therapeutic interventions. The emergence of antimicrobial resistance along the food chain is also a major public health issue, with several studies having reported food animals and products being colonized and/or infected and contaminated by resistant genes such as extended spectrum beta lactamase (ESBL) Enterobacteriaceae. In this study the antibiotic susceptibility profile of the selected microflora obtained from street vended foods in Maseru Lesotho was assessed using the Kirby Bauer disc diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI) on Mueller Hinton agar (MERCK, SA). The following antibiotics were used amoxicillin (30 µg), cefoxatime (30 µg), cefpodoxime (10 µg), nalidixic acid (30 µg), chloramphenicol (30 µg), gentamicin (10 µg), tetracycline (30 µg) and penicillin (10 µg). The results showed 100% resistance to tetracycline and 100% susceptibility to both chloramphenicol and gentamicin. All isolates were multidrug resistant (MDR) as they had resistance to at least two or more of the antibiotics. *E.coli* had the highest resistance (75%), then *B.versicularies* and *S.paucimobilis* showed the lowest resistance (25%). Although the occurrence of MDR is a natural phenomenon, failure to prudently use antibiotics, poor infection control and inappropriate food handling contributes to the emergence and the spread of MDR pathogens. Moreover resistance to third generation cephalosporins,

observed in this study is of great concern hence the need to regulate and constantly assess the quality of street vended food.

Keywords: Antibiotic resistance, multidrug resistance, food chain

4.2 INTRODUCTION

Antibiotics have not only improved the prognosis of patients over the years but also reduced their morbidity and mortality (Wojkowska-Mach *et al*., 2018). Although their existence has been highlighted as one of the greatest accomplishments in modern medicine, antimicrobial resistance currently presents a global public health challenge and a need to develop new therapeutic interventions (FSA, 2015). Antimicrobial resistance occurs when microorganisms develop the abilities, through various mechanisms, to defeat the drug developed to kill them and continue to grow (CDC 2018, Founou *et al.,* 2016). However the risk of resistance can be avoided or even delayed if they are used prudently (Lee *et al.,* 2013).

The high demand of antimicrobials due to the frequent infections of both humans and animals is undoubtedly a leading factor in the growing problem of antimicrobial resistance (CDC, 2018)**.** It is well established that bacteria cells, whether drug resistant or not, can be transferred amongst humans, animals and the environment in more ways than one and many similar antimicrobial agents are used for treating both humans and animals (da Costa *et al*., 2013). Moreover, food handlers may be reservoirs and contribute foodborne outbreaks among the general population (Campos *et al*., 2015). In the food chain, food may become contaminated with antimicrobial resistant bacteria from food animals, contaminated fertilizer or irrigation water, environmental factors such as food preparation surfaces and storage facilities, or poor food handler practices and poor hygiene (CDC 2018; CAC 2011; WHO 2011).

4.3 PUBLIC HEALTH SIGNIFICANCE OF ANTIMICROBIAL RESISTANCE

The modern age of antibiotics all began decades ago with the discovery of penicillin by Sir Alexander Fleming in 1928 (Sengupta *et al*., 2013). Initially, antibiotics were only used to treat serious infections in the 1940s (CDC, 2013). However, microorganisms have developed resistance over the years and this has been attributed to drug overuse, inappropriate prescribing, extensive agricultural use and the lack of availability of new antimicrobials (Ventola, 2015). The public health concern of antimicrobial resistance is mainly in relation to the therapeutic antimicrobial agents, that is, those antimicrobials used in low concentrations and with little or no toxic effects to the animal or human cell for treating or preventing infections. These antimicrobials have different mechanism actions that lead to the destruction or growth inhibition of bacterial cells, these include; inhibition of cell wall synthesis, inhibition of protein synthesis and DNA synthesis inhibition (FSA, 2015).

E. coli is part of the normal flora in the intestine of humans and animals. Moreover, it has been found to be the most frequent cause of community and hospital acquired urinary tract infections and a leading causative agent of foodborne infections worldwide (Bélanger *et al*., 2011). Infections with *E. coli* usually originate from the person affected and strains with a particular resistance or disease-causing properties have been found to be transmitted from animals through the food chain which may carry transferable resistance genes (Sunde and Norstrom, 2006). There are a number of mechanisms by which antimicrobial resistance in foodborne bacteria can increase the burden of illness

and these include rendering infections more difficult or expensive to treat thus enhancing virulence.

In the developing world, where biosecurity and food safety measures are limited along the farm-to-fork continuum and where humans interact intimately with animals and the environment, the public health risk is likely to be associated with both the direct and indirect transmission of antibiotic-resistant bacteria (Padungtod *et al*., 2008). The human health consequences of these resistant organisms include more serious infections and increased frequency of treatment failures. For example, the emergence of beta-lactamases is an indication that risks to human health include the possibility of horizontal transfer of resistance genes (Kaesbohrer *et al*., 2012).

4.4 STREET VENDED FOODS AND ANTIMICROBIAL RESISTANCE

The role of street foods as vectors of pathogenic bacteria transmission to humans has been extensively described (de Olivieira *et al*., 2011).The nature and operational aspects of the street food trade creates an ideal environment for cross contamination. This alone renders street vended foods as potential reservoirs for antimicrobial resistant pathogens. For example, Akinyemi *et al*., (2013) showed that strains of *E. coli, S. Typhimurium* and *K. Pneumoniae* which were isolated from street vended foods were multi drug resistant. Multidrug resistance is described as the phenomenon of resistance to more than one therapeutic drug presented by a certain specific organism (Nikaido, 2009).

4.5 TRANSMISSION ROUTES OF ANTIMCROBIAL RESISTANCE ALONG THE FOOD CHAIN

Antimicrobials are in most countries used for food animal treatment and animal growth than mainly for humans (Bartlett *et al*., 2013; Spellburg *et al*., 2014). This further creates problems due to the fact that microorganisms do not have boundaries and are thus easily transported across borders, meaning that antimicrobial resistance can quickly spread to neighbouring countries. The spread of antimicrobial resistance is possible along the food chain through direct or indirect contact. Occupationally exposed workers such as veterinarians, farmers, abattoir workers and food handlers, as well as those directly in contact with animals, are at high risk of being colonized or infected with antibiotic-resistant bacteria (Marshall and Levy, 2011). Additionally, human population may be indirectly exposed to antimicrobial resistant bacteria through consumption of contaminated food products (e.g. meat, eggs and dairy products).

Foods of non-animal origin (fruits and vegetables) can also carry antibiotic resistant bacteria due to extensive use of antibiotics such as streptomycin in the prevention of apple and pear tree diseases (Ventola, 2015). Therefore fruit and vegetable consumption without proper washing and disinfection could render them to be vehicles for antibiotic resistant bacteria (Prestinaci *et al*., 2015). Figure 4.1 outlines the indirect transmission of antimicrobial resistance through the food chain.

