Foodborne pathogens and their antibiotic resistance profiles in ready-to-eat meat sold around Johannesburg Central Business District, Gauteng Province

M.E Tshipamba

Student ORCID number: 0000-0003-4469-3200

Previous qualification (Doctor in veterinary medicine)

Dissertation submitted for the degree of Master of Science in Agriculture in Animal Health at Mafikeng Campus of the North-West University,

Promoter: Professor Mulunda Mwanza

Co-Promoter: Doctor Lubanza Ngoma

Graduation October 2018

Student number: 26997355

DECLARATION

I, TSHIPAMBA MPINDA EDOUARD, hereby declare that the dissertation entitled "Molecular characterisation of foodborne pathogens and their antibiotic resistance profiles in ready-to-eat meat sold around Johannesburg Central Business District, Gauteng Province, hereby submitted for the degree of Master of Science in Animal Health has not previously been submitted by me for a degree at this or any other university. I further declare that this is my work in design and execution and that all materials contained herein, have been duly acknowledged.

Signed.....this..2nd..day of .....16.....2017

DEDICATION

I dedicate this study to the following:

- My late father, MPINDA BAKAMANA FRANÇOIS, for his love, teachings and for constantly reminding me that in life, one has to work hard in order to serve or assist others (may your soul rest in peace);

My mother, Jeanne Mutombo, sisters and brothers, François Mpinda, Fabien Mpinda, Cedrick Mpinda, Nadine Mpinda, Jeanne Mpinda, Higuette Mpinda and Gracia Mpinda, for their moral and spiritual support during my studies; and I also wish to thank my family and friends and bride for their support and prayers.

I thank also all the members and staff of the department Animal Health and all the post-graduate students, for making me feel at home during my studies.

ACKNOWLEDGMENTS

I wish to express sincere gratitude to the Almighty, for His continuous blessings and for providing me with the strength and energy to undertake this study.

I extend sincere thanks to Prof. Mulunda Mwanza, who served as my main supervisor during my studies. His concern for his students was extraordinary, and his enthusiasm regarding the field of food safety and microbiology in Animal Health was very inspiring and enriching. He was an excellent mentor, and it was truly a blessing to work with such an amazing person.

I am also grateful to my co-supervisor, Dr. Lubanza Ngoma, for his assistance during my time in the laboratory. He was always there to offer advice and answers to my questions and concerns.

ABSTRACT

The aim of this study was to identify and characterise foodborne pathogens and their antibiotics resistance profiles in ready-to-eat meat sold around Johannesburg Central Business District, Gauteng Province. To achieve this objective, a preliminary observation study was performed, in order to assess the general practices of street-vended meats in terms of food hygiene and safety using the pre-structure check list form. Data from the observational study was analysed using the Statistical Package for the Social Sciences (SPSS) version 20.0. The study revealed unhygienic practices was more occurred in varying degree in the tree Streets under-study. It was observed during this study that 90.63, 77.42, and 68.89% of vendors were exposing meats to dust and flies. It was also observed during the survey that 94.4, 92.31 and 87.5% of vendors were handling money while serving food, while the presence of stagnant water around vending locations was observed at MTN taxi-rank (21.88%) and Hancock Street (55.56%).The frequency of the presence of stagnant water ($P>0.05$), exposure of food to flies and dust ($P>0.05$) and of use of polythene bags for serving food ($P<0.05$) was not significantly different across the three streets sampled. The results ratter revealed poor hygiene practices were more assessed.

A total of 115 samples from street-vended foods that included chicken meat, chicken gizzard, beef intestines, beef head meat and wors were randomly collected across the different streets sampled during the study. Meat samples were analysed for microbial contamination using the conventional biochemical test (Gram staining, catalase test, oxidase test, voges proskauer test, Indole test and IPI-staph) as well as molecular methods based on 16S rRNA (DNA extraction, PCR amplification, and sequencing). The total bacterial count of all meat samples was ranged from 9.9×10^2cfu/g to 1.1×10^2cfu/g, while the total coliform counts of all meat samples was ranged from 2.9×10^2cfu/g to 1.0×10^2cfu/g. The mean bacterial count was significantly different across (MTN-taxi rank) and Bree Street (corner Plein Street) ($P< 0.05$). The mean bacterial count was also significantly different between chicken meat and beef head meat, chicken gizzard and chicken meat, and chicken gizzard and wors ($P< 0.05$). No statistically significant difference in the mean coliform count across the type of meat ($P>0.05$).

Molecular characterisation revealed the contamination of almost all meat samples with different bacteria such as *Kurthia* sp (7.14%), *Staphylococcus aureus* (25.0%), *Bacillus cereus* (10.71%), *Macrococcus caseolyticus* (14.29%), *Bacillus sp* (7.14%), *Bacillus thurigiens* (3.57%), *Staphylococcus vitulinus* (3.571%), *Bacillus subtilis* (3.57%), *Planomicrobium glaciei* (3.57%), *Planococcus antarcticus* (3.57%), *Citrobacter* sp (3.57%), *Staphylococcus equorium* (3.57%), *Enterococcus faecium* (3.57%) and *Enterococcus faecalis* (3.57%). This could be a potential public health danger. Some of the isolated bacteria are well-known to be causative agent of food-borne diseases such as *Staphylococcus aureus*, *Bacillus cereus* and *Bacillus* spp. The presence of *Staphylococcus aureus* in food may be considered as an indication of poor handling practices among street vendors and the degree of ignorance relating to proper hygienic practices.

Isolated bacteria were evaluated for their antibiotic resistance profiles against eight common antibiotics (ampicillin, tetracycline, chloramphenicol, erythromycin, ciprofloxacin, streptomycin and sulphonamides), using the disc diffusion method as described by Kirby-Bauer and the interpretation of the break point zone as specified in the guideline of antibiotic resistance according to the clinical laboratory institute (2011). The antibiotic resistance test revealed that most isolates were resistance to 2 or 3 antibiotics tested against such as *Kurthia* spp was resistant to ampicillin (18%) and tetracycline (29%), *Staphylococcus aureus* (ampicillin (20%), tetracycline (50%), sulphonamides (50%), streptomycin (100%), chloramphenicol (50%) and erythromycin (50%), *Bacillus cereus* ampicillin (29%), tetracycline (17%), and erythromycin (25%) .

Conclusion: The study revealed the contamination of street foods with different bacteria, and some of them are known to be implicated in food poisoning such as *Bacillus cereus*, *Bacillus* sp., and *Staphylococcus aureus*. The bacteria isolated in this study revealed different rates of resistance to different antibiotics. The surveillance of antimicrobial resistance needs to be strengthened on food pathogens. There is, therefore, a need to enforce training in terms of street-vended food. The study also revealed that there is a need for good hygiene practices, proper handling of food, as well as a clean vending place to ensure good quality and safe food.

LIST OF ABBREVIATIONS AND ACRONYMS

API	Analytic Profile Index
CFU	Colony forming units
CDC	Centres for Diseases Control and Prevention
CBD	Central Business District
CLIS	Clinical Laboratory Institute Standards
DNA	Deoxyribonucleic Acid
ETA	Exfoliative toxin
et al.	And others
FAO	Food and Agriculture Organisation
FB	Foodborne
FBD	Foodborne Diseases
I	Intermediate
PCR	Polymerase Chain Reaction
rmp	Rounds per minutes
R	Resistant
SAgs	Staphylococcal Super antigens
S	Susceptible
SEs	Staphylococcal enterotoxins
WHO	World Health Organisation

LIST OF SYMBOLS

>	Greater than
<	Less than
%	Percentage
/	Per
°C	Degree Celsius
G	Gram
mL	Milli litre
mm	Milli metre
µL	Micro litre

LIST OF FIGUR

LIST OF TABLES

CONTENTS

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Street foods are very well patronised in many developing countries since they are affordable, easily accessible and also serve as an important source of income (Monney *et al.*, 2013). However, these street foods largely do not meet proper hygienic standards and can, therefore, lead to morbidity and mortality due to foodborne illnesses, and concomitant effects on trade and development (Belluco *et al.*, 2013; DeWaal & Robert, 2005). In developing countries such as South Africa, the migration of people from rural to urban areas, as a result of unemployment, has led to street foods becoming an increasingly important part of their daily diet (Cortese *et al.*, 2016; Hanashiro *et al.*, 2005; Hill, 2016). Street vendors are conveniently situated, either in living areas, near workplaces or in street with thousands of commuters, and provide a source of inexpensive, convenient and comparatively nutritious food (Patricia V. Azanza, 2000; Tinker, 1997). Foodborne illnesses are a growing public health concern worldwide and result from eating food contaminated with pathogenic microorganisms, mycotoxins or chemical hazards (McCabe-Sellers & Beattie, 2004; WHO, 2002). The number of reported outbreaks of foodborne illnesses is high, both in developed as well as developing countries (Osaili *et al.*, 2013; Vos *et al.*, 2013). However, the problem is exacerbated in developing countries due to poverty, lack of adequate health care facilities, and the dearth of data regarding foodborne diseases (Organization, 2007). The safety of street or vended foods is, therefore, one of the most pressing health and safety issues facing most developing countries since it leads to both public health and social-economic consequences (Osaili *et al.*, 2013). Food contamination in developing countries is caused by many factors, including traditional food processing methods, inappropriate holding temperatures and poor personal hygiene of food handlers (Feglo & Sakyi, 2012). Furthermore, the prevalence of foodborne illnesses in developing countries is intertwined with other economic and developmental issues, namely, legislation, infrastructure and enforcement mechanisms. Specific examples include inadequacy of food safety laws, laxity in regulatory enforcements, and lack of education for food handlers (Monney *et al.*, 2013). The incidence of food and waterborne diseases is estimated at 3 to 4 episodes per child per year in Africa and food and waterborne diarrhoeal diseases are estimated to cause between

450.000-700.000 deaths in Africa annually, with many more sporadic cases going unrecorded (Santos *et al.*, 2008; Simpson *et al.*, 2007). In most of these cases, pathogens such as *Escherichia coli, Bacillus cereus, Salmonella, Hepatitis, Shigella, Brucella, Staphylococcus aureus, Campylobacter, rotavirus* and enteric bacteria are identified (Feglo & Sakyi, 2012; Monney *et al.*, 2013).

In Ghana, as well as in many other countries in the African continent, there is abundance of national legislation but limited resources to control street food safety (DeWaal & Robert, 2005; Monney *et al.*, 2013). Institutions such as the Ghana Standards Authority and Food and Drugs Board are committed to regulating food standards and training the general populace on food safety issues. However, improvement in food safety systems has not been fully realised and this is observed in recent reports on foodborne illness and/ or contamination of street foods with enteric bacteria in various parts of the country (Feglo & Sakyi, 2012).

Foodborne illnesses of microbial origin are a major international health problem associated with food safety and an important cause of death in developing countries (Hird *et al.*, 2009; Newell *et al.*, 2010). Many countries have now realised that foodborne diseases constitute a major public health issue (Schlundt, 2002). Foodborne diseases represent a widespread and growing public health problem, both in developed and developing countries (Rocourt *et al.*, 2003). This problem has a large impact on the health and economy of developing countries, globally 1.8 million people die from diarrheal diseases annually (Jahan, 2012). More than 200 known diseases transmitted through food are caused by a variety of agents such as bacteria, fungi, viruses and parasites (Oliver *et al.*, 2005a). It is estimated that 76 million people get sick, more than 300 000 get hospitalised while 5 000 die each year from foodborne illnesses (Oliver *et al.*, 2005b; Widdowson *et al.*, 2005).

The risk of foodborne illnesses has increased markedly over the last 20 years, with nearly a quarter of the population at higher risk of illness today, consequently, preventing illness and death associated with foodborne pathogens remains a major public health challenge (Shiferaw *et al.*, 2004;Oliver *et al.*, 2005a; Chang *et al.*, 2009; Quinlan, 2013). Therefore, it is generally accepted within the scientific community, that the true incidence of foodborne diseases is unknown (Andargie *et al.*, 2008; Rocourt *et al.*, 2003). The incidence of foodborne illnesses is increasing worldwide, and is probably the result of profound changes in food production, preservation, storage, and consumption, as well as the globalisation and liberation of food trade and importation of foods (Abdelgadir *et al.*, 2009).

One of these profound changes is consumer demands for convenience, such as the increase in ready-to-eat foods (Cho *et al.*, 2011). Street food is defined as food and beverages prepared and/or sold by vendors in streets and other public places for instant consumption or consumption at a later time without further processing or preparation (Mosupye & von Holy, 1999; Von Holy & Makhoane, 2006). Those who sell street foods are considered as micro-entrepreneurs and constitute part of the informal sector (Chukuezi, 2010b; Martins, 2006).

In some countries, the types of foods purveyed are not documented, yet they are often unique and are an important source of nutrients for the population (Muzaffar *et al.*, 2009; Tambekar *et al.*, 2011). Street foods, therefore, contribute to the food security of the low-income urban population and provide a source of livelihood for a large number of potential workers who, would otherwise, would be unable to establish a business for want of capital (Gadaga *et al.*, 2014). However, street foods are frequently associated with foodborne illnesses due to their exposure to contamination (Barro *et al.*, 2006; Gadaga *et al.*, 2008). Street foods such as meats, beverages and snacks, show a large dissimilarity in their ingredients, handling, selling methods and consumption which frequently reflect the local traditional culture as a role of convenience. Street food provides an important fraction of urban diet, mainly in developing countries (Winarno & Allain, 1991). The street food industry plays a very significant role in meeting food requests of customers and urban-dwellers in numerous cities and towns of developing countries, as it feeds thousands of people every day with a large variety of foods that are relatively cheaper and easily accessible (Tambekar *et al.*, 2008). Whereas, street foods provide undoubted benefits from the food security and socio-economic standpoints, the growing population and fast development in developing countries lead to problems of environmental and food sanitation that also impact on street foods. The inadequate provision of potable water and lack of basic infrastructure and services (such as waste removal and water supply) may unfavorably affect street food structures (Muinde & Kuria, 2005; Omemu & Aderoju, 2008; Rane, 2011).

In developing nations, such as South Africa, street vended food is a common part of city lifestyle due to high joblessness and restricted work opportunities (Bryan *et al.*, 1988; Von Holy & Makhoane, 2006;Martins, 2006). Street foods are not only loved for their single flavours, suitability and the character which they display in the cultural and social legacy of societies, they are also significant and needed for preserving the nutritional status of the population (Tambekar *et al.*, 2008; Tambekar *et al.*, 2011; Muzaffar *et al.*, 2009 Rane, 2011; Okojie & Isah, 2014; Onyeneho & Hedberg, 2013).

Several observational studies have revealed that street foods are frequently held at inappropriate temperatures, excessively handled by food retailers and sold in very dirty environments (Agbodaze *et al.*, 2005; Barro *et al.*, 2006; Muinde & Kuria, 2005). Therefore, concerns have been raised by the Food and Agriculture Organisation (FAO) and other institutions about these foods and their probable cause of outbreaks of food poisoning (Oranusi *et al.*, 2013; Rane, 2011). Even though data on foodborne diseases in Africa is very rare (DeWaal & Robert, 2005), studies have shown that the following pathogens are principal causes of food poisoning: *Campylobacter, Salmonella, Shigella, Hepatitis, Bacillus Cereus, Escherichia* coli and *Rotavirus* (Rane, 2011). Numerous reports have revealed the risks associated with consuming contaminated street-vended foods that have high levels of coliform bacteria and pathogenic bacteria, such as *Salmonella spp., Staphylococcus aureus, Escherichia coli, Bacillus cereus, Clostridium perfrigens* and Vibrio *cholera* (Cho *et al.*, 2011; Elobeid *et al.*, 2014; Mankee *et al.*, 2005). In addition, consumption of food containing above organisms could contribute to the occurrence of multi-drug resistance in consumers, hence posing a real risk to the public health of the general population (Guven *et al.*, 2010; Harakeh *et al.*, 2005). Antimicrobial resistance is presently the greatest challenges to public health worldwide. It decreases the effectiveness of drugs that decrease morbidity and mortality associated with serious and life-threatening infections and thus, compromising human health (Collignon *et al.*, 2016; Guven *et al.*, 2010). Since the last decade, the prevalence of antimicrobial resistance among foodborne pathogens has increased (Hakim *et al.*, 2015; Landers *et al.*, 2012; Van *et al.*, 2007).

1.2 Justification of the study

Foodborne diseases represent a major concern in developing countries and South Africa is no exception. Informal meat food preparation is frequently unhygienic and meat is easily contaminated. According to the World Health Organisation (WHO, 2000), it is crucial to gain more information on the attitudes, knowledge and practices of food handlers. The present study contributes to these aspects by investigating the bacteriological quality, food handling and hygienic practices of street-vended meat around Johannesburg central business district (CBD).

1.3 Research questions

Most street vended meat, sold read to eat meat in open areas, exposing meat to flies and dust sometimes serving that meat to consumers without proper hygienic measures taken. The questions raised in this study were: 1) what is the microbiological quality of the meats sold around Johannesburg CBD?

1.4 Hypothesis

Informal street vended meat is increasingly growing all over the world due to unemployment and hold by people who are not qualified for selling meat or food. It was observed that there are poor hygienic methods applied by vendors when preparing and serving food. Furthermore, the environment where the food is prepared and served is not proper for selling food and can lead to bacterial contamination.

1.5 Problem statement

There is a strong movement of people on a daily basis in Johannesburg CBD. These people eat meat sold on the street. The consumption of street-vended meats is done without taking into consideration hygienic conditions under which they are prepared. There is a risk of contamination through common food-borne bacteria and diseases. To date, there is need to assess the microbiological quality of ready-to-eat meat sold on the streets of Johannesburg CBD. Furthermore, developing nations face high occurrences of the eruption of food poisoning, with palpable economic consequences while foodborne illnesses persist across the world.

1.6 Significance of study

This study will provide baseline information on the microbiological profile of ready-to-eat meats sold in streets around Johannesburg, informal markets and the predominant isolates of bacterial serotypes for their toxi-genicity and antimicrobial resistance profiles. The study will also provide information on different bacterial isolates from meats and food products sold in streets across different geographical areas around Johannesburg CBD, Gauteng Province, South Africa.

CHAPTER TWO

Literature review

2.1 Introduction

Street foods are described as ready-to-eat foods and beverages prepared and sold by vendors or hawkers in streets and other public places (Okojie & Isah, 2014; Osorio Ospina, 2012; Rane, 2011; Tinker, 1997), for immediate consumption or consumption at a later stage without further processing or preparation (Von Holy & Makhoane, 2006). Street foods are largely appreciated for their flavours, convenience, low cost and their cultural and social heritage links (Chukuezi, 2010b; Ekanem, 1998; Samapundo *et al.*, 2015). Street foods contribute significantly to the diets of many people in the developing world (Derbew *et al.*, 2013; Ekanem, 1998). Worldwide, it is estimated that 2.5 billion people consume street foods every day (Samapundo *et al.*, 2015; Samapundo *et al.*, 2016). In addition to this, the street food sector is, in most cases, informal and not strictly regulated (Samapundo *et al.*, 2015; Samapundo *et al.*, 2016). Mosupye & Von Holy, (2000) maintain that street foods increase health problems and are regularly unsafe for human ingesting. These authors examined 132 samples of street foods from two street vendors in South Africa and found an significant number of *Escherichia coli* spp. Cardinale *et al.*, (2005) support the notion that street foods are dangerous and could raise health risks. These researchers also examined samples of foods from 148 street-restaurants in Dakar and found the presence of harmful bacteria in the food samples, the problem was traced back to poor personal hygiene of workers (Cardinale *et al.*, 2005).

Microbiological studies carried out on street vending in several developing countries have reported high bacterial counts in food (Bryan *et al.*,1997; Umoh and odoba, 1999). Previous studies found that street-vended foods in Johannesburg (South Africa) contains *Bacillus cereus*, *Clostridium perfrigens, Salmonella* spp. and *Escherichia coli* (Mosupye & von Holy, 1999). New investigation reports from South Africa document *Salmonella enterica* serotypes Typhimurium and enteritidis as the two most regularly isolated salmonella serotypes. The public health laboratory in KwaZulu-Natal province irregularly receives food samples collected by public health officials, but epidemic research is not routinely done (Niehaus *et al.*, 2011). Three eruptions of foodborne gastroenteritis that occurred in 2008 in the Ulundi Municipality were not further explored. One of these eruptions involved 38 high school children who consumed beef soup and porridge left at room temperature for 24 hours

(Niehaus *et al.*, 2011). Although outbreaks of foodborne diseases are common in South Africa, they are under-reported. The health-care authorities are often alerted late in the course of an outbreak, negating the impact of timely, comprehensive epidemiology investigations (Niehaus *et al.*, 2011). In 2006 and 2007, two epidemics were associated with *salmonella* contaminated fresh basil. However, fresh herbs are unusual products to be consumed alone; they are regularly used as ingredients in a variety of retail-prepared or home-based ready-to-eat products (Zweifel & Stephan, 2012).

The high occurrence of diarrheal amongst newborns and young children is an indication of the poor food hygiene situation in Africa (DeWaal & Robert, 2005). Although epidemics of acute poisoning are frequent in Africa, individual countries have done little to implement investigation systems for food-borne illnesses. Due to its less industrialised public health division, the role of mass media in outbreak reporting becomes relevant and significant for assessing the public health influence of contaminated food or water (Organization, 2008). Foodborne illnesses are illnesses resulting from absorption of bacteria, toxins and cells shaped by microorganisms present in food (Addis & Sisay, 2015; Clarence *et al.*, 2009). Issues of foodborne diseases are well documented worldwide (Hazariwala *et al.*, 2002). Foodborne illnesses is a major international health problem with economic consequences (Duff *et al.*, 2003). In the United States, seven pathogens commonly found in animal products were *Escherichia coli* O157:H7, *Listeria monocytogenes, Campylobacter jejuni, Clostridium perfringens, Salmonella spp., Toxoplasma gondii* and *staphylococcus aureus* account for approximately 3.3-12.3 million cases of foodborne illnesses and a record of 3900 deaths each year (Buzby & Roberts, 1996; Clarence *et al.*, 2009).

2.2 Importance of ready-to-eat food in urban areas

A large number of ready-to-eat food sold in streets feed approximately 2.5 billion people in both developing and developed countries (Fellows & Hilmi, 2011). Street foods have become a part of the life in big cities or urban areas (Riet, 2002). It is also reported that the phenomenon of street food is on the rise, especially in developing countries due to increasing levels of poverty (Riet, 2002). Street food feeds millions of people daily because the food is cheap and is easily accessible (Latham, 1965; Riet, 2002). Street foods play a vital role for consumers, particularly in middle and low income populations (Dardano, 2003; Mensah *et al.*, 2002). FAO reports that street foods provide nutritionally balanced diets, sufficient in quantity and present options for variety and choice for consumers, particularly from middle

and low income sectors of the population who depend heavily on such foods (Rask & Rask, 2011). In a study conducted in Indonesia, it was observed that, it is possible to obtain nearly half of the recommended daily allowance of protein, iron, vitamin A and vitamin C from a street food meal (FAO, 2000). Another study in Bangkok, Thailand, revealed that street foods provide around 39%, 40% and 44% of total energy, protein and iron intake correspondingly (FAO, 2004). The nutritional importance was even greater in children aged 4 to 6 years, who obtained 80% of their energy, protein, fat and iron intake from the street (FAO, 2000). An additional important aspect of street vended food is the conservation of the local food culture (Proietti *et al.*, 2014; Proietti *et al.*, 2013).

2.3 Socio-economic value of street food

The significant socioeconomic contribution of street food with respect to revenue and as a possible source of work, predominantly for women, is widely recognised (da Silva *et al.*, 2014). Street food vending is also believed to make food available at inexpensive prices to low-income groups. Therefore, as a function of its low cost, street food represents an easily accessible nutritional and dietary option, based on the physical availability and social points of view (da Silva *et al.*, 2014; Husain *et al.*, 2015; Muzaffar *et al.*, 2009). The role of street food vending in urban livelihood and food provision has been recognised and investigated over many years (Chukuezi, 2010a; Njaya, 2014). The greater job opportunities in towns and metropolitan areas lead to a high rate of daily commuting from rural to urban areas migration (FAO, 2007). In most cases, the work place is distant from home and lacks cooking facilities, thus, most workers are used to eating out (FAO, 2007). Limited resources (budget and time) available for eating result in many workers preferring cheap ready-to eat meals prepared and by street vendors (FAO, 2007).

Changes in socio-economic status of many developing countries and the increase in the urban population have led to an increase in the number of people who consume street-vended food (Omemu & Aderoju, 2008; Samapundo *et al.*, 2015). Consequently, as the number of people who consume this food increases, so does the number of consumers who are potentially exposed to foodborne hazards (Adam *et al.*, 2014). With an increase in the number of consumers of street food, it is crucial that food safety measures be taken by consumers (Asiegbu *et al.*, 2016). This can play an essential role in the prevention of foodborne diseases as they constitute the last link in the food chain (Losasso *et al.*, 2012).

In general, street food represents an important source of nourishment (Ohiokpehai, 2003; Pang & See Toh, 2008; Toh & Birchenough, 2000). In Africa, for example, street food enables 80% of urban populations to feed themselves easily and at low prices and represented around 40% of food expenditure in urban settings in the 1990s (IFPRI, 2000). The sale of street foods also contributes to the standard of living for families involved in food vending (FAO, 2007).

The contribution of street food to the economies of developing countries has been considerably underestimated and even ignored (FAO, 1991; 2007). Nevertheless, the trade of street food generates income and employment (FAO, 2005). In 2003 in Zambia, the sale of street food provided employment to around 16 000 people, mostly women with minimal education, for whom the sector offers a unique possibility of working and earning (FAO, 2005). The social value of the sale of street food is important, particularly for women (Proietti *et al.*, 2014). Female who head households account for the majority of street vendors in many countries for instance, women are involved in 90% of street food business in the Philippines, 81% in Zimbabwe, 67% in Nigeria and 53% in Senegal (Chukuezi, 2010a; Gadaga *et al.*, 2005; Ohiokpehai, 2003; Proietti *et al.*, 2014).

From the economic perspective, street food corresponds to the informal sector and has shown extraordinary development in the last periods as a function of worldwide socio-economic changes, urbanisation and population growth revealed by some states (Chukuezi, 2010a; Omemu & Aderoju, 2008). Therefore, as a function of its low cost, street food symbolises an easily reachable nutritional and dietary option, based on the physical availability and social points of view (Muzaffar *et al.*, 2009).

2.4 Food safety aspect of street-vended food

Poor food safety remains a major threat to human health, and outbreaks of foodborne diseases have occurred worldwide in recent years (Kuo *et al.*, 2009). Five important foodborne pathogens, *Salmonella*, *L. monocytogenes*, *S. aureus*, *E. coli* O157 and *B. cereus*, are a major concern for food safety in terms of frequency and seriousness of the disease (Yu *et al.*, 2016). In this context, typical Gram-positive pathogens such as *B. cereus* (Choma *et al.*, 2000), *Listeria monocytogenes* (Pichler *et al.*, 2009) and *Staphylococcus aureus* (Dagnew *et al.*, 2012) have been categorised as both spoilage and *E. coli* O157 are typical Gram-negative pathogens which cause gastroenteritis (Burnens *et al.*, 1992; Yu *et al.*, 2016). Therefore,

these five pathogens have always been important hazards to monitor in food supervision and inspection (Yu *et al.*, 2016).

Up to 2 million people (most of them children) die each year due to the consumption of contamination of food and water (Asiegbu *et al.*, 2016). Controlling and ensuring the safety of street-vended food in many countries is a big challenge, considering the fact that this is often less expensive, and prepared and sold in streets by local food vendors (Asiegbu, 2015). Street-vended food constitutes the primary source of food for low and middle income consumers outside their home (Asiegbu, 2015). The safety of street food can be affected by several factors, such as the quality of raw materials, preparation conditions, handling and storage conditions (Mafune *et al.*, 2016), as well as the operation of businesses in locations that do not meet all food safety requirements (Aluko *et al.*, 2014). Street food vendors often operate unregulated without being monitored by any relevant authority (Bhattacharjya & Reang, 2014). As a result, street-vended food has the potential to become contaminated, thus exposing people who consume such food, to the potential risk of foodborne diseases such as Salmonellosis, listeriosis, typhoid fever, Cholera, and diarrhea, among others (Manguiat & Fang, 2013).

The availability of safe food is a basic human right and contributes to sound health, productivity, and a platform for sustainable development and poverty alleviation (Asiegbu, 2015). The safety of street foods is dependent on the quality of raw materials, food preparation, handling and storage practices (Aluko *et al.*, 2014). Food handling and safety is a critical issue globally and every person at risk of foodborne illnesses (Aluko *et al.*, 2014). Poor food handling procedures applied by street food vendors, expose food to cross contamination (Ekanem, 1998). There is, therefore, a general perception that street foods are unsafe due to poor hygienic conditions under which they are prepared, sold and consumed (Muinde & Kuria, 2005).

There has been an international call to increase the safety of food sold on streets, which encompasses all stages of the food production chain (Rane, 2011). However, available literature has predominantly uncovered inadequate sanitary conditions in venues where street food is sold (Nunes *et al.*, 2010). Focusing on the challenges of food safety at all levels, the World Health Organisation dedicated the theme of the world health day, *"from farm to plate"*, in order to ensure food safety and the protection of people against the hazard of unsafe food (Kumar Singh *et al.* 2016; WHO, 2015). The safety of street food has become one of the major concerns for public health since the potential for unsafe or unsanitary food handling by

food vendors is substantial (Burt *et al.*, 2003; WHO, 2010). Ready-to-eat foods, particularly those composed of meat/poultry and salads, prepared and sold by vendors in streets, have been recognised as potential vehicles of microbial foodborne bacteria (e.g. *Salmonella, Listeria monocytogenes*, and entero-pathogenic *Escherichia coli*) (Cho *et al.*, 2011; El-Shenawy *et al.*, 2011).