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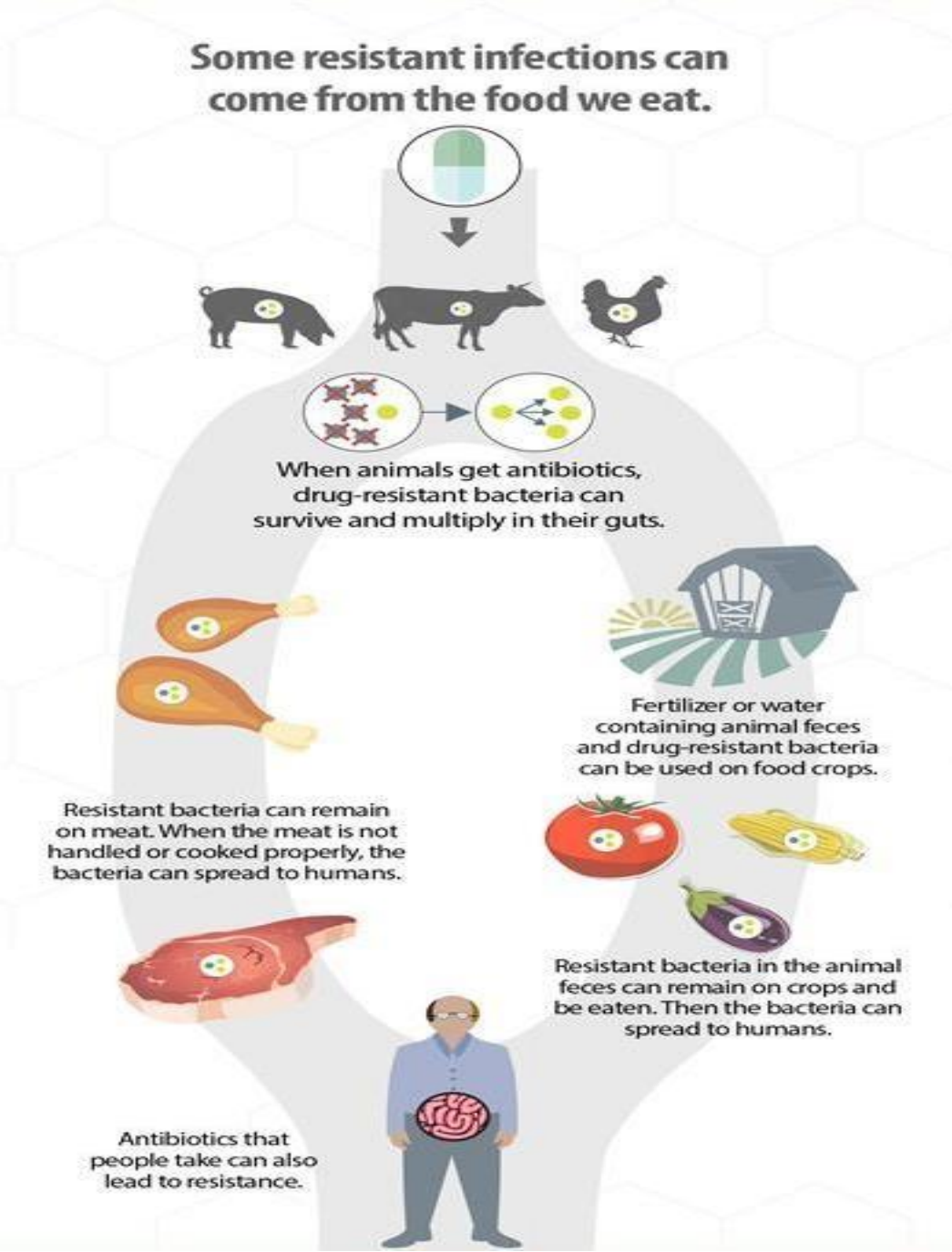


Figure 4.1 Possible routes and sources of infection with antibiotic-resistant bacteria in the food chain (CDC, 2018)

4.6 MATERIALS AND METHODS

Antibiotic susceptibility test for the bacterial isolates was carried out by using Kirby Bauer disc diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2013), on Mueller Hinton agar (MERCK, SA). The antibiotic powders selected were the following: Amoxicillin (30 µg), Cefxatime (30 µg), Cefpodoxime (10 µg), Nalidixic acid (30 µg), Chloramphenicol (30 µg), Tetracycline (30 µg), Gentamicin (10 µg) and Penicillin (10 µg). These antimicrobial agents were selected based on the availability and frequency of prescription for the treatment of bacterial infections in South Africa. Briefly, the selected bacterial strains (*E. coli, E.Cloacae, E. Asburiae, E.Cancerogenous, C.Freundii, S.Paucimobilis* and *B. Versicularies*) were streaked on PCA to obtain pure colonies and subsequently inoculated into 5 ml of sterile nutrient broth for incubation at 37°C for 24h. Thereafter, 0.1ml of bacterial suspension (0.5 McFarland turbidity) was spread on the Muller Hinton agar plates and sterile swab was used to completely flood the agar surface and this was done in duplicate. Antibiotic discs were applied to the agar surface at constant distances and the plates were incubated at 37°C for 24h. At the end of incubation period zones of inhibition were measured as (mm).

Concurrently the isolates were further tested for ESBL production using the double disc synergy test (DDST). Cefoxatime (30 µg), cefpodoxime (30 µg) and amoxicillin/clavulanic acid (amoxicillin 20 µg and clavulanic acid 10 µg) were used for ESBL detection. Amoxicillin/clavulanic acid and third-generation cephalosporin discs were placed at a distance of 20 mm from centre to centre on lawn culture of Muller-

Hinton agar plates. The plates were incubated at 37ºC overnight. Any enhancement in zone of inhibition of cephalosporins towards the amoxicillin/clavulanic acid disc was considered positive results for an ESBL. The reference strain *Escherichia coli* (ATCC 13762) was used as control to check the potency of antimicrobial disks.

4.7 RESULTS AND DISCUSSIONS

The antimicrobial susceptibility profile of selected microbial strains isolated from street vended foods are depicted in Table 4.1 below.

Table 4.1 Antimicrobial susceptibility of selected organisms from street vended foods

ORGANISM	***AMX***	***CTX***	***CPDX***	***NA***	***C***	***TE***	***CN***	***P***
E. Cloacae	S (25)	R(18.8)	S(25.6)	R(10.9)	S(24.2)	R(9)	S(27.8)	I(24.6)
C. Freundii	R(10.6)	S(37.5)	R(16.2)	R(13.9)	S(28.4)	R(0)	S(19.6)	R(0)
E. Coli	R(12.7)	R(11.5)	R(8.4)	R(11.1)	S(24.1)	R(6.5)	S(21.1)	R(0)
S. Paucimobilis	I(19.6)	S(32.8)	S(25)	S(21.5)	S(25.6)	R(0)	S(20.1)	R(0)
E. Asburiae	R(0)	S(32.5)	R(19.7)	I(17.6)	S(28.7)	R(0)	S(20.5)	R(0)
E. Cancerogenous	S(24.1)	R(20.5)	S(27.4)	I(17.2)	S(25.4)	R(8.8)	S(34)	I(24.4)
B. Versicularies	S(19.2)	S(33)	I(23.1)	S(21.7)	S(25.4)	R(0)	S(21.3)	R(6.5)

R- resistant, I- intermediate, S- susceptible, (mm)
AMX=Amoxicillin, CDPX=Cefpodoxime, CTX=Cefoxatime, NA=Nalidic Acid, C=Chloramphenicol, TE=Tetracycline, CN=Gentamicin, P=Penicillin

The *Enterobacter cloacae* specie are mainly comprised of an extremely diverse group of bacteria that are associated with plants, soil and humans. According to the results *E.cloacae* was susceptible to both amoxicillin (25 mm), cefpodoxime (25.6 mm), chloramphenicol (24.2 mm) and gentamicin (27.8 mm), however it was found to be resistant to cefoxatime (18.8 mm), nalidixic acid (10.9 mm) and tetracycline (9 mm). This susceptibility profile was somehow expected since this organism *E.cloacae* has been shown to be multidrug resistant to third generation (cefoxatime) cephalosporins (de Almeida *et al*., 2017). These results are similar to a study (de Almeida *et al*., 2017), wherein shrimp *E.cloacae* isolates were resistant to cefoxatime. *Citrobacter frendii* is a facultative anaerobic gram-negative bacteria that belongs to the family Enterobacteriaceae and is known to cause a number of opportunistic infections. The results of this study show *C.freundii* to be resistant to five antibiotics, namely amoxicillin (10.6 mm), cefpodoxime (16.2 mm), nalidixic acid (13.9 mm), tetracycline (0 mm) and penicillin (0 mm); studies (Ramos *et al.,* 2009, Liu *et al.,* 2018), reported resistance of *C.freundii* to both tetracycline and penicillin. However, cefoxatime and chloramphenicol were effective against *C.freundii* as shown by inhibition zones, 37.5 mm and 28.4 mm. Interestingly, *C.freundii* is known to be mainly resistant to cefoxatime, however in this study *C.freundii* was found to be susceptible to cefoxatime: A study (Liu *et al*., 2018), also showed *C.freundii* to be susceptible to cefoxatime.

Escherichia coli are abundant in the human gastrointestinal tract and can serve as carriers/reservoirs of antimicrobial resistant genes. In this study *E. coli* was resistant towards amoxicillin (12.7 mm), cefoxatime (11.5 mm), nalidixic acid (11.1 mm), tetracycline (6.5 mm) and penicillin (0 mm). These results were expected as *E. coli* has been found to be resistant to a number of antibiotics including some third generation cephalosporins (Chauvin *et al.,* 2013). Gentamicin is however recommended as one of the best treatments for *E. coli* infections (Kibret *et al*., 2011) and in this study it was effective against *E. coli* strains. *Sphingomonas paucimobilis* is an opportunistic gram-negative bacilli that is regarded to be of minor clinical significance (Ryan *et al*., 2010), however humans can acquire it through community or hospital settings (Nandy *et al*., 2013). It is however important that such microorganisms not be disregarded as their evolution can occur daily. In this study *S.paucimobilis* was found to susceptible to 63% of the antibiotics used, and resistance was only found in tetracycline (0 mm) and penicillin (0 mm). According to (Lugito *et al.,* 2016), *S.paucimobilis* is usually susceptible to carbapenems, aminoglycosides but mainly resistant to penicilins and first generation cephalosporins.

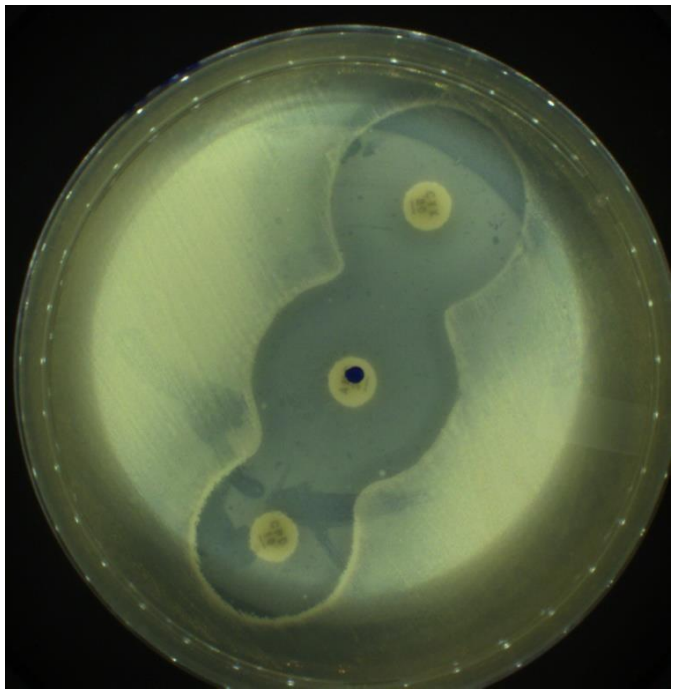
Enterobacter asbiriae is a gram-negative, facultative anaerobic species of the Enterobacteriaceae family that has been isolated from soil, water and food products (Lau *et al.,* 2013). According to the results of a study (Brenner *et al*., 1986, Mardaneh *et al*., 2016), *Enterobacter asbiriae* was found to be resistant to tetracycline and penicillin, but rather showed susceptibility to gentamicin, cloramphenicol and nalidixic acid. These results are similar to those of this study whereby susceptibility was found in gentamicin and chloramphenicol with the inhibition zones (20.5 mm) and (28.7 mm) respectively.

Nalidixic acid did however showed intermediate results (17.6 mm) against *E.asburiae.* On the other hand, *Enterobacter cancerogenous* is usually known as an opportunistic human pathogen. In this study , *E.cancerogenous* was found to be susceptible to amoxicillin (24.1 mm), cefpodoxime (27.4 mm), chloramphenicol (25.4 mm) and gentamicin (34 mmm). *Brevundimonas versicularies*, an emerging global opportunistic pathogen was found to be resistant to tetracycline (0 mm) and penicillin (6.5 mm). Chloramphenicol and gentamicin in this study were found to be the most effective as all the isolates were susceptible to them, while tetracycline was regarded as the least effective as all isolates showed resistance towards it.