It is widely acknowledged that street vendors in these countries often operate under conditions which are unacceptable for the preparation and selling food, mostly with inadequate layout and equipment, frequently associated with poor environmental sanitation, improper food handling, and storage practices, as well as low quality of raw materials (Aluko *et al.*, 2014; da Silva *et al.*, 2014; Manguiat & Fang, 2013; Muyanja *et al.*, 2011). There are some serious concerns regarding the safety of street food (Muinde & Kuria, 2005). Moreover street-vended foods have dually been implicated in the outbreaks of foodborne illnesses around the world (Aluko *et al.*, 2014; Bryan *et al.*, 1992). In different studies conducted to assess the food safety knowledge and attitudes of street food vendors, it was observed that street food vendors generally have poor levels of food safety knowledge (Rane, 2011), with demographic characteristics such age and gender not associated with knowledge on food safety by street food vendors (Annor & Baiden, 2011; Samapundo *et al.*, 2015).

Contrasting results have been reported on the relationship between the level of education of street food vendors and their knowledge on food safety (Soares *et al.*, 2012). It has noted that, there is a positive correlation between the level of education and knowledge of food safety (Soares *et al.*, 2012). However, have not reported any correlation between the two (Omemu & Aderoju, 2008). To date, knowledge on food safety, attitudes and practices of food handlers, including street food vendors in several countries, have been reported in several studies in Turkey (Baş *et al.*, 2006), Bangkok, Thailand (Cuprasitrut *et al.*, 2011), Shijiazhungcity, China (Liu *et al.*, 2014b) and in South Africa, City of Johannesburg (Lues *et al.*, 2006).

The safety of food is affected by several common factors, from the quality of the raw materials to food handling and storage practices. In most of the cases, running water is not continuously supplied for hand and dish washing, cooking or drinking, thus leading street vendors to store water under vulnerable conditions (subject to contamination). Street foods are exposed to aggravating environmental conditions, such as the presence of insects, rodents, other animals and air pollution (Lucca & da Silva Torres, 2006).

A number of observational studies have shown that street foods are sometimes held at improper temperatures, excessively handled by food vendors and sold at very dirty surroundings (Hanashiro *et al.*, 2005;Ghosh *et al.*, 2007). Studies conducted to assess the quality of different street foods in several countries have shown that these foods were positive vectors of foodborne illnesses (Nunes *et al.*, 2010; Omemu & Aderoju, 2008). The main factors that determine food hygiene are: handling; preparation techniques; and storage practices (Ifediora *et al.*, 2006). The presence of specific microorganisms such as *Escherichia coli, Staphylococcus aureus* and *Salmonella* in foods served by street-vendors, is an indicative of the degree of ignorance on the part of handlers towards proper hygiene practices (Lues *et al.*, 2006). However the conditions of preparation and selling are sources of contamination, especially by germs involved in poisoning food such *salmonella, E.coli, Staphylococcus aureus* (Bukar *et al.*, 2010). Diseases resulting from the consumption of street food are a major problem of food safety and a cause of mortality in developing countries (Bukar *et al.*, 2010).

In Ghana, many street foods have been found not to conform to food safety standards and have, therefore, been linked to the outbreaks of some food-borne diseases (Omari *et al.*, 2013). The annual out-patient reported cases of foodborne illnesses related to food safety, such as diarrhea, typhoid and cholera, stood at about 420,000, with an annual death rate not less than 65,000 (Ministry of Health, 2013). In 2006 alone, a total of 90,692 people died from food and personal hygiene related illnesses in the country (Omari *et al.*, 2013).

2.5 Knowledge and attitude of street food vendors to food safety practices

Most food vendors do not observe good food handling practices, exposing foods to dangerous conditions such a cross contamination, unsafe storage and poor time temperature conditions (Ekanem, 1998). Poor knowledge and improper handling of food by street vendors in terms of basic food safety measures, poor knowledge and awareness among consumers on potential hazards associated with certain foods, could clarify the health and safety matters that street foods may pose (Rane, 2011).

The majority of outbreaks of diseases related to street food have been linked to the negligence of food handlers (WHO, 2002). About 15 cases of foodborne disease outbreaks observed in Zhapo, a coastal resort of Guangdong, China, from 2008 to 2011, were traced to the negligence of food vendors (Liu *et al.*, 2015).

Some researchers in developing countries have made efforts to study the depth of food safety knowledge, a disposition to food safety issues and practices of street food vendors (Choudhury *et al.*, 2011; Liu *et al.*, 2014b; Muyanja *et al.*, 2011). In their studies, they identified education, food safety training, race and vending environment as factors that affect knowledge and attitude of food vendors to food safety practices. Toh & Birchenough,(2000) established interdependence of knowledge and attitude of street food vendors to food safety practices with strong linear relationship ($r = 0.000$, $p < 0.01$). Several authors have reported that education and training enhance hawkers' knowledge and attitude to food safety practices (Subratty *et al.*, 2004).

2.6 Street-vended food and vending location

In South Africa, Johannesburg Municipality, street-vended food is often prepared and sold under improper hygienic conditions as vendors usually concentrate in overcrowded areas such as taxi ranks, railway stations and busy street pavements (Mosupye & Von Holy, 2000). Where there are high numbers of potential customers. Such areas usually provide limited access to basic sanitary facilities, such as garbage disposal and clean toilets and running water making it difficult for vendors to apply standard sanitary practices (Kubheka *et al.*, 2001). In such areas, large amounts of garbage accumulate and provide harborage for insects and animal pets (Mosupye & von Holy, 1999). Such conditions have given rise to many concerns regarding the sanitary standards of street vending operations, especially because consumers are concerned about the price of food rather than its safety and hygiene in many cases (Bryan *et al.*, 1988; Ekanem, 1998). At the vending location, foods are usually not protected from dust and flies which may harbour foodborne pathogens and safe food storage temperature is difficult to maintain. In most cases there are no toilet facilities, and vendors sell beside garbage (which harbours pests and rodents). Such contagion may result from contact with the animal or their faeces (Campbell, 2011; Jahan, 2012). However, the greatest human contagion is created from the ingesting of contaminated water, or raw, or undercooked food (Jahan, 2012).

2.7 Street food vendors and food handling

Poor hygiene practices and operation in unsanitary environments are considered as the major risk factors leading to the production of microbiologically unsafe foods (Mosupye & Von Holy, 2000). Serving utensils used at vending sites are often contaminated with *staphylococcus* spp., which could originate from the hands of vendors when they touch food

preparation areas, dishes, cloths, and water during dish washing and hand washing, which lead to cross contamination between dish-water. Food preparation surfaces and the food itself consequently pose a key public health risk (Das *et al.*, 2010; Mensah *et al.*, 2002).

2.8 Microbial quality of street food and challenge

The microbiological quality of street-vended food is an important concern (Al Mamun *et al.*, 2013). Microbiologically contaminated food and drinking water are considered as important vehicles for the transmission of foodborne diseases throughout the world (Al Mamun *et al.*, 2013;Organization, 2011a Organization, 2011b).

Microbial pathogens in food may cause spoilage and contribute to foodborne diseases (Miladi *et al.*, 2013). Various reports have identified the risks associated with consuming contaminated street-vended foods that have high levels of coliform bacteria and the presence of pathogenic bacteria, such as *Escherichia coli, Salmonella* spp., *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens*, and *Vibrio cholera* (Cho *et al.*, 2011; Hanashiro *et al.*, 2005; Manguiat & Fang, 2013).

The contribution of street foods to food security should, be considered alongside several food safety issues (Proietti *et al.*, 2014). Food prepared and exposed for sale may become contaminated by pathogenic micro-organisms as well as hazardous chemicals (Proietti *et al.*, 2014). Several studies on microbiological hazards in street-vended foods have been carried out in a number of developing countries (Chukuezi, 2010a; Ghosh *et al.*, 2007; Madueke *et al.*, 2014; Muinde & Kuria, 2005).

Among the microorganisms found in street-vended food, *Salmonella, Staphylococcus aureus, Bacillus cereus* and *Clostridium perfrigens* are the most common ones (Mosupye & Von Holy, 2000). Microorganisms present in street-vended foods, come from different sources and practices. Improper food handling can lead to the transfer of pathogens such as *Salmonella*, *E.coli* and *S. aureus* from the body and the environment into foods (Rane, 2011). Improper waste disposal have been associated with the transmission of enteric pathogens such as *E.coli*, Fecal *streptococci*, *Salmonella* and *Vibrio cholera,* while vegetables and spices are associated with the introduction of spore formers like *Bacilli* and *Clostridium* and pathogens bacteria such as *L. monocytogenes*, *Shigella* and *Salmonella* (Rane, 2011).

Improper storage temperatures and reheating of food have been associated with the production of heat stable toxins produced by pathogens such as *Clostridium perfrigens* and

Bacillus cereus (Rane, 2011). In several studies conducted in Brazil, foodborne bacteria and high microbial counts were been found in different street foods in the country (Hanashiro *et al.*, 2005). In another study conducted in Johannesburg, *B. Cereus* was the most prevalent bacteria detected in 23 samples (17%) out of 132 street food samples (Mosupye & Von Holy, 2000). In the same study, *Clostridium perfrigens* was detected in one raw chicken sample, *S. aureus* in two beef and two stew samples of street-ended foods (Mosupye & Von Holy, 2000). In a study conducted on street foods in Zaria, 26.3% and 15% of samples were contaminated with *Bacillus cereus* and *Staphylococcus aureus* respectively (Umoh & Odoba, 1999).

In a study on street foods in 13 towns, the samples did not meet the bacteriological criteria and were contaminated with *Escherichia coli, Bacillus cereus* and *Staphylococcus aureus* respectively (Garin *et al.*, 2002). Previous studies have revealed high bio-load with vast array of microorganisms of public health concerns in street-vended foods (Omemu & Aderoju, 2008; Wada-Kura *et al.*, 2009). The isolated microorganisms include *Escherichia coli, Salmonella* spp., *Clostridium* spp., *Proteus* spp., *Pseudomonas* spp., *Klebseilla* spp., *Citrobacter* spp., *Staphylococcus* spp. and *Bacillus* spp. These microorganisms are portals for potential pathogenic bacterial and foodborne illnesses. Street foods in some African countries have been tested for various microorganisms of public health concern, including faecal coliforms, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* species and *Bacillus cereus*. Escherichia coli and *Staphylococcus aureus* were recovered in a significant proportion of food, water, hands and surface swabs tested in different countries in Africa (Gitahi *et al.*, 2012; Pswarayi *et al.*, 2014).

In North West Ethiopia, the bacteriological study conducted on street vending potato chips in Gondar town revealed that samples were contaminated with microorganisms such as *Escherichia coli*, *Staphylococcus, Salmonella* and *Shigella* (Bizuye *et al.*, 2014). In Ghana, microbiological study conducted on the quality of food sold on and around Koforidua polytechnic campus, were found contaminated with *Escherichia coli, Salmonella* and *Staphylococcus* these was traced to poor hygienic methods (Amissah & Owusu, 2012).

In Nigeria, bacteriological studies conducted on street-vended foods in Delta state, isolated nine bacterial species from the foods sampled. The microorganisms were *Escherichia coli, Bacillus* sp, *Staphylococcus aureus*, *Enterococcus faecalis*, *Citrobacter* sp, *Proteus* sp, *Klebseilla* sp and *Salmonella* sp. In this study, it was revealed that street foods are potential

vehicles for transmitting foodborne illnesses, thus the need to develop practical strategies geared towards the safety of street food. In India, the identification of microbiological hazards and the safety of ready-to-eat food vended in streets of Amravati city, revealed *Aeruginosa*, *S. aureus*, *Salmonella* spp. and *Proteus* sp. It was concluded in the study that food contamination in the streets of the city of Amravati was mainly due to poor water quality and hygiene during food preparation, washing of utensils and poor personal hygiene (Samuel, 2013).

In Egypt, street-vended food, especially meat products may represent a hazard due to the bad condition of their production and the use of raw materials of poor quality, inadequate personnel hygiene of vendors and holding for long periods, thus leading to contamination of food with pathogenic microorganisms. Such contamination may render the product of inferior quality or unfit for human consumption (Gundogan *et al.*, 2005).

In Senegal, a study on factors responsible for the contamination of ready-to-eat street vended poultry dishes in Dakar, conducted in 148 street restaurants, it was found that *Campylobacter and Salmonella. Hadar, Salmonella enteritidis* and *Salmonella brancaster* are also predominant in live poultry (Cardinale *et al.*, 2005). These finding suggests that these *salmonella* serovars could survive during cooking or could be brought back on to chicken through cross contamination (Cardinale *et al.*, 2005).

A study conducted in Mauritius revealed the first outbreak of salmonellosis caused by the consumption of contaminated marlin mousse (Issack *et al.*, 2009). Between 29 October and 5 November, at least 53 persons developed diarrheal, all with a history of eating marlin mousse. *Salmonella* was isolated from the stool of 26 affected patients and blood culture from one patient. The same type of *salmonella* spp. was isolated in three samples of marlin mousse manufactured on 27 October 2008. All isolates belonged to *salmonella* serovar Typhimurium and were susceptible to all antimicrobials tested (Issack *et al.*, 2009).

2.9 Street food and foodborne diseases

There are growing interests worldwide on the significance of street food as part of an overall concern for food security and well-being (Canet & N'diaye, 1996; Nicolas *et al.*, 2007). Numerous studies on the sanitary quality of street food in Africa, Asia and Latin America, have revealed the transmission of pathogens (Abdallah & Mustafa, 2010; Mosupye & Von Holy, 2000; Yuli *et al.*, 2008), as a function of consequent public health risks. The United

Nations Food and Agriculture organisation (FAO) and the World Health Organisation (WHO) established international guidelines that have been adopted on various continents based on feasibility to ensure the safety of food sold and consumed on streets (Sezgin & Şanlıer, 2016).

Street foods might increase health problems and such foods are often dangerous for human ingesting (Mosupye & Von Holy, 2000). Other researchers have specified that street foods are unsafe and could increase health risks (Cardinale *et al.*, 2005).

Foodborne illnesses (universally known as food poisoning), is a main public health concern often triggered by pathogens that enter into a host through the consumption of contaminated food samples (Cho *et al.*, 2015). According to the Centre for Diseases Control and Prevention, each year, 1 in 6 Americans gets sick due to food poisoning associated with notorious pathogens such as *Escherichia coli* O157: H7, *Salmonella typhimurium*, *Salmonella enteritidis* and *Listeria monocytogenes*. These pathogens have also been responsible for the greatest number of cases of hospitalisation and deaths arising from foodborne sickness (DeWaal *et al.*, 2012).