Resistance to beta lactams continues to be confounded by target organisms, despite their long history of use in the treatment of infectious diseases (Poole, 2004). This resistance of gram-negative pathogens to beta lactams threatens their viability. Some of the gram negative pathogens such as *E.coli* and *Klebsiela* sp. are able to evade killing by penicillin, cephalosporins and carbapenems, therefore presenting a sophisticated multi drug resistance phenotype (Thomson *et al*., 2005). Some multi drug resistant pathogens are also extended spectrum beta lactamase (ESBL) producers. ESBL is an enzyme that hydrolyses the beta lactam ring of third generation cephalosporins, altering the antibiotic structure, thereby presenting resistance (Shrestha *et al*., 2017). Bacterial species that carry the ESBL gene are said to be normal inhabitants of the gastrointestinal tract, and they are commonly associated with food (Overdevest *et al*., 2011).

In this study disc diffusion demonstrated that 57% of the isolates presented the ESBL phenotype. Figure 4.2 depicts the ESBL production among *E.cloacae* and *C.freundii* isolates. Additionally, the extended spectrum β-lactamase activity was found in all the isolates with the exception of *S.paucimobilis, B.versicularies and E.asburiae* (Figure 4.4 and 4.5).

A



B

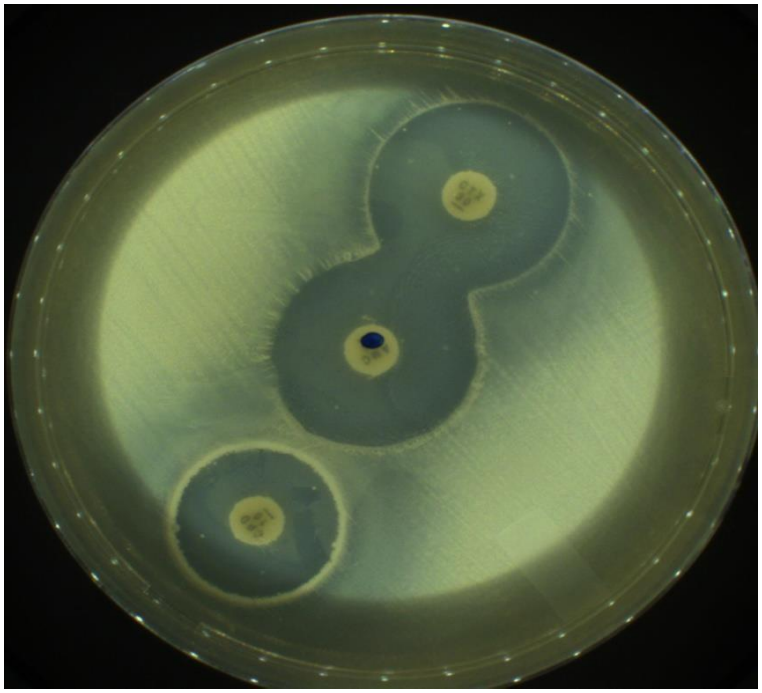


Figure 4.2 ESBL production by *E.cloacae* (A) and *C.freundii* (B). Antibiotic discs contain cefoxatime (30 µg), cefpodoxime (30 µg) and amoxicillin/clavulanic acid (amoxicillin 20 µg and clavulanic acid 10 µg)

A

B

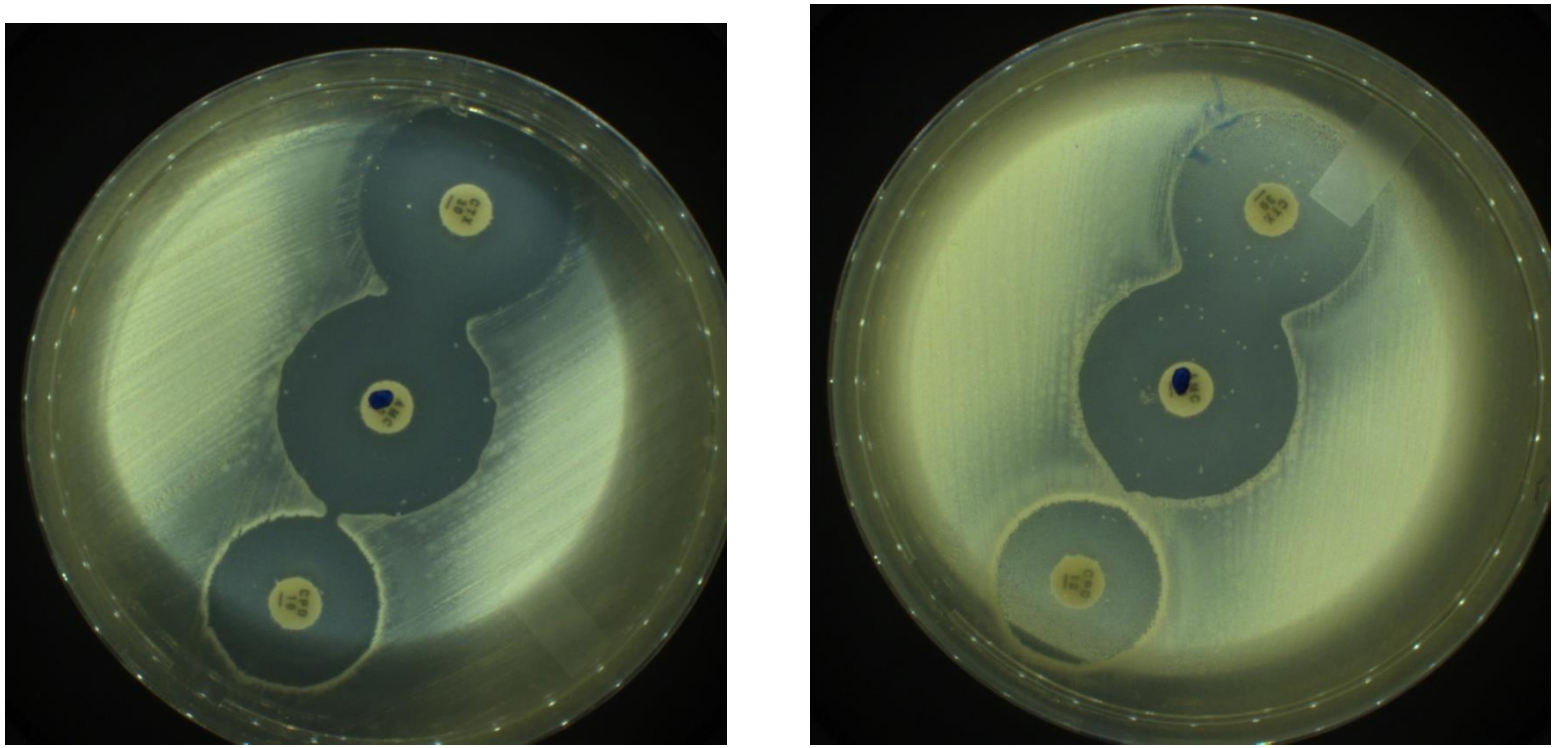


Figure 4.3 ESBL production by *E.coli* (A) and *E.cancerogenous* (B). Antibiotic discs contain cefoxatime (30 µg), cefpodoxime (30 µg) and amoxicillin/clavulanic acid (amoxicillin 20 µg and clavulanic acid 10 µg) A

B

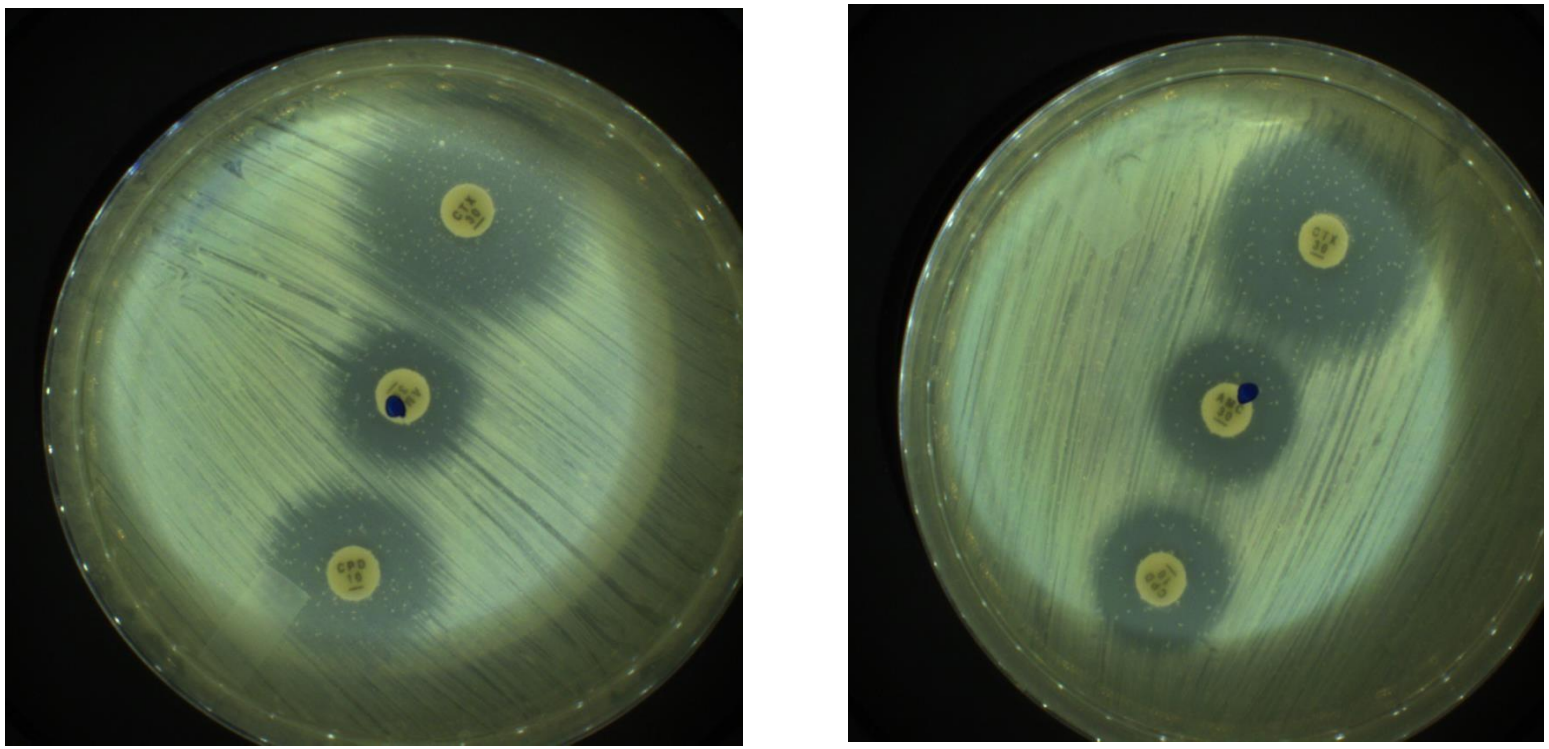


Figure 4.4 Negative ESBL production by *E.asburiae* (A) and *S.paucimobilis* (B)

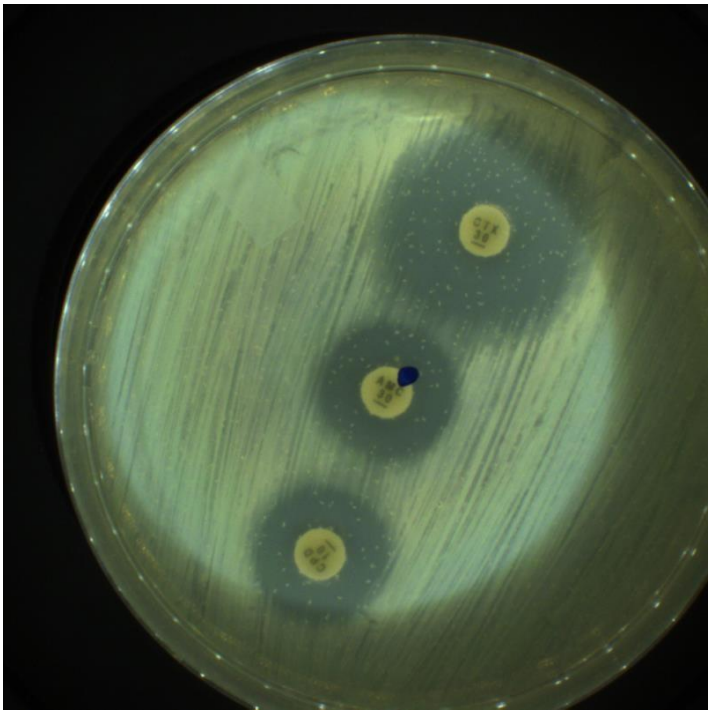


Figure 4.5 Negative ESBL production by *B.versicularies*

ESBL producers are intrinsically resistant to all the cephalosporins although they may exhibit an in vitro susceptibility (Kaur *et al*., 2013). The resistance presented by ESBL producing strains poses a threat of treatment failure and also reduces therapeutic choices. B-lactam inhibitor combination have been recommended for the treatment of ESBL producing bacterial infections, however, the downside to this is that these drugs must be given in high doses (Gold *et al*., 1996).