Foodborne disease is a condition where a person experiences diarrhea, vomiting, abdominal cramps, fever and/or bloody stool (Nsoesie *et al.*, 2014). The illness occurs following the consumption of contaminated food (Xue & Zhang, 2013). Foodborne disease is a global issue and has been reported worldwide. Incidence rates have been reported to be 1210 cases per 100,000 inhabitants in France, 26000 cases per 100,000 in the United Kingdom, and more than 25000 cases per 100,000 inhabitants in Australia and the United States (Teisl & Roe, 2010). Malaysia, however, reported a low incidence rate of 48 cases per 100,000 inhabitants (Ministry of Health, Malaysia. 2014). However, cases of foodborne illnesses in Malaysia usually go unreported because a chain of events need to be addressed first before it is brought to the authority (Soon *et al.*, 2011). Therefore, the actual rate is likely higher. In addition, the diarrhoeal disease has contributed 3% mortality globally (Mutalib *et al.*, 2015).

Each year, up to one-third of the population in developed countries is affected by foodborne illnesses (Bhattacharjya & Reang, 2014; Isara *et al.*, 2010; Webb & Morancie, 2015). Previous studies have found that a considerable proportion of street-vended food is of poor microbiological quality and has the potential to cause diseases (Oguttu *et al.*, 2014).

There are increased interests worldwide on the importance of street foods as part of the general concern for food security and health (Canet & N'diaye, 1996). The incidence of foodborne diseases is rising in developing countries as well as in the developed world (Redmond & Griffith, 2003). It has been proved that street-vended foods have been involved in the eruption of illnesses throughout the world (Bryan *et al.*, 1992; Canet & N'diaye, 1996).

In 1988, 14 deaths were reported in Malaysia caused by food-borne sicknesses related to street foods. In the same year, some 300 persons became ill in Hong Kong after consuming street-vended foods. In 1981, a cholera epidemic in Pune, India was related to ingesting of street-vended juice. In 1987, in Singapore, an outbreak of Cholera was also attributed to street food (Food & Nations, 1990).

Foodborne sicknesses are usually mild and self-limiting, severe cases can occur in high risk groups resulting in high mortality and morbidity. High risk groups for foodborne diseases include infants, young children, the elderly and immunocompromised persons (Fleury *et al.*, 2008). The population in developing countries is more prone to suffer from foodborne illnesses because of multiple reasons, including lack of access to clean water for food preparation, inappropriate transportation and storage of food, and lack of awareness regarding safe and hygienic food practices (Havelaar *et al.*, 2015). Moreover, most developing countries have limited capacity to implement rules and regulations regarding food safety. There is also lack of effective surveillance and monitoring systems for foodborne illnesses, inspection systems for food safety, and educational programs regarding awareness in terms of food hygiene (Havelaar *et al.*, 2015).

2.9.1 Incidence of *salmonella* foodborne pathogens

Salmonella is a leading cause of foodborne diseases worldwide (Scallan *et al.*, 2011; Shao *et al.*, 2011). To date, more than 2600 serotypes have been reported (Guibourdenche *et al.*, 2010). Globally, 94 million cases of gastroenteritis due to *Salmonella* are estimated each year, and 85% of these cases are food-related (Deng *et al.*, 2012; Majowicz *et al.*, 2010). Currently, the consumption of RTE foods remains as well documented as the major source of Salmonellosis, with undercooking and cross contamination considered as recognised risk events (Carrasco *et al.*, 2012; YU *et al.*, 2010). It is also noted that *salmonella* is a major cause of foodborne illnesses in humans (Adzitey *et al.*, 2012; Yang *et al.*, 2016). Farm animals and the origin of foods are important sources of human *Salmonella* infections (Adzitey *et al.*, 2012; Yang *et al.*, 2016). *Salmonella Typhimurium and Enteritidis* are the

most frequently reported serovars associated with human foodborne illnesses (Suresh *et al.*, 2006).

Non-typhoidal *Salmonella* spp. are the second largest cause of foodborne illnesses after *Campylobacter* spp. (Mead *et al.*, 1999). *Salmonella* spp. can cause systemic infections, especially in children and immune-compromised individuals, while healthy individuals suffer from symptoms such as fever, diarrhoea, nausea, abdominal pain, vomiting and occasional septicemia (Coburn *et al.*, 2007). *Salmonella* is also a pathogen of significant importance in worldwide animal production and the emergence of antibiotic-resistance strains (Forshell & Wierup, 2006). The incidence of *Salmonella* spp. in food of animal origin has been documented. Outbreaks of salmonellosis associated with eating beef were reported by several researchers (Mahmoud, 2011; Silva *et al.*, 2014).

2.9.2 Incidence of *E. coli* foodborne pathogens

Escherichia coli are members of the genus *Escherichia* which belongs to the family of Enterobacteriaceae. Members of this family are widely distributed in the environment. *E. coli* are usually a non-pathogenic member of the animal intestinal flora. As long as these bacteria do not acquire genetic elements encoding for virulence factors, they remain benign commensals. References obtained from the European Centre for Diseases Control and Prevention and the WHO Organisation's epidemiological updates, indicate that health officials and the WHO are concerned about the unusually virulent outbreak of foodborne illnesses caused by E. coli (Borgatta *et al.*, 2012). The epicentre of the outbreak was Northern Germany, from where it spread throughout Germany and beyond, to other European countries (Beutin & Martin, 2012; Frank *et al.*, 2011; Wu *et al.*, 2011). Strains of *E. coli* that cause enteric diseases are termed entero-virulent or diarrhoeagenic. However, certain strains have acquired virulence factors and may cause a variety of infections in humans and animals (Stenutz *et al.*, 2006). Cattle and sheep represent a major reservoir for the entry of pathogenic *E. coli* into the human population through the food chain (Pradel *et al.*, 2001). Ruminants, especially cattle, are the most frequent direct and indirect sources of E. coli O157 (Bolton, 2011).

Escherichia coli are an important indicator of faecal contamination in food and are commonly used to assess the hygienic quality of food products. Entero-toxigenic *E. coli*

(ETEC) is a major cause of traveller's diarrhoea (TD) and young children's diarrhoea in developing countries (Fletcher *et al.*, 2013; Rivera *et al.*, 2013).

Escherichia coli O157: H7 infection is transmitted by the faecal-oral route through contaminated food or water. Shiga-toxin producing strains have a high potential for person-to-person transmission since a very low infective dose is required and ingestion of as few as 10 organisms may be sufficient to cause infection (Parry & Salmon, 1998). The rate of secondary household transmission for sporadic Shigatoxin producing strains has been estimated to range from about 4% to 15% (Parry & Salmon, 1998). Institutional outbreaks due to person-to-person transmission have also been reported in nursing homes, schools and day care centres for children (Su & Brandt, 1995). The consumption of contaminated food remains a major source of ETEC infection, and outbreaks of foodborne diseases caused by ETEC have been reported in many countries (Cho *et al.*, 2014; Gonzales-Siles & Sjöling, 2016).

Enterotoxigenic *E. coli* (ETEC) is one of the most common causes of infantile and adult diarrhoea and traveller's diarrhoea in low income endemic countries and is estimated to cause 2.5 million cases and 700 000 deaths in children below 5 years of age (Lamberti *et al.*, 2014; Liu *et al.*, 2012; Qadri *et al.*, 2005; Walker *et al.*, 2010). Enterotoxigenic *E. coli* diarrhoea is associated with poverty, lack of safe drinking water and inadequate sanitation (Curtis *et al.*, 2000). In addition, ETEC may also cause outbreaks of food-borne gastroenteritis (Sjöling *et al.*, 2015). The hallmark of ETEC is the expression of one or two plasmid borne enterotoxins, the heat labile toxin (LT) and/or the heat stable toxin (ST) that both mediate deregulation of membrane ion channels in the epithelial membrane (Fleckenstein *et al.*, 2010).

It was estimated during the period of 1982- 2002, in the United States of America that the incidence of *E. coli* O157:H7 causes 73,000 illnesses annually. The incidence of *E. coli* O157 outbreaks reported to Centers for Disease Control and Prevention (CDC) to better understands the epidemiology of *E. coli* O157.

Escherichia coli O157 outbreaks were ranged up to 2 cases of *E. coli* O157 infection with a common epidemiologic exposure, reported to CDC from 1982 to 2002. In that period, 49 states reported 350 outbreaks, representing 8,598 cases, 1,493 (17%) hospitalizations, 354 (4%) hemolytic uremic syndrome cases, and 40 (0.5%) deaths. Transmission route for 183 (52%) was traced to foodborne, 74 (21%) unknown, 50 (14%) person-to-person, 31 (9%) waterborne, 11 (3%) animal contact, and 1 (0.3%) laboratory-related. The food vehicles for

75 (41%) foodborne outbreaks were ground beef, and for 38 (21%) outbreaks, produce (Josefa *et al.,* 2005).

2.9.3 Incidence of Listeria foodborne pathogens

Listeria monocytogenes is a foodborne pathogen that is widely dispersed in the environment; it is found in soil, water, and plant material, and can grow at refrigeration temperature and at unfavorable conditions of pH (up to pH 4.7) and salt (up to 10%). It can persist in harsh conditions of the food processing environment and can contaminate food. Infection with *L. monocytogenes*, can be mild but the ability of the pathogen to cross the epithelial barrier of the intestinal tract, the blood brain barrier, and the feto-placental barrier, can also result in more severe illness, including bacteremia and meningitis or spontaneous miscarriage (Jooste *et al.*, 2016). *L. monocytogenes* is the main causal agent of listeriosis, a well-documented foodborne illness (Rodrigues *et al.*, 2017; Thomas, 2016; Vázquez-Boland *et al.*, 2001). Infections often have high risks of hospitalization, and human mortality is also high, ranging between 20 % and 30 % worldwide (de Noordhout *et al.*, 2014) and with a hospitalisation rate of >95% (Scallan *et al.*, 2011), and up to 80–99 % in the vulnerable groups such as people with compromised immunity (e.g., HIV/ AID patients), pregnant women, neonates, and elderly people (Ayaz & Erol, 2010) it ranks as the third most serious foodborne disease (Schlech & Acheson, 2000). *Listeria*, *Campylobacter*, *Salmonella* spp. and *E. coli* O157: H7 have been isolated from the majority of outbreaks of food-borne diseases (Chemburu *et al.*, 2005).

Several listeriosis outbreaks have been reported throughout the world, including many multistate outbreaks in the United States. In 2003, mortality from listeriosis was about 500 people from a reported ~2500 illnesses (Mead et al., 1999). However, listeriosis is underreported in Africa Boukadidda *et al.,* (1994) because food-processing industries are still evolving. In Nigeria, *L. monocytogenes* was isolated from some patients that showed clinical signs of listeriosis, and the infection produced a mortality rate of 27 % (Onyemelukwe *et al.,* 1983). But in Morocco, human listeriosis is uncommon (Benomar *et al.,* 2000). Chintu and Bathirunathan. (1975) reported 85 cases of listeriosis in Zambia, while Hohne *et al.,* (1975) reported that the outbreak serotypes (1/2a and 4b) of *L. monocytogenes* were isolated in slaughtered cattle in Togo.

Listeria species cause listeric infections in a variety of animals, including man. They are ubiquitous bacteria widely distributed in the natural environment and in vegetal foods (Low

& Donachie, 1991). *L. monocytogenes* is frequently present in raw foods of both plant and animal origin (including fish), and can be found in cooked foods due to post-processing contamination. Thus, it has been isolated from foods such as raw and unpasteurised milk, cheese, ice cream, raw vegetable, fermented meats and cooked sausages, raw and cooked poultry, raw meats, and raw and smoked seafood. In addition, its ubiquitous presence also leads to the potential for contamination of the food processing environment, where occurrence and persistence of *L. monocytogenes* are frequent (Jami *et al.*, 2014; Nakari *et al.*, 2014; Vongkamjan *et al.*, 2013).

2.9.4 Incidence of campylobacter foodborne pathogens

Campylobacteriosis is the most significant bacterial foodborne disease in the developed world. Ingesting of chicken meat, beef or raw milk, direct contact with ruminants and exposure to contaminated surface water or even consumption of tap water, have been identified as risk factors for human diseases (Indikova *et al.*, 2015). However, the most important risk factor is consumption of and/or handling of contaminated chicken (Indikova *et al.*, 2015). *Campylobacter jejuni* is the most important bacterium that produces foodborne infections in the developed world. Infection with this pathogen leads to severe economic loss in industrial countries and it is estimated that 1% of the population in Europe is infected per year (Humphrey *et al.*, 2014). In the United States, over 6,000 cases of *campylobacter* infection were reported in 2009 alone, but many cases were not reported to public health authorities. A 2011 report from the CDC estimates that *campylobacter* causes approximately 845,000 illnesses in the United States each year. Most of these *Campylobacter* infections are sporadic. Outbreaks, when a group of individuals is affected, have been primarily traced back to raw or incompletely pasteurised milk and water (Evans *et al.*, 1996; Fernandes *et al.*, 2015; Palmer *et al.*, 1983).

2.9.5 Incidence of Staphylococcus food borne pathogens

S. aureus can cause a wide spectrum of infections, from superficial skin infections to severe, and potentially fatal invasive diseases (Lowy, 1998). *S. aureus* is a significant cause of FBD, causing an estimated 241000 illnesses per year in the United States (Scallan *et al.*, 2011).

Several studies have reported incidences of *S. aureus (Hennekinne et al., 2012; Klein, 2007; Schlecht et al., 2015).* These authors maintain food commonly associated with staphylococcal enterotoxins poisonings, are meat and meat products, as well as dairy products. Between

1993 and 1998, 0.9 % (the Netherlands) and 13.6 % (France) of outbreaks of food poisoning recorded in Europe were caused by *S. aureus*. In Italy, about 1.8% of cases were reported in 1998 and attributed to this micro-organism. In the United States, it has been estimated that there are 185000 cases of food poisoning caused by *S. aureus* enterotoxins every year, resulting in 1750 hospital admissions annually (Wieneke *et al.*, 1993).

2.9.6 Incidence and public health impact of foodborne diseases

Foodborne illnesses are prevalent (Hoffman *et al.*, 2005) but the magnitude of illnesses and associated deaths are not accurately reflected by the data available in both developed and developing countries. The WHO has taken initiatives to estimate the global burden of foodborne illnesses (Kuchenmüller *et al.*, 2009). The WOH and the US CDC report, every year, a large number of people affected by foodborne illnesses (Busani *et al.*, 2005). Globally, an estimated 2 million people die from diarrheal diseases in 2005; approximately 70% of diarrheal diseases are foodborne. It is estimated that up to 30% of the population suffer from foodborne illnesses each year in some industrialised countries (Organization, 2014). According to 2011 estimates of CDC, annually, 48 million Americans get sick, there are 128000 hospitalisations and 3000 deaths due to foodborne illnesses in the USA (CDC, 2011). In Canada, an estimated 1.3 episodes of the enteric disease occur per person each year (Fleury *et al.*, 2008). In New Zealand, there are an estimated 119.320 episodes of foodborne illnesses each year, accounting for 3241 per 100.000 people (Scott *et al.*, 2000).