4.8 CONCLUSIONS

The principles that are applied to the prevention and control of the spread of pathogenic bacteria via food will also contribute to the prevention and spread of antimicrobial-resistant pathogenic bacteria. Due to the escalated public health hazard posed by antimicrobial resistance in foodborne pathogens and commensals, additional control measures for antimicrobial resistance are critically important. Improved hygiene at all steps of the food chain, including primary production, is effective in reducing the number of foodborne pathogens in food. Moreover, here is a need for increased awareness of public health aspects related to antimicrobial resistance. There is also need for surveillance of both resistance and antimicrobial use, and the importance of improved food safety activities throughout the farm-to-folk continuum.

Failure to implement appropriate interventions for combating antibiotic resistance coupled with the pacing advent of new resistance mechanisms results in the failure of microbial response to standard treatments, thereby prolonging illness and increasing health care expenditure. It is also paramount that when providing food safety training for food handlers, issues of drug resistance are incorporated as means of further emphasizing the need for proper food handling practices and infection prevention/control. In the current study the obtained resistance to some third generation cephalosporins poses a great concern in public health and further provides evidence that without proper caution, intensive surveillance and regulation of street vended foods, the possibility of foodborne outbreaks of antibiotic resistant bacteria is eminent.

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CHAPTER 5

General conclusions

5.1 INTRODUCTION

Many studies have elaborated on the cause of food borne illnesses, which include amongst others negligence of personal hygiene, improper storage and preparation practices. Negligence of all these aspects provides room for the proliferation and multiplication of microbial pathogens. Therefore food handlers have the responsibility of assuring that microbes are not introduced in the food chain and consumers equally have the responsibility of washing hands before eating the food in order to prevent contamination. Proper storage of food either before or after preparation is fundamental and can help prevent contamination. Furthermore, when storing different types of food, temperature should be considered as it allows for growth of microbes and spoilage of food if not correctly monitored. Moreover, food handlers should consistently adhere to good personal hygiene and this include infrastructure in which food is prepared. Consequently this will also reduce microbial load on food preparation surfaces and chances of contamination.

It is therefore in this line that the objectives of this study were as follows:

- To evaluate the knowledge, attitudes and practices of street food vendors in relation to food safety
- To identify, characterize and enumerate microorganisms in street vended foods and preparation surfaces.
- To assess the antimicrobial susceptibility profile of organisms isolated from the street vended foods

This study was therefore organized into the following chapters

Chapter 1: Review of literature

Chapter 2: The assessment of food handler knowledge, attitudes and practices and consumer perceptions on street vended foods

Chapter 3: The assessment of bioburden on food samples, food preparation surfaces and food handler hands

Chapter 4: Antimicrobial susceptibility profile of microflora isolated from food samples

Chapter 5: General conclusions

Concluding remarks

Chapter 1 literature review focused on the current trends in street food vending issues such as the benefits of street food vending which include cheap and easily accessible diets to consumers. This form of trade also provides a source of income and livelihood for vendors and their families as well as contribution to the economy globally. However, due to the unsatisfactory conditions under which street foods are prepared, they are often associated with microbial contamination and food poisoning. Furthermore, consumers are less interested in safety and quality of the food but rather their convenience (Mensah *et al.,* 2002). Factors contributing to contamination of street vended foods may include lack of education of food handlers and consumers about food safety, lack of basic water supply and proper infrastructure, temperature abuse and inadequate cleaning of utensils (Proietti *et al*., 2014, Rane 2011). Street food contamination has in previous studies been associated with organisms belonging to the

genus *Bacillus*, *Stapylococcus*, *Clostridium*, *Vibrio*, *Campylobacter*, *Listeria* and *Salmonella* (Rane 2011, Khairuzzaman *et al*., 2014, Alimi 2016). Organizations such as the WHO and FAO have developed strategies in an attempt to reduce microbial loads commonly responsible for foodborne illnesses. Therefore with the implementation of such strategies coupled with clear regulations for the street food trade and cooperation between governments, there is a potential that outbreaks due to street vended foods can be reduced.

In chapter 2 the knowledge, attitudes and practices of street food handlers including the perceptions of consumers on street food vending in Maseru Lesotho were evaluated. Food handlers and consumers both assume an important role in the safety and quality of street vended foods. Furthermore, it is important that they both understand and execute their responsibilities concerning food safety, failure of which could result in hospitalization or mortality due to food poisoning. Consumers have the choice to voice their opinions about food safety interventions employed in food industries, standards and any other activity the government and food handlers may apply regarding safe food production and handling. Food handlers also play an important role in the entire food production process. In this study, there was substantial proportion of vendors were operating without food safety training and some even demonstrating poor food safety handling practices, which is a potential risk that may compromise the safety of food. Hence it is important that food handlers have good food safety knowledge, attitudes and practices. Limited knowledge, or negative attitudes and practices of food safety compromise the health of the consumer and food security. The minimal knowledge of food safety exhibited by food handlers in this study highlights the significant gaps that

have to be addressed by the government and Maseru City Council. It is also necessary that good habits should be adopted by vendors in order to attain sustainable food safety and security. This can be achieved by gradual and continuous food safety training, monitoring and improvement of resources and the environment. Aspects of this study that require more attention are, but not limited to; thawing of frozen foods, transmissible foodborne illnesses, detergent and disinfection use and storage temperatures. Another risk factor identified during the course of the study was the poorly constructed stalls and the lack of proper sewage and waste disposal facilities. Although this study focused on bacterial foodborne pathogens, viruses and fungi could also be a threat and further investigations needs to be done to assess the situation further.

Chapter 3 focused on the assessment of hygiene status (Total Viable Count) of food contact surfaces and food handler hands. Food preparation surfaces and food handler hands are important because they come in direct contact with food. Therefore it is of the utmost necessity that they are always kept clean and free of contamination. Based on the results obtained, it was apparent hygiene compliance still remains a concern among food handlers and this in turn places the consumers at risk of food poisoning due to cross contamination. It can therefore further be concluded that, based on the findings obtained regarding practices, the following aspects need to be improved; frequent and proper hand washing, use of clean water and soap for cleaning surfaces and utensils, use of protective wear such as gloves when handling food and other extensive precautionary measures for preventing cross contamination. In this study high microbial counts were detected on tables than on hands, and it was speculated that it could have been due to infrequent or inadequate cleaning of the tables. However, there were some

food preparation surfaces which had no detectable microorganisms and this could mean that the vendors were adequately cleaning the surfaces.

Consistently, the microbial quality of food samples in this study indicated that they harbour indicator organisms such as *E. coli* and Enterobacteriaceae, which although may not inherently be a hazard, can be an indication of poor practices which may include, use of spoiled or poor quality of raw ingredients, undercooking of food, improper storage of food before or after preparation. The results further highlighted that food storage practices and use of cooler boxes for temperature control including personal hygiene need immediate attention to reduce chances of microbial growth and contamination. The conditions under which the food is prepared can shed light regarding the microorganisms found on the food, for example, stalls are of poor construction making it difficult to clean food preparation surfaces and wash hands especially with pests and flies that are often seen in the vicinity: Moreover, the environment around the stalls is dusty and cars are freely roaming about. In light of the afore mentioned reasons, it can also be speculated that most of bacteria detected on the food originates from the dust in the environment around the stalls.

Regarding chapter 4, the focus was the assessment of antimicrobial susceptibility profile of isolates from street vended foods. Dissemination of antimicrobial resistant bacteria, including multidrug resistant organisms is a globally increasing problem and a serious concern for human health. In the food chain, drug resistant bacteria have been detected in both meat and fresh produce (Doyle, 2015). Food handlers may also be sources of contamination with drug resistant bacteria if proper food handling practices are not followed. In this study all organisms were resistant to at least two or more antibiotics,

and this is considered as multidrug resistance. The implications are that should consumers get infected upon consuming the meal, there will be increased treatment costs coupled with high rates of morbidity and mortality. A study (Howard *et al.,* 2003), highlighted that resistance also impacts on the treatment of patients/persons infected with non-resistant organisms as treatment regimens are usually changed by physicians or governments in high resistance areas. In most cases multiqdrug resistant gram negative bacteria produce the ESBL enzyme. This enzyme allows them to be resistant towards penicilins, azreonam as well as first, second and third generation cephalosporins (Lin *et al*., 2017). In this study organisms that tested positive for the ESBL enzyme showed resistance to at least one or more of the broad-spectrum antibiotics. Although there are no known cases of infection with ESBL producing bacteria from street vended foods in Maseru that have resulted in mortality, further analysis of the results obtained in this study leads to the conclusion that consumers are at risk of infection and possible foodborne outbreak. This presents with a challenge which requires the invention of new therapeutic interventions. Furthermore, it is imperative to develop strategies to prevent new infections and further development of resistance.

5.5 CONCLUSIONS

The goal of food safety is to prevent injury or illness during handling, preparation and storage of food. With the current parameters such as climate change, growing and aging population, urbanization and increased affluence, food safety is compromised and presents a challenge for all stakeholders involved, including consumers. Pathogenic microorganisms are also opportunistic thereby leading to foodborne outbreaks globally. This is not different regarding street food vended foods since literature and evidence presented in this study support the fact that there is an urgent need for improvement in the efficiency and effectiveness of food safety interventions in order to improve consumer health. It can be further concluded that the food preparation and storage facilities used in Maseru street food vending are not suitable for the handling and storage of food. Furthermore, the current state of poor infrastructure creates conducive environment for microbial growth and therefore pose microbial hazard. Additionally, street food vendors operate outside or in open stalls and they are therefore subjected to extrinsic factors such as relative humidity and temperature, which greatly influence the microbial quality of food. Additionally, poor sanitation conditions such as lack of clean and safe water supply, access to toilets with wash facilities and proper sewage and waste disposal could be a contributing factor to the high levels of contamination on food handler hands and food preparation surfaces.

Retailers supplying vendors with raw materials also require attention and should be advised to take food safety into account as they also play a prominent role in the food chain. The development of a fully functioning Foodborne Disease Outbreak Surveillance System is also a necessity as most cases of foodborne outbreaks go unnoticed, with the

risk of occurrence. This initiative could be a collaboration between various divisions and disciplines to make it more efficient and effective. In conclusion, priority should be given to food safety training of food handlers, consumer education about food safety and their role in it, microbial testing and food safety interventions.

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APPENDICES

OBSERVATION CHECKLIST

CONSTRUCT	NO		YES	
	N#	(%)	N#	(%)
FACILITIES				
Food preparation surface material				
Plastic	**0**	**(0%)**	**1**	**(2%)**
Wooden	**0**	**(0%)**	**46**	**(79%)**
Zinc/iron	**0**	**(0%)**	**11**	**(19%)**
Where is the food prepared?				
At home	**0**	**(0%)**	**1**	**(2%)**
On site	**0**	**(0%)**	**57**	**(98%)**
Is the vending stall protected from the sun, dust and wind?	**37**	**(64%)**	**21**	**(36%)**
ENVIRONMENT AROUND THE STALL				
Are animals, pest (flies etc.) evident around the stall?	**22**	**(38%)**	**36**	**(62%)**
Is the vending stall maintained in a clean condition?	**32**	**(55%)**	**26**	**(45%)**
There is portable water at the site or close to the site	**23**	**(40%)**	**35**	**(60%)**
Are adequate hand washing facilities available?	**58**	**(100%)**	**0**	**(0%)**
Are adequate waste (water or food) disposal facilities available?	**33**	**(57%)**	**25**	**(43%)**
Is the environment around the stall clean: far from rubbish, waste water, toilet facilities, open drains and animals?	**57**	**(98%)**	**1**	**(2%)**
PERSONAL HYGIENE				
Does the food handler wash their hands in clean water each time before handling, preparing and serving food?	**46**	**(79%)**	**12**	**(21%)**
Does the food handler wash their hands each time after visiting the toilet?	**0**	**(0%)**	**58**	**(100%)**
Are the food handler's clothe clean and presentable?	**3**	**(5%)**	**54**	**(95%)**
Does the food handler use an apron when handling, preparing and serving food?	**24**	**(41%)**	**34**	**(59%)**
Does the food handler handle food with bare hands?	**0**	**(0%)**	**58**	**(100%)**
Does the operator have clean short nails?	**6**	**(10%)**	**52**	**(90%)**
Is the hair of the operator covered when handling, preparing and serving food?	**12**	**(21%)**	**46**	**(79%)**
Does the food handler handle money while serving food?	**1**	**(2%)**	**57**	**(98%)**
If yes, are the hands washed after handling money before handling food again?	**57**	**(100%)**	**0**	**(0%)**

Does the food handler wear jewelry?	**35**	**(60%)**	**23**	**(40%)**
If yes, is the jewelry adequately covered?	**20**	**(87%)**	**3**	**(13%)**
Does the food handler blow air into the polythene bag before use?	**53**	**(91%)**	**5**	**(9%)**
Is dirt/dust removed by means of apron, dirty cloth or bare hands?	**44**	**(76%)**	**14**	**(24%)**
Is dust removed by blowing?	**57**	**(98%)**	**1**	**(2%)**
Does the vendor smoke during handling/preparation of food?	**58**	**(100%)**	**0**	**(0%)**
Does the food handler use the same utensils (knives, boards etc.) to prepare raw and cooked food products or to cut raw vegetables and fresh meat and poultry?	**33**	**(57%)**	**25**	**(43%)**
Does the food handler have any unhygienic behaviors like blowing nose or coughing and continuing to prepare food?	**56**	**(97%)**	**2**	**(3%)**
FOOD STORAGE				
Is the food displayed/stored				
Openly in stalls	**0**	**(0%)**	**19**	**(33%)**
In sealed containers	**0**	**(0%)**	**39**	**(68%)**
Are raw and cooked food kept separately?	**10**	**(17%)**	**48**	**(83%)**
Are previously cooked foods kept in an ice box or refrigerator?	**58**	**(100%)**	**0**	**(0%)**
UTENSILS				
Utensils are cleaned with				
Warm soapy water	**0**	**(0%)**	**34**	**(59%)**
Cold soapy water	**0**	**(0%)**	**17**	**(29%)**
Dirty water	**0**	**(0%)**	**7**	**(12%)**
Are utensils covered	**46**	**(79%)**	**12**	**(21%)**
Are utensils adequately cleaned every time after use?	**35**	**(60%)**	**23**	**(40%)**

CONSENT FORM

I acknowledge that I understand all the information that was provided by the researcher and I agree to participate in the intended study. I also understand that I am free to end my participation at any time and I will not be forced to do anything that may feel uncomfortable to me, harm my business in any way or put me in any danger. I am also entitled to total confidentiality throughout the study.