Foodborne illnesses comprise a broad spectrum of diseases and are responsible for substantial morbidity and mortality worldwide. It is a growing public health problem in developed countries, and it is difficult to determine the exact mortality associated with foodborne illnesses (Helms *et al.*, 2003). However, worldwide, an estimated 2 million deaths occurred due to gastro-intestinal illness during 2005 (Fleury *et al.*, 2008). Although the majority of cases of foodborne illnesses are mild and self-limiting, severe cases can occur in high risk groups resulting in high mortality and morbidity in this group (Fleury *et al.*, 2008). The population in poor countries is more prone to suffer from foodborne illnesses due to the following reasons: lack of access to clean water for food preparation; inappropriate transportation and storage of foods, and lack of awareness regarding safety and food practices (Jahan, 2012).

2.8.7 Economic impact of food-borne diseases

Health economists have acknowledged that it is very difficult to estimate the full cost of foodborne diseases (Sockett, 1991) and estimates of the financial cost vary across publications. Foodborne diseases create an enormous burden on the economy. Consumer costs include medical, legal and others as well as absenteeism at work and from school. For many consumers who live at subsistence level, there is loss of income due to foodborne diseases.

In the USA, direct cost of bacterial foodborne illnesses is about US $23 billion. For instance, salmonella alone is estimated at US$3.91million (Sockett & Roberts, 1991), Australia is between $ 487 million and $1900 million (Havelaar *et al.*, 2013; Kuchenmüller *et al.*, 2013; Organization, 2016). Every illness has an economic cost and such is the case with foodborne illnesses; however, the economic cost of health losses has not been extensively studied. Each year, almost 2.2 million people, mostly children in developing countries, die from diarrheal diseases (Havelaar *et al.*, 2013; Kuchenmüller *et al.*, 2013; Organization, 2016). Foodborne diseases are multi-sectoral public health risks linked with agricultural and animal health (Kuchenmüller *et al.*, 2013).

Although foodborne illnesses cause substantial morbidity in developed countries, the main burden is borne by developing countries. These illnesses are an obstacle to global development efforts and in the achievement of Sustainable Development Goals (SDG) (Jahan, 2012). There is an impact of foodborne illnesses on four out of the eight MDGs, these include SDG1 (eradication of extreme poverty); SDG3 (reduction in child mortality); SDG5 (improvement of maternal health); MDG6 (combating HIV/AIDS and other illnesses) (Jahan, 2012).

2.9 Antimicrobial agents and antimicrobial resistance

About 90% of antimicrobials used in animal husbandry are for growth promotion and prophylaxis (Pitout & Laupland, 2008; Sarmah *et al.*, 2006). With the majority of them being excreted unchanged into the environment, concerns about their potential impact on the aquatic environments keep increasing over time (Sarmah *et al.*, 2006).

The intensive uses of the antibiotic agent in industrial animal husbandry have spread into developing countries, and the negative impact on human health and food safety has often followed (Casewell *et al.*, 2003; Silbergeld *et al.*, 2008). The impact may vary considerably between countries and regions influenced by the interaction between human population, land

use, contaminated water source, animal demography, national policies (Production, trade, food security, animal health) and international trade (WHO, 2012). Hence, antibiotic resistance is a major global societal problem (Mellon *et al.*, 2001), involving many different sectors such as medicine, veterinary medicine, animal husbandry, agriculture, environment and trade (Wise *et al.*, 1998).

Epidemiological studies have demonstrated a correlation between antibiotic use and antimicrobial resistance (Beerepoot *et al.*, 2011; den Heijer *et al.*, 2012). Furthermore, antibiotic resistance has been given a low priority in most developing and many developed countries. Most important developing countries, such as South Africa, have received limited attention regarding this problem (Okeke *et al.*, 2007; Winters & Gelband, 2011). Development of anti-microbial resistant foodborne bacterial pathogens can potentially compromise human drug treatments (Cattaneo *et al.*, 2008; Peterson, 2009).

2.9.1 Antimicrobial resistance of *Campylobacter* spp.

Antimicrobial resistance of *Campylobacter* spp. to fluoroquinolones, which are generally used for the empiric treatment of bacterial gastro enteritis, has increased during the past two decades, mainly as a result of the approval of this group of antimicrobials for use in food producing animals (Han *et al.*, 2009; Ommi *et al.*, 2016). The use of antibiotics is not usually indicated for the treatment of uncomplicated cases of Campylobacteriosis; however, severe systemic or chronic infections necessitates antibiotic therapy (Silva *et al.*, 2011). Therefore, the increase in antibiotic resistance of *campylobacter* isolates is an issue of concern, particularly resistance to macrolides, fluoroquinolones, and gentamicin, which are the drugs of choice for serious Campylobacteriosis (Duarte *et al.*, 2014). Resistance to fluoroquinolones is mainly due to a mutation (Thr-86-ile) in quinolone resistance determining the region (QRDR) of the gyr A gene (Iovine, 2013). As in other bacteria, in *Campylobacter* spp., point mutations in the peptidyl-encoding region in domain V of the 23s rRNA gene at the base position 2074, 2075 or both, have been associated with a high level of macrolide resistance (Vacher *et al.*, 2005).

2.9.2 Antimicrobial resistance of *Salmonella* spp.

Ciprofloxacin and cefotaxime are the most commonly used antibiotics for the treatment of invasive *salmonella* infections in humans (Bertrand *et al.*, 2006; Whichard *et al.*, 2007). However, multidrug resistant *salmonella* strains are becoming a real worldwide threat

(Bouchrif *et al.*, 2009a; Bouchrif *et al.*, 2009b; Egorova *et al.*, 2007; Espié *et al.*, 2005). Since 2002, in Europe, an emergence of ciprofloxacin resistant *Salmonella* spp. isolates have been reported in travellers returning from Northeast and East Africa (Adzitey *et al.*, 2012; Collard *et al.*, 2007). In African countries, multidrug-resistance *Salmonella* spp. strains exhibiting resistance to ciprofloxacin have been reported in several studies (Bouchrif *et al.*, 2008; Molla *et al.*, 2006; Sodagari *et al.*, 2015). For these reasons, food-borne diseases caused by *Salmonella* spp. are a significant public health concern around the world (Havelaar *et al.*, 2015; Oueslati *et al.*, 2016; Tauxe *et al.*, 2010). *Salmonella* spp are becoming increasingly resistant to antibiotics, making it more difficult to treat a patient with severe infections. This makes *Salmonella* serovars that are resistant to multiple antibiotics a continuous and an important subject area of research, and a major concern for food safety. For instance, Willford *et al.,* (2007) tested 21 strains of *salmonella* enterica serovar Newport and found 20 to be resistant to amoxicillin-clavulanic acid, ampicillin, cefoxitin, ceftiofur, cephalothin, chloramphenicol, streptomycin, tetracycline and sulfamethoxazole. Among these 20 isolates, some were also resistant to gentamycin, kanamycin and trimethoprim-sulfamethoxazole. One isolate exhibited complete resistance to all antibiotics tested (Willford *et al.*, 2007). Several studies have also shown that *salmonella* exhibits multi-drug resistance patterns (Adley *et al.*, 2011; Singh *et al.*, 2010; Suresh *et al.*, 2006; Yildirim *et al.*, 2011). Mutation in gyrase and isomerase genes have also been reported to be associated with fluoroquinolone resistance in multi-drug resistant *salmonella* isolates recovered from retail meats (Adzitey *et al.*, 2012; Yang *et al.*, 2012). Patients infected with *Salmonella* at the extremes of age and those who are immune-compromised, need an appropriate antimicrobial therapy (White *et al.*, 2001). This is because resistance to conventional antibiotics has increased, extended spectrum cephalosporin's and fluoroquinolones have been chosen to treat infections caused by multi-drug resistant *salmonella* (Su *et al.*, 2004).

2.9.3 Antimicrobial resistance of *Staphylococcus aureus*

For the past years, this organism has established as a frequent multiple antibiotic resistant (MAR) bacteria, particularly to methicillin and vancomycin that may cause nosocomial or community infections (Abdulgader *et al.*, 2014; Abdulgader *et al.*, 2015 Boucher & Corey, 2008 ; Tong *et al.*, 2015; Vandenesch *et al.*, 2003). The emergence of MAR *S. aureus* spp. poses therapeutic challenges to health, veterinary professionals and dairy cattle producers (Hiramatsu *et al.*, 2014). In recent years, a systematic growth in the number of antibiotic resistant staphylococcal strains in the human environment has been observed (Fijałkowski *et*

al., 2016). Most research concerning antibiotic resistance of staphylococci isolated from food focuses on *S. aureus* spp., whereas less attention is paid to other species (Gao *et al.*, 2012). Antibiotic resistant strains other than *S. aureus* spp. were also found in food (Guran & Kahya, 2015) and genes encoding microbial resistance to tetracycline, erythromycin and ß-lactams have been detected in CoNS (Coagulase-negative staphylococci) isolated from starter cultures, probiotic bacteria, fermented food and meat (Chajęcka-Wierzchowska *et al.*, 2014; Guran & Kahya, 2015). Different species of Staphylococci have also been suggested as a reservoir of antibiotic resistance genes (Chajęcka-Wierzchowska *et al.*, 2014), which can be transferred to *S. aureus* spp., making it resistant to multiple agents (Al-Masaudi *et al.*, 1991).

2.9.4 Antimicrobial resistance of *Escherichia coli*

Antimicrobial resistance among pathogens has become an emerging threat to human and veterinary medicine. This is due to the excessive and indiscriminate use for treatment, prophylaxis or growth promotion. With increased multi drug resistance, *Escherichia coli* has now become a major public health issue, both in developed and developing countries (Chandran *et al.*, 2008; Okeke *et al.*, 2005). *E. coli* has been the foremost indicator of faecal contamination and possible incidence of waterborne/ food-borne diseases that are most injurious to health and a significant reservoir of genes coding for antimicrobial resistance and, therefore, is a useful indicator of resistance in bacterial communities (Bucknell *et al.*, 1997; Organization, 2001; Tadesse *et al.*, 2012). *E. coli* has been reported to acquire and transfer virulence and antimicrobial resistance genes to enteric pathogenic and normal flora bacteria in the environment through horizontal transfer of resistance plasmids, transposons and integrons (Ozgumus *et al.*, 2009).

CHAPTER 3

OBSERVATIONAL STUDY

3.1 INTRODUCTION

Food contamination is a result of several factors such as storage, manipulation and handling by vendors. In addition, the attitude and practices of vendors as well as consumers has a great impact on the quality of food. It has been argued that observations, as opposed to self-reports, provide the best descriptive data concerning the food preparation practices of food workers (Clayton & Griffith, 2004). Hence, in this study, observations were done in order to provide detailed information about the hygienic behaviour of street vendors during food handling and hand washing activities. Such information is valuable for improving public health by reducing food contamination. It also allows for the identification of areas in need of improvement and subsequent targeted interventions. In this study, particular focus was on the attitude and practices of vendors on the main streets of Johannesburg Central Business District (CBD) in order to assess possible public health risks for consumers.

Data collection was done through observation of twenty critical aspects related to the general hygiene attitude and practices of street-venders selling meat and meat products as well food hygiene and safety. Observation was done using structured check list used in previous studies (Campbell, 2011; Chukuezi, 2010b; Muinde & Kuria, 2005; Organization, 2002).

3.2 OBJECTIVE OF THE STUDY

This chapter provides an assessment of the general practices and attitudes of street-venders selling ready-to-eat meat in preparation for sampling for microbial analysis.

3.3 RESEACH METHODS

3.3.1 Study area

The study was conducted in three different areas around Johannesburg CBD. The central Business District, commonly called Johannesburg CBD, is one of the main business centres of Johannesburg, South Africa. It is the most dense collection of skyscrapers in Africa, however due to white flight and urban blight, many of the buildings are unoccupied as tenants have left for more secure locations in the Northern Suburbs, in particular Sandton and Rosebank. The Central Business District is located on the southern side of the prominent ridge called the Witwatersrand (Afrikaans: White Water's Ridge) and the terrain falls to the north and south. By and large the Witwatersrand marks the watershed between the Limpopo and Vaal rivers as the northern part of the city is drained by the Jukskei River while the southern part of the city, including most of the Central Business District, is drained by the Klip River. The north and west of the city has undulating hills while the eastern parts are flatter. The city enjoys a sunny climate, with the summer months (October to April) characterised by hot days followed by afternoon thundershowers and cool evenings, and the winter months (May to September) by dry, sunny days followed by cold nights (www.wikipedia.org/wiki/johannesburg). Temperatures in Johannesburg are usually fairly mild due to the city's high elevation, with an average maximum daytime temperature in January of 25.6 °C (78.1 °F), dropping to an average maximum of around 16 °C (61 °F) in June. The UV index for Johannesburg in summers is extreme, often reaching 14-16 due to the high elevation and proximity to the equator (www.wikipedia.org/wiki/johannesburg). Winter is the sunniest time of the year, with mild days and cool nights, dropping to 4.1 °C (39.4 °F) in June and July.

The study was conducted in this Street listed below:

- MTN-taxi rank (considered as the main research site);
- Bree Street, considered as the second main site; and
- Hancock Street, corner Claim, considered as the third main research site. The three streets were study was conducted can be seen in figure 1, 2 and 3

Figure A showing Plein Street in red

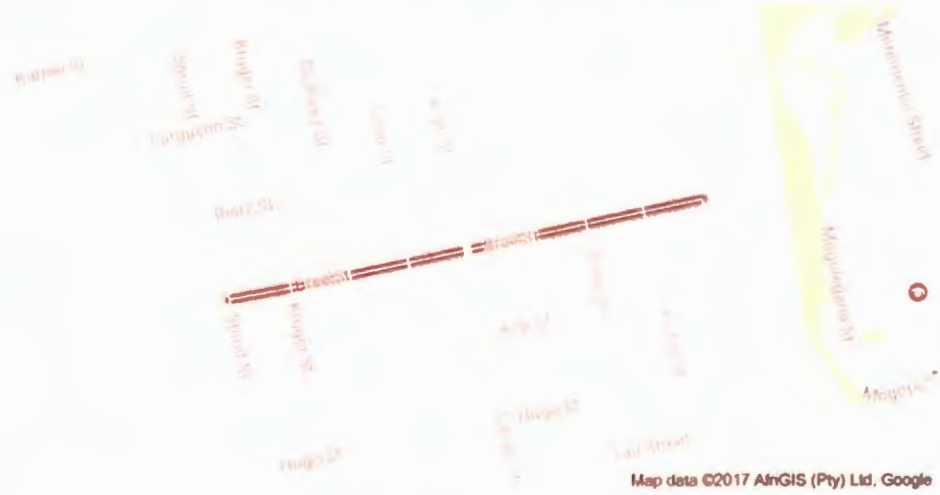

Figure B showing Bree Street in red

Figure C showing Hancock Street in red

3.3.2 Study design

A cross-sectional observational descriptive study was conducted around Johannesburg CBD, South Africa from December 2015 to April 2016 in order to assess and evaluate the general hygiene practices of vendors of street-vended foods towards the food safety of street-vended ready-to-eat meats sold in streets around Johannesburg CBD. This design was chosen as it would provide information on hygienic practices of Street vended meat.

3.3.3 Data collection and instrument used

The observations were done using a pre-structure check list form to assess food safety practices of street-vended food by adapting different check lists used in previous studies (Campbell, 2011; Chukuezi, 2010b; Muinde & Kuria, 2005). One hundred and fifteen participants were assessed in order to determine the hygiene practices. Recordings were done of all observations at the vending sites included food safety practices (see appendix 1). Among aspects taken into consideration during this study were:

a) Hygiene at vending sites

Vending sites were assessed for the presence or absence of the following:

- Stagnant water around the vending sites;
- Flies; and
- Salubrious.

If one of these aspects was found, a tick (or yes) was made on the list, if nothing was found; a cross (or no) was made on the list.

b) General hygiene practices of street vendors

Hygiene practices were assessed by observing for the following practices at time of sampling:

The observations focused on:

- Washing of hands before handling foods;
- Washing of hands after touching money;
- Washing of hands after handling waste foods;
- Handling money while serving food;
- Touching money with gloves;
- Washing of hands with cold water only;
- Washing of hands with cold water and soap;

- Presence of food debris on vendor's hands;
- Wearing of gloves during preparation; and
- Touching money with gloves.

If washing of hands was observed, the answer was (yes), if not, the answer was (no).

c) Assessment of food hygiene

Food hygiene was assessed by observing the following hygiene practices:

- Exposure of food to flies and dust;
- Storage of food in a cooler box;
- Using of polythene bags for serving food; and
- The presence of foods debris on vendor's hands.

During sample collection, if the foods were found to be exposed to flies or dust (not covered), the answer was (yes), if not, the answer was (no).

d) Assessment of the serving utensils

To assess the hygiene status of serving utensils the following were observed:

- Cleaning dishes with clean water and soap;
- Cleaning dishes with the same water;
- Changing water to clean dishes;
- Cleaning tables with a clean cloth; and
- Cleaning tables with the same cloth.

If any aspect was applicable, the answer was (yes), if not applicable, the answer was (no).

3.4 STATISTICAL ANALYSIS

Data was analysed using the Statistical Package for the Social Sciences (SPSS) version 20.0. Bar chats, pie charts, and histograms were used to summarise the percentage frequencies of Cochran's Q test was used to determine the difference between the three streets in terms of the following variables: hygiene practices at each street; and types of meat sold per street. Cochran's test was preferred because it is meant to determine the change in categorical data (with a dichotomous trait) over a number of observations (Pett, 2015). Hence, it was suitable for the variable 'Hygiene practices' because it had a dichotomous trait (1 = yes and 2 = no).

3.5 RESULTS

It was observed during the survey that 90.63, 77.42 and 68.89 % of vendors at MTN-taxi rank, Bree Street and Hancock Street respectively were found exposing their meats to dust and flies as a result of not selling in suitable containers with aseptic covers (Figure 3.1 to .3). It was also observed that 94.4, 92.31 and 87.5% of the vendors handles money while serving food at MTN taxi-rank (Figure 3.1), Bree Street (Figure 3.2) and Hancock Street (Figure 3.3) respectively. Furthermore, Results obtained from observational study show that salubrious around MTN-taxi rank was observed at 65.63% (Figure 3.1) and 55.56% at Hancock Street (Figure 3.3) while no salubrious was observed in Bree Street, Also, 37.5,7.14 and 41.67% of the vendors were observed to have food debris on their hands at the various sales points respectively. The presence of stagnant water around vending sites was also observed at the MTN taxi-rank (21.88%) and Hancock Street (55.56%) while stagnant water was not found in vending site at Bree Street. It was observed that only few percentages (16.67%) of the vendors at the MTN taxi-rank (Figure 3.1) were washing plates with clean water and soap while others in the two streets did not clean their plates at all with clean water. Only 2.2% of vendors change water when cleaning dishes while only 22.2 % of them uses a clean cloth to clean tables after serving customers. Other hygienic practices observed were washing of hands after handling waste food, washing of hands with only water or with water and soap, use of polyethylene for serving meats was very high 90.32% at Bree street corner Plein street (Figure 3.2) and all the results of observational study are presented in figure (3.1) for MTN taxi-rank, (3.2) for Bree Street and (3.3) for Hancock Street respectively. Statistical comparisons between the three streets of observation using the Cochran's Q test revealed that there existed a significance difference in some hygienic practices such as presence of stagnant ($P=0.016$), exposure of foods to flies and dust ($P=0.015$) and usage of polythene bags for serving foods ($P=0.000$) at the 95% confidence level ($P<0.05$) among food vendors in the three streets, while there were not significant difference in all other hygiene practices among the food vendors.

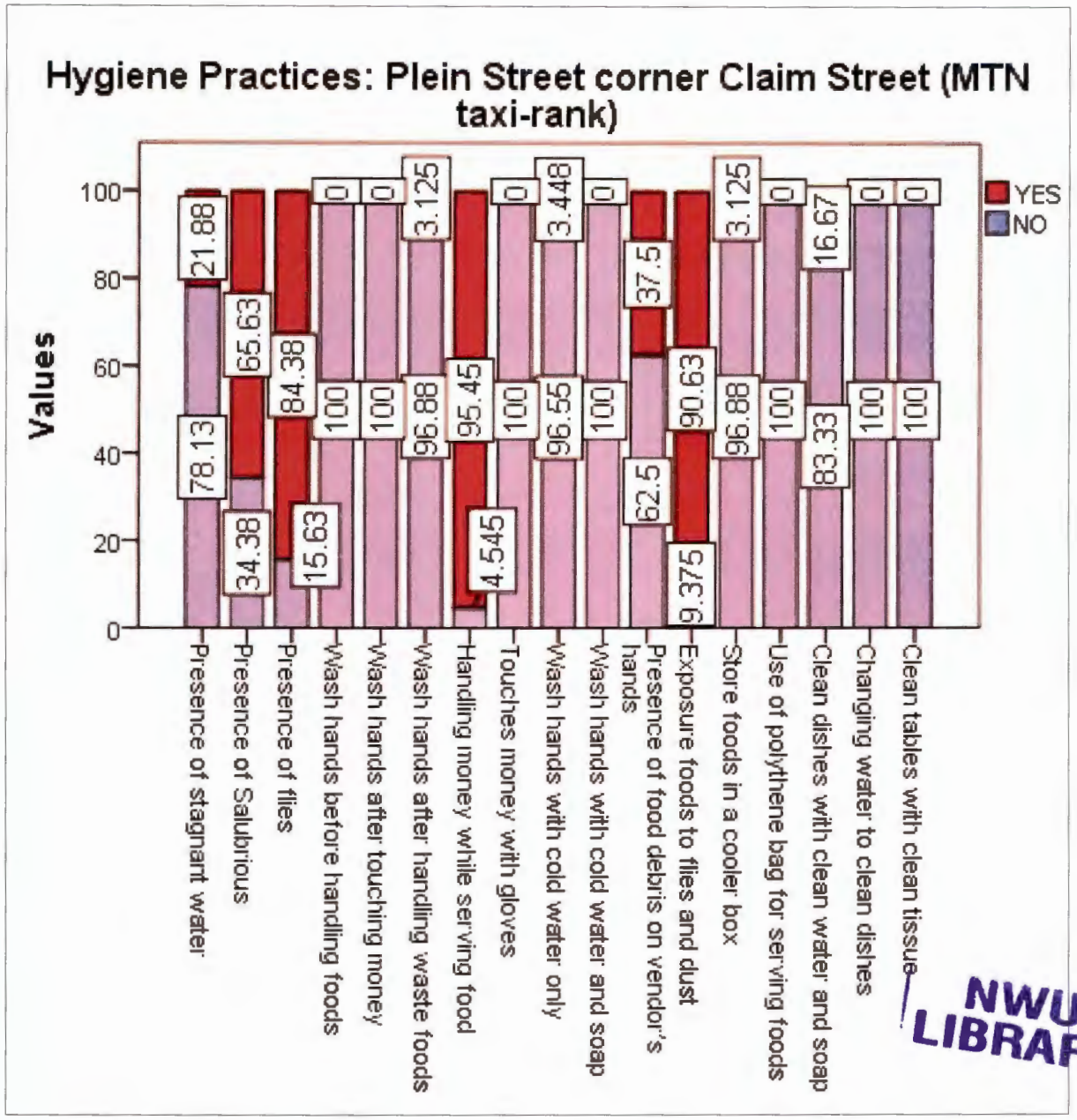

Figure 3.1: Summary of hygiene practices around MTN taxi-rank

The Figure shows that there was a frequent presence of salubrious (65.63%), a frequent presence of flies (84.38%), many occasions of handling money while serving food (95.45%), frequent exposure of food to flies and dust (90.63%), some degree of presence of food debris on the hands of vendor (37.5%) and some degree of presence of stagnant water (21.88%), washing of hands after handling waste food (3.125%), washing of hands with cold water only (3.448%), storing of food in a cooler box (3.125%) and washing of dishes with clean water and soap (16.67%) around MTN taxi-rank.

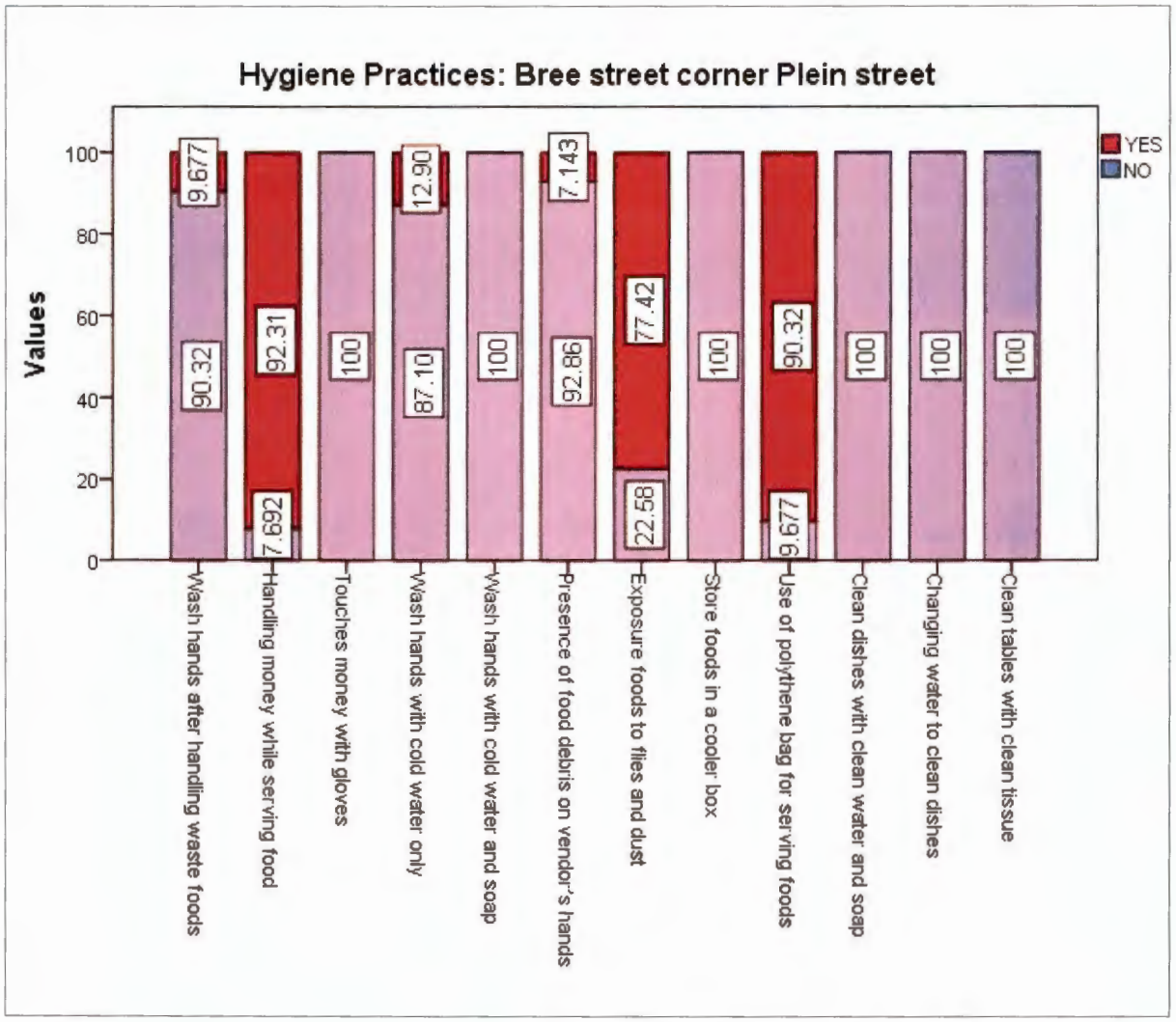

Figure 3.2: Summary of hygiene practices around Bree Street (corner Plein Street)

The Figure shows that washing of hands after handling waste foods represented 9.67%, washing of hands with cold water only stood at 12.9% and the presence of food debris on the hands of vendors represented 7.14%. Handling of money while serving food was represented 92.31%, exposure of food to flies and dust stood at 77.42% while frequent use of polythene bags for serving meats stood at 90.32%.