Signed …… on this day …… at ……..

THE SESOTHO TRANSLATION STATES:

Mona ke nka kano ea hore ke utloisisa tsohle tseo monga lipatlisiso a nthlaloselitseng tsona, mme ke lumela ho nka karolo boithutong ba hae ele hona ho mo thusa. Ke utloisisa hore e lokolohile ho emisa ho nka karolo litabeng tsena kaofela nako eohle ha ke utloa ke se ke sa thabele kapa ho se ho sena ts'ebelisano mmoho. Ka hare ho litaba tsena kaofela monga lipatlisiso o itlama ho boloka boitsebiso baka ele lekunutu hofihlela a qetile ka boithuto ba hae.

Tekeno …… ka la…… sebakeng sa ……

FOOD HANDLER QUESTIONNAIRE

Food safety training YES/ NO

Gender: ……

Age: ……

FOOD SAFETY KNOWLEDGE	YES	NO	DO NOT KNOW
Washing hands before work reduces the risk of food contamination			
Using gloves while handling food reduces the risk of food contamination			
Proper cleaning and sanitization of utensils increase the risk of food contamination			
Eating and drinking in the work place increase the risk of food contamination			
Food prepared in advance reduces the risk of food contamination			
Reheating cooked foods can contribute to food contamination			
Washing utensils with detergent leaves them free of contamination			
Children, healthy adults, pregnant women and older individuals are at equal risk for food poisoning			
Typhoid fever can be transmitted by food			
AIDS can be transmitted by food			
Bloody diarrhea can be transmitted by food			
Abortion in pregnant women can be induced by food borne diseases			
Salmonella is among food borne pathogens			
Hepatitis A virus is among food borne pathogens			
Staphylococcus is among food borne pathogens			
Can swollen cans contain microorganisms			
Microbes are in the skin, nose and mouth of healthy individuals			
During infectious disease of the skin, it is necessary to take leave from work			

FOOD SAFETY ATTITUTES	YES	NO	DON'T KNOW
Thoroughly cooked food are free from contamination			
Proper hand hygiene can prevent food-borne diseases			
Can a closed can/jar of cleaning product be stored together with closed cans and jars of food products			
Raw and cooked foods should be stored separately to reduce the risk of food contamination			
It is necessary to check the temperature of refrigerators/freezers periodically to reduce the risk of food contamination			
Defrosted foods can be refrozen			
The health status of food handlers should be evaluated before employment			
The best way to thaw meat is in a bowl of cold water			
Wearing masks is an important practice to reduce the risk of food contamination			
Wearing gloves is an important practice to reduce the risk of food contamination			
Wearing caps is an important practice to reduce the risk of food contamination			
The ideal place to store raw meat in the refrigerator is on the bottom shelf			
Eggs must be washed after purchase			
Dish towels can be a source of contamination			
Knives and cutting boards should be properly sanitized to prevent cross contamination			
Food handlers who have abrasions or cuts on their hands should not touch food without gloves			

FOOD SAFETY PRACTICES	YES	NO	DON'T KNOW
Do you use gloves during the distribution of unpackaged foods? (if no, ask question 3)			
Do you wash your hands properly before or after using gloves?			
Do you wear an apron while working?			
Do you wear a mask when you distribute unwrapped food?			
Do you eat or drink while preparing food to be sold?			
Do you wear nail polish when handling food?			
Do you prepare the meals in advance?			
Do you properly clean the food storage area before storing new products?			
Do you use the sanitizer when washing service utensils(plates, mugs, spoons etc)			
Do you used the sanitizer when washing fruits?			
Do you check the shelf life of foods at the time of delivery?			

CONSUMER QUESTIONNAIRE

Age: ……

Gender: ……

Occupation: ……

Please answer all the questions accordingly

1. **Do you ever eat or buy street vended foods?**
2. **If not please state your reasons why**
   ……
3. **How often do you eat street foods?/ O ja lijo tsa seterateng na?**

Never/ hohang	Once in a while/ hoane le hoane	Almost every day/ kamehla

4. **Why do you prefer street vended foods above the rest?/ hobaneng o khetha hoja tsona hofeta ho reka tsa mabenkeleng?**

……
……

5. **Have you ever gotten ill from eating street vended foods?/ o kile oa kulisoa ke lijo tsa seterateng?**

Yes	No

6. **If yes, please describe the symptoms you had/ haeba ho joalo, o ne o na le mats'oao afeng?**

……
……

7. **Do you know where to report such incidences?/na o tseba moo o lokelang ho tlaleha taba ea mofuta oo teng?**

Yes	No

8. **Did you report any of the encounters you had with street foods to the relevant authorities?/ na o kile oa tlaleha mathata ao o kopaneng le onna lijong tsa seterateng ho lekala le nepahetseng?**

Yes	No

9. **If not, please state your reasons/ebang hose joalo, mabaka a hao ke afeng?**

……..

10. **During your observation as a consumer, do the food handlers use the followingwhen preparing and serving food?/ ho ea ka tsebo ea hao ole moreki, na batho ba phehang ba sebelisa tse latelang ha ba pheha kappa ba le ngoathela lijo?**

i) Nose mask

Yes	No

ii) Hair nets/covers

Yes	No

iii) Plastic gloves

Yes	No

11. **Do food handlers wash their hands before and after handling food?/ na batho ba phehang ba hlapa matsoho pele le kamora ho ts'oara lijo?**

Yes	No

12. **Is good personal hygiene important when handling food?/ na bohloeki bo bohlokoa ha motho a tshoara lijo?**

13. **Do you feel there are improvements than need to be done in street food vending?/ na hona le lintlafatso tseo o boning li hlokahala lijong tse pheuoang seterateng?**

14. **Please list them below/ e ka ba lifeng?**

    a) **……**

    b) **……**

    c) **……**

    d) **……**

END OF QUESTIONNAIRE!! THANK YOU!!