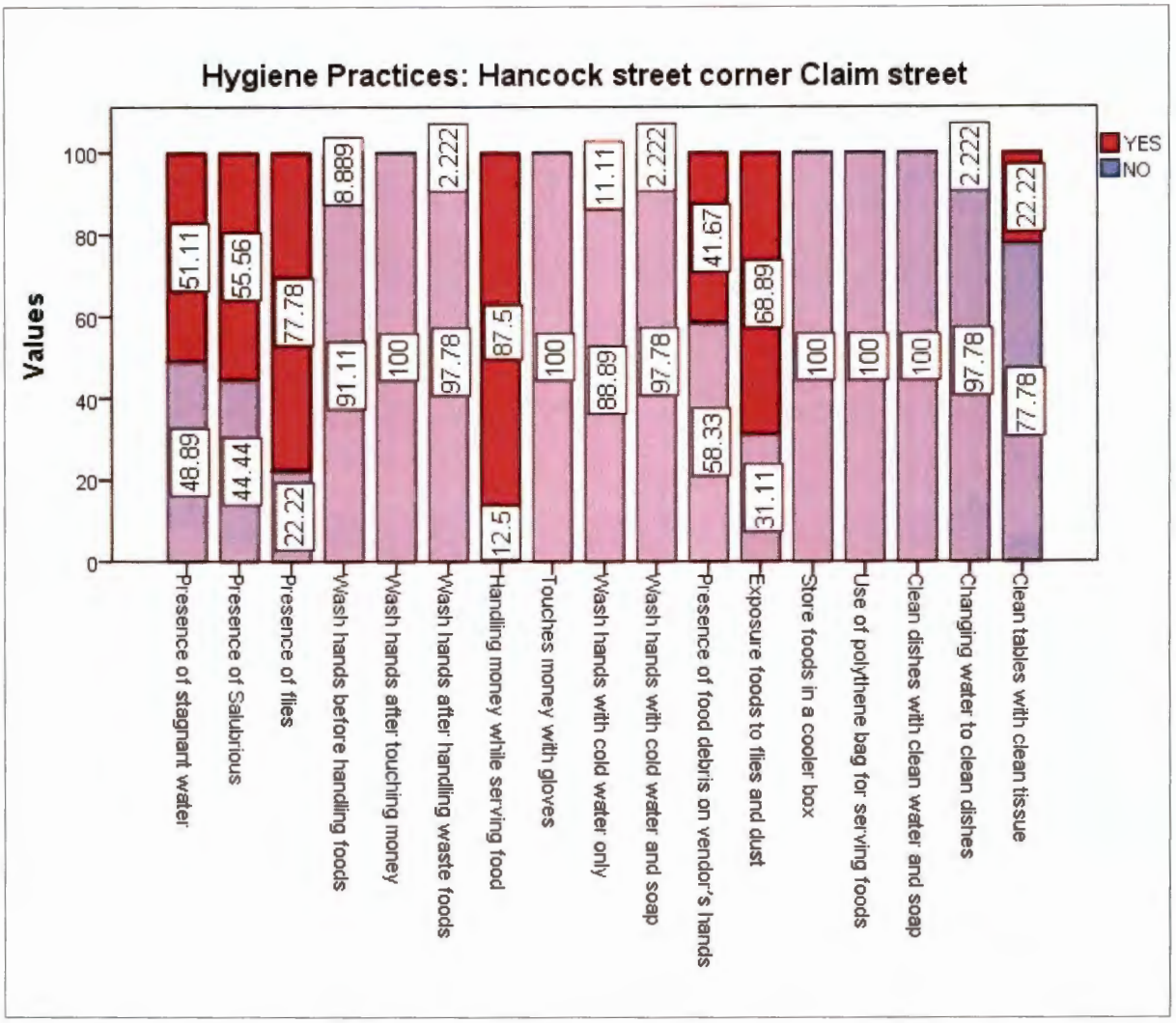

Figure 3.3: Summary of hygiene practices around Hancock Street (corner Claim Street)

Results presented in Figure 4.3 summarises the hygiene practices of vendors of street food around Hancock Street (corner Claim Street). The results show that the cleaning of tables with a clean cloth is done by 22.2 % of vendors; only 8.9% of vendors wash their hands before handling food; while 2.2% wash their hands after handing waste food. In addition, the results obtained reveal that 11.1% of vendors wash their hands with cold water while only 2.2% of them use clean water and soap to wash their hands. 2.2% of vendors pour in clean water to clean dishes around Hancock Street (corner Claim Street) while 87.5% of vendors handle money while serving food to customers. In addition to hygiene practices, it was also observed that 55.5%; 55.56 % and 68.7% respectively of vendors sold food around stagnant water, where there is the presence of salubrious, and in areas exposed to flies and dust.

Table 3.1: Statistical comparison of observation frequencies between the three sampling areas

Statistical comparisons (Table 3.1) between the three areas of observation using the Cochran's Q test revealed that there was statistical significance for the presence of stagnant water (P=0.016) between the three sites and the exposure of foods to flies and dust (P=0.015). The usage of polythene bags for serving foods (P=0.000) were significantly different across the three Streets while all other hygiene practices did not show any frequency correlation between the areas of observation.

	Test statistics	
Presence of stagnant water	Cochran's Q	8.273
	P-Value	0.016
Presence of salubrious	Cochran's Q	2.889
	P-Value	0.236
Presence of flies	Cochran's Q	1.263
	P-Value	0.532
Wash hands before handling foods	Cochran's Q	2
	P-Value	0.368
Wash hands after handling waste foods	Cochran's Q	1.6
	P-Value	0.449
Wash hands with cold water only	Cochran's Q	2
	P-Value	0.368
Expose foods to flies and dust	Cochran's Q	8.444
	P-value	0.015
Store foods in a cooler box	Cochran's Q	2
	P-value	0.368
Use of polythene bags for serving foods	Cochran's Q	56
	P-value	0.00

3.6 Discussion

The aim of this study was to assess hygienic practices related to food safety of street vendors around Johannesburg CBD before sampling. The result of this survey revealed that unhygienic practices during food preparation, sales and even within the vending environment were occurred more among street vendors.

It was observed higher percentage of salubrious around MTN-taxi rank (65.63%) and Hancock Street (55.56%) which can lead to the contamination of street meat due to it accumulation. However, results obtained in this study are in agreement with the findings of other studies conducted by different researchers (Bryan *et al.*, 1997; Hanashiro *et al.*, 2005; Lues *et al.*, 2006; Mosupye & von Holy, 1999; Muinde & Kuria, 2005; Umoh & Odoba, 1999). These researchers concluded that the accumulation of garbage which provides harborage for insects and pest around vending sites may expose food to contamination. Mensah *et al.* (2002) and Muinde *et al.* (2005) who conducted similar studies on street foods in Nairobi, Kenya, and Accra, Ghana, recommends that foods should be prepared in a place set aside exclusively for such purposes, while the place of preparation should be kept clean at all time and should be far from any sources of contamination which was not a case in this study.

It was also observed that washing of hands before handling foods and washing with soap and water is not a common practice among the food vendors .Less than 3% of the vendors in Hancock Street washed their hands with water and soap before handling foods while none of the vendors in MTN taxi-Rank and Bree street practice washing with water and soap. In addition, most of the vendors collect money while handling or selling their foods. Handling money while serving foods was ranged at 95.45% (MTN taxi rank), 87.5% (Hancock Street) and 92.31% (Bree Street). Usually, money as a means of exchange is transferred from one person to another and in the process deposition of microbes on the currency might have occurred. So handling money while selling food is an unhygienic practice which could lead to contamination of food. Washing of hands with soap and water should be a usual practice of both the vendors and the consumers as this could help prevent a lot of food borne diseases and infection. E.g., during the outbreak of Ebola virus in Nigeria in 2015, one of the recommendations of WHO was constant washing of hands with soap and water which really helped in curbing the spread of the disease in the nation.

In addition, Ferro *et al.* (1993) and Muinde & Kuria, 2005 discovered that the hands of food vendors are usually the most critical means of transmitting pathogens from contaminated

places and items, and hence could result in cross contamination upon contact with foods. Such is the case when vendors use their hands to handle money from consumers. This could further aggravate the situation due to the possible accumulation of dirt on the money. In addition, the low level of sanitation and poor hygienic practices of food vendors could be due to lack of proper and formal education of the vendors on acceptable and ideal hygienic practices. Singh *et al* (2016) in his study on the impact of health education intervention on food safety and hygiene of street vendors commented that education of street vendors is essential in maintaining hygiene and hence safety of food. Behling *et al.* (2010) also specified that contact of the hands with foods represents a potentially important mechanism through which pathogens may enter the food supply. These results are also similar to the findings of Rane (2011) who observed that improper food handling can lead to the transfer of pathogens bacteria such as *Salmonella*, *E. coli* and *S. aureus* from the human body and the environment into food. This result is in line with the findings of Kumar Singh *et al* (2016). In their study on the impact of health education intervention on food safety and hygiene of street vendors, the authors noted that education of street vendors is essential in maintaining hygiene and hence, safety of food.

The high prevalence of exposure of foods to flies and dust was observed at MTN taxi rank (90.63%), Bree Street (77.42%) and 68.89% around Hancock Street. Flies were found present in the entire sales environment and in addition the meat samples were exposed to flies and dust especially at the MTN taxi- rank where over 80% of the vending environment was filled with flies. Flies are vector agents for most of these pathogenic organism and also aids in spreading of food borne infections like cholera, dysentery etc. The presence of flies around vending place is an indication of poor environmental and sanitation condition and it could also be as a result of poor waste management. The flies after perching on the decayed food from the dust bins gets in contact with the microorganisms in the decayed food and will later deposit the microbes on the uncovered meat. These results are consistent with the findings of Mayanja *et al.* (2011) who also noted that uncovered foods can be subjected to airborne diseases and microbes which may be pathogenic if allowed to settle on prepared food surfaces filled with dust. This concurs with the recommendations of the Food Agriculture Organisation (FAO) that food must be adequately protected from flies and dust to avoid any food safety threats (Chukuezi, 2010b; Cuprasitrut *et al.*, 2011). These results also correlate with the findings of several other studies conducted on food hygiene by several researchers (Ashenafi, 1994; Bryan *et al.*, 1992; Derbew *et al.*, 2013; Sobukola *et al.*, 2009; Rayza *et al.*, 2015). These authors specified in their study that open areas, close to the roadside which

expose food to dust and flies become contaminated either by spoilage or pathogenic microorganisms.

It was also noticed that 3.12% of vendors store foods in cooler boxes at MTN taxi rank while 0% of vendors at Bree and Hancock Streets. Instead food was kept in plastic buckets and casseroles. In some instances, the food was already cooked and ready to be eaten. This situation can increase the risks of multiplication of microorganisms in ambient temperatures. Holding food at ambient temperature for long periods of time have been reported to be a major contributor to the occurrence of food poisoning (Rane, 2011). This result is in line with the findings of several other authors (Ekanem, 1998; Lues *et al.*, 2006; Martínez-Tomé *et al.*, 2000; Unnevehr, 2000; Walker *et al.*, 2003). Who in their study specified that lack of cooler boxes to keep food could adequately pose a health risk, especially in warmer seasons when food is displayed for a long period of time.

It was observed during the survey that vendors do not clean dishes with clean water and do not use soap as well during the cleaning process. Only 2.2% of vendors change water when cleaning dishes while 22.2 % applied unhygienic methods (clean utensils with cloth). These findings correlate with another study conducted on food hygiene by Bryan *et al.* (1998). The authors found that washing utensils with only cold water without regularly changing water promotes cross contamination from utensils to cooked foods. This is largely caused by greasy and dirty water used to rinse dishes, thus posing a public health risk to consumers. Bryan *et al.* (1988) reported also that major contamination of street food that occurs at vending sites are due to cross contamination, during chopping, cutting, cooking and serving.

The majority of vendors (90.32%) used polythene bags when serving customers at Bree Street (Figure 3.2). Mensah *et al.* (1997) specified that polythene bags are usually contaminated by food handlers and pathogens may invade the interior surface of polythene bags during packaging and poor handling practices of vendors, hence the use of polythene bags for serving ready to eat meat or food could increase the risk of food contamination exposing consumers at risk (Mensah *et al.*, 1997; Nicolas *et al.*, 2007). Poor storage of polythene bags contributes to their contamination with microorganisms from the environment (Nicolas *et al.*, 2007). Studies on the use of high density polythene (HDP) and low density polythene (LDP) bags for packaging of sliced watermelon showed that bacteria and fungi survived and increased in both packages when stored at ambient temperatures (Nwachukwu *et al.*, 2008). It was further discovered in the study that the frequency of the presence of

stagnant water (P=0.016), exposure of food to flies and dust (P=0.015) and of the usage of polythene bag for serving foods (P=0.000) were significantly different across the three areas.

3.7 CONCLUSION

The observation study revealed that bad hygiene practices occurred more frequently than good hygiene practices, the survey also revealed the poor hygienic conditions under which street vendors manipulate and prepare meat and the quality of the surrounding environment in which food was sold. These findings show that, there is an imperative need for local public health authority to get involved in the continuous training and education of vendors on issues around food safety and hygiene and environmental implications in food quality.

CHAPTER FOUR

MICROBIAL PROFILE OF READY TO EAT MEAT SOLD IN THE INFORMAL MARKET

4.1 INTRODUCTION

Consumption of unclean food can cause gastrointestinal diseases. The number of bacterial in food determines the quality of the food. Consumption of contaminated food can cause diarrhea, nausea, vomiting, fever, headache or even death.

4.2 Aim of the study

The main aim of this chapter was to use qualitative and quantitative approaches to determine the food-borne pathogens in meat sold in the streets around Johannesburg CBD by using conventional biochemical test and molecular examination (DNA extraction, PCR and sequencing)

4.3 Objectives of the study

The specific objectives of this chapter were to:

- Analyse ready-to-eat meat sold in street of Johannesburg CBD for bacterial contamination;
- Determine the microbiological profile of ready-to-eat meats sold in streets around Johannesburg Central Business District (CBD);
- Identify bacterial isolates using preliminary biochemical tests (Gram staining, Catalase, Oxidase, voges proskauer, indole, and APIs) and to confirm isolates using PCR and sequencing;
- Classify and genotype the isolates bacteria

4.4 Significant of the study

This study provided information on microbial quality of ready-to-eat meats sold in the three streets of Johannesburg CBD under the study and their antimicrobial resistance profiles.

4.5 Materials and methods

4.5.1 Study site

Meat samples were collected from three different streets around Johannesburg Central Business District (CBD) namely:

- MTN-taxi rank;
- Bree Street (corner Plein street); and
- Hancock Street (corner Claim).

All those three streets have high numbers of vendors, and there are many potential customers for vendors.

4.5.2 Study design

This was cross-sectional study conducted around Johannesburg CBD, South Africa from November 2015 to April 2016 in order to evaluate microbial quality of street-vended ready-to-eat meats sold in streets of Johannesburg Central Business District (CBD).

4.5.3 Collection of samples

The targeted study sample for this study was 100 for the all three Streets, in order to be representative and able to draw good conclusions. Hundred and fifteen (115) meat samples were purchased randomly twice a week for 12 consecutive weeks (Table 4.0). On the day of sampling, meat samples were collected during the day between 12:00 to 14:00 hours. All samples were immediately placed in a cooler box and transported to the Microbiology Laboratory at the Animal Health Department, Mafikeng Campus, North-West University for further analysis.

Table 4.0: Summary of sample collection sites and quantity

Meat samples	Collection sites/ streets	Quantity per street
Chicken meat	Bree Street (corner Claim St)	25
Chicken gizzard	MTN-Taxi rank	21
Beef head meat	Hancock Street (corner Claim Street)	24
Beef intestines	MTN-Taxi rank	20
Wors	Bree Street (corner Claim St)	25
Total	For the three Streets	115

4.5.4 Sample preparation and homogenisation

Meat samples were wrapped and minced using sterilised knives and forceps. Twenty five grams (25g) of meat samples were weighed aseptically under biological safety cabinet class II (Model, 4BI02, Serial, 4BI020158. South Africa) with a balance bel engineering (Model A1b1001. Italy) and added to 225 ml of nutrient broth in order to obtain one part of sample and nine parts of nutrient broth (1:10). The inoculum were incubated aerobically at 37°C for 24 hours in a checker incubator Labcon (Model, fsies p024, serial C2421, South Africa) for enrichessment (Apha, 1998).

a) Serial dilution

From the homogenised inoculum (original solution), 1 ml was pipetted and added in test tube containing 9 ml of sterile nutrient broth and homogenised using vortex mixer (Lab net int So 200-230 V-EU, Serial #: Z 0061666 USA) at 260 rpm (revolution per minutes) for 2 minutes. The diluted inoculum was then used as base solution to make an additional dilution (10^{-2}), from 10^{-2} 1m was pipetted and added to 9 ml of sterile nutrient broth to make 10^{-3}, the processes was repeated in a range of concentrations until 10^{-5}. In order to avoid any contamination, the process was performed inside Biological safety cabinet class II.

4.5.5 Bacterial isolation and total bacterial counts

From the 10-fold dilutions of the homogenate inoculum, 1ml of 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} dilutions were pipetted from test tubes (15ml) and plated on nutrient agar, using L-shape sterile glass rod for surface spread method. The process was performed inside Biological safety cabinet class II to avoid any contamination. Plates were incubated aerobically at 37°C

for 48 hours (Apha, 1998) in a room incubator (Ecr manufacturing, model, Su 131h, Serial 128538. South Africa). After incubation for 48 hours at 37°C, colonies were counted using colony counter (Model #81-520-000 Bioresearch supplies: Perry hall, USA). The counts for each plate were expressed as colony forming units per gram (CFU/g). Coliform were isolated using MacConkey agar (Apha, 1998). For control and quality assurance, 1 ml of sterile nutrient broth was plated on sterile nutrient Agar and MacConkey agar, the plates were incubated aerobically at 37°C for 48 hours using room incubator. After the incubation, the absence of growth on the control plate confirmed the sterility of the environment where samples were cultured. Isolated bacteria were sub-cultured, on fresh nutrient agar and the plates were aerobically incubated at 37°C for 24 hours. In order to obtained pure culture (Figure 4.1) bacteria were sub-cultured 3X.

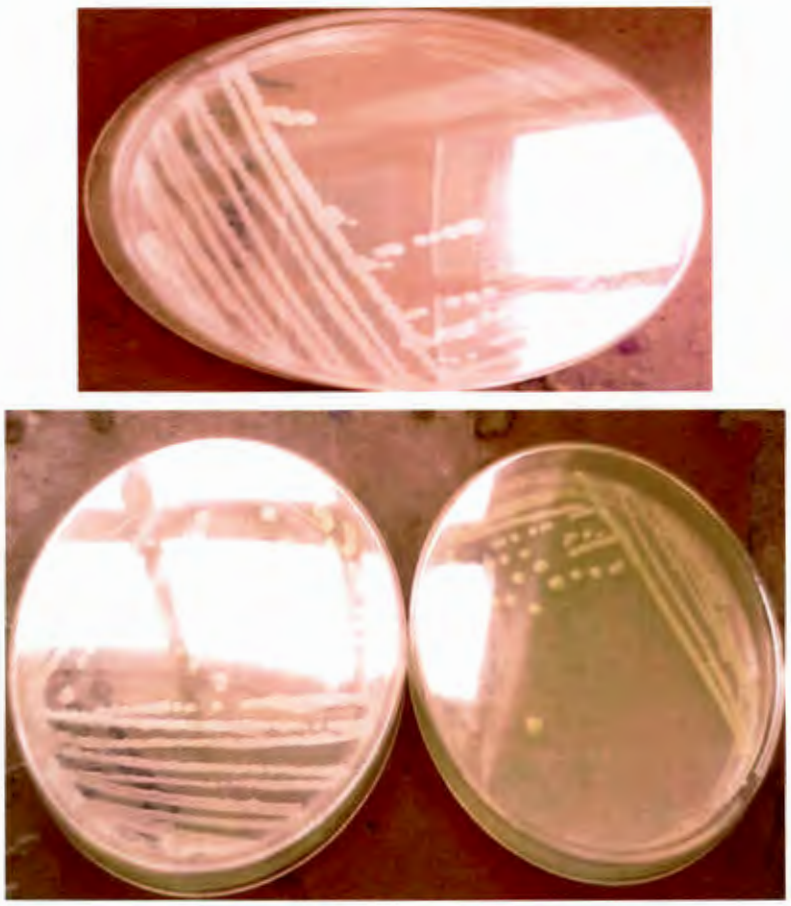

Figure 4.1: Showing pure isolated bacteria on nutrient agar

4.5.6 Biochemical test

a) Gram's staining

Gram's staining was performed on each colony using standard methods as follows: the single colony was picked up from a pure culture with a sterile wire loop and smeared on the slide with few drops of running tap water and fixing on the slide by softly heating for 30-45 seconds; and crystal violet was applied on the stain for one minutes followed by washing the slide with sterile water. Few drops of iodine solution were then applied on the stain for one minute and the slide washed again with running tap water. After, a few drops of ethanol 95% were applied on the smear slide till to decolorise the slide, and then the slide was washed again with running tap water. Finally, few drops of safrim solution were dropped on the slide as a counter stain for 1 minutes, the slide was then washed with running tap water, blotted and dried in air and examined by a light microscope (Axio star plus, Serial. 3108001956) at 400x using immersion oil (Fawole & Oso, 2004; Purkayastha *et al.*, 2010).

b) Catalase test

A catalase test was performed to differentiate bacteria that produce the enzyme catalase called, catalase positive like, *Staphylococci*, *Enterobacteriaceae* from non-catalase bacteria like streptococci. The catalase test was fallowing a method described by Montso & Ateba. (2014). From a pure culture, a single bacterium was collected with a sterile wire loop and smeared on the glass slide, then 2 to 3 drops of hydrogen peroxide 100 Vol (SAAR3063820LP) were dropped on top of the bacteria. If the isolates were catalase positive, bubbler of oxygen were released after 10 seconds, if the isolates were catalase negative, no gas was produced (Cheesbrough, 2006).

c) Oxidase test

An oxidase test was performed following the method described by Ateba and Setone, (2011) to identify bacteria that produce cytochrome oxidase. The isolates were subjected to sub-culturing on fresh nutrient agar and incubated at 37°C for 24 hours, using a room incubator (ecr manufacturing, model su 131, serial 128538. South Africa). After 24 hours of incubation, a single isolate from the pure culture, were smeared over oxidase strips paper (Microbact identification kits, MBO266A). The result was read after 5 to 10 seconds. A positive result was indicated by an intense blue coloration after 5 to 10 seconds. A delayed positive result

appeared within 10 to 60 seconds and a negative result was observed by the absence of coloration or by later coloration after 1 minute.

d) Voges proskauer test

Voges Proskauer test was performed as described by Manga & Oyeleke .(2008). Bacterial cultures were inoculated in MR-VP medium, and incubated at 37°C overnight. After incubation, 20 drops of Barritt's reagent A and Barritt's reagent B were added to the inoculum and shaken carefully. The presence or appearance of red colour within 15-20 minutes was an indication of a positive result.

e) Indole test

Indole test was performed as described by Hassan *et al.* (2010). This test was performed using Kovaco's reagent. Tryptone broth were inoculated with the test organism and incubated at 37°C for 24–28 hours. After 24 hours, 3–5 drops of Kovac's reagents were added and the red colour ring at the upper layer of the liquid (Figure 4.2) was an indication of indole positive while yellow/no colour was an indication of indole negative.

Figure 4.2: Picture showing an indole positive test

4.5.7 Analytic profile index (API staph)

Twenty-five bacterial isolates suspected according to conventional biochemical tests results were re-examined using API-staph idem system (BioMérieux, France). API-staph was used to identify Gram positive such as *staphylococcus* spp. for confirmation according to Downes & Ito (2001). The tests were performed according to the manufacturer's instruction. From a fresh nutrient agar culture, pure colonies were picked up with a sterile wire loop to make bacterial suspensions and mixed with API staph medium provided. The microtubules were inoculated with the suspensions as instructed. The strips were incubated using a room

incubator for 24 hours. Results were read with the addition of reagents. Indices were generated for the different isolates and used to determine their identities using the IPI web identification software.

4.6 Molecular identification of bacterial isolates

Molecular identification has provided powerful tools to investigate microbial communities at the level of species. This technique provides informative insights about bacteria, possible kind of bioactive compounds, and if it novel or not (Donate-Correa *et al.*, 2005). The identification of bacterial isolates in the present study was based on 16S-rDNA gene sequence analysis. Numerous methodologies were developed in this study to examine isolated bacteria and microbial diversity (Fakruddin & Mannan, 2013; Kirk *et al.*, 2004). These techniques involved DNA extraction, PCR amplification, Agarose gel electrophoresis and phylogenetic investigation.

4.6.1 Extraction of genomic DNA

The extraction of genomic DNA was performed as described by Ngoma *et al.*, (2013). Pure bacteria isolated from nutrient agar (after sub-culture for 24 hours aerobically) were inoculated into 5 mL of nutrient broth and incubated aerobically at 37°C for 24 hours. The inoculum product was then transferred into 15 mL conical tube and centrifuged at 15000 rpm for 10 minutes, the supernatant (pellets) were collected and used for DNA extraction using zymo Research kit (Zymo-Research fungal/Bacterial Soil Microbe DNA, D6005 USA supplied by Bio lab, South Africa) as follows: pellets were suspended in 750 µL lysis solution, and disrupted with disruptor gene (Inquaba biotech, mode SID258, USA) and vortexed at 14.000 rpm for 14 minutes followed by centrifugation at 10.000 rpm for 1 minute. Four hundred µL of the upper aqueous phase was aliquoted into a new zymo-spin IV™ and centrifuged at 7000 rpm for 1 minute. 12.000 µL of buffer was added to the filtrate and 800 µL of the mixture was transferred to a new collection tube (Zymo-spin IIC™) and centrifuged at 10.000 rpm for 1 minute. The filtered DNA was pre-washed by adding 200 µL DNA pre-wash buffer and centrifuged at 10.000 rpm for 1 minute. 500 µL of DNA wash buffer was added to the new collection tube (Zymo-spin IIC™) and centrifuged at 10.000 rpm for 1 minute. Finally, 100 µL of DNA elution buffer was added to elute the DNA in a sterile 1.5 mL micro-centrifuge tube.

4.6.2 Amplification of 16S rDNA

The amplification of 16S rDNA gene was performed using polymerase chain reaction with the help of an Engine DYAD Peltier thermal cycler (Bio Rad. USA). A reaction volume of 25 µL containing 12 µL PCR Master Mix, 1 µL template DNA, 10 µL nuclease free water and 1µL of each oligonucleotide primer were prepared and mixed in PCR tubes (Ngoma *et al.*, 2013; Ngoma *et al.*, 2014). For the amplification of 16S rDNA gene, the procedure was performed according to (Ngoma *et al.*, 2013). The PCR was conducted using the Universal primers, forward 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and the Reverse 1492R (5'-TGA CTG ACT GAG ACG TTG CGA-3'). These primers were commercially synthesised by Inqaba biotechnical Industrial (Pty) Ltd (Pretoria, South Africa). The thermocycling (Bio-Rad T100™ thermal cycler, Singapore) conditions consisted of an initial denaturation step at 95°C for 5 minutes followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 61°C for 30 seconds and extension at 72°C for 5 minutes, followed by single and final extension step at 72°C for 7 minutes in peltier thermal cycler (DNA engine DYAD™, Bio-Rad, South Africa).

4.6.3 Agarose gel electrophoresis

Electrophoresis of PCR products was performed according to the methods and procedure described by Ngoma *et al.*, (2013). The extraction of genomic DNA product was determined by analysis for the presence of DNA by 1% of agarose gel. The agarose gel was prepared as follows: 100g of agarose gel was weighed and mixed to 100 ml of sterile water plus 10 ml of TE buffer; Agarose was then dissolved by using the microwave for 5 minutes; the gel was allowed to cool to about 40°C and ethidium bromide 0,5ml was added for staining; and the gel was cast and allowed to set. After gel had set inside the electrophoresis chamber, 5 µL DNA and 5 µL of loading buffer were mixed and transferred to one of the walls in the gel electrophoresis tank. Electrophoresis conditions were set 90 minutes at 80 voltages and 250 MA. Gel was then visualised under UV light at 420 mm wavelength using a Bio-Rad Chemi DOC™MP imaging system (Bio-Rad. USA). The imaging system (Bio-Rad Chemi Doc™MP imaging system, UK) was used to capture the presence of DNA bands (version 6.00.22) software. The presence of a single bright band (DNA bands) for each sample indicated a successful amplification. After, the PCR products were sent to Inquaba, biotechnology in Pretoria, for sequencing.

4.6.4 DNA sequencing

DNA sequencing was performed according to the methods suggested by (Ngoma *et al.*, 2013). The purified PCR fragment of the 16S rDNA of the isolated bacteria was sent to INQABA biotechnology, in Pretoria, South Africa for sequencing. The sequences and chromatograms were observed with Bio-systems; forward and reverse sequences were compared and corrected for conformity. Blast program tools were applied to search for the sequences according to (Altschul *et al.*, 1997) to find the closest match for each in the Gen-Bank. The closest sequences were then downloaded and aligned with the original sequences using clustal (Thompson *et al.*, 1994) and edited *via* Bio-edit for all to have the same length. The edited sequences were compared using maximum parsimony in the program DNA pair (Felsentein *et al.*, 1989). Results were then showed as bootstrapped (1000 boot strap).

4.6.5 Phylogenic tree

Sequencing results obtained were analysed and corrected with Bio-Edit sequence alignment editor according to Ngoma *et al.* (2013). Multiple alignments of the sequences were performed with Mafft program 6.864 against corresponding nucleotides sequences retrieved from Gen-Bank. Evolutionary distance matrices were created according to the methods developed by (Tamura *et al.*, 2011). The aligned 16S rDNA gene sequences were used to construct a phylogenetic tree and the establishment of phylogenetic relationship at the genus and species level neighbor joining analysis was implemented with MEGA 5.10 package (Tamura *et al.*, 2011) for minimum evolution; maximum likelihood (Fitch, 1986); unweighted pair Group Method with Arithmetic Mean (UPGMA) and maximum parsimony (Rzhetsky & Nei, 1992). Bootstrapping was done with 1000 replications (Saitou & Nei, 1987). Putative chimeric sequences were identified with the chimera Buster 1.0 software. Manipulation and tree editing were performed using tree view (Page, 1996).

4.7 Statistical analysis

Bacteria counts as well as the prevalence of bacteria according to type of meat and the overall prevalence of bacteria in meat was done per street. The unbalanced analysis of variance (ANOVA) was used to determine the difference between the three streets in relation to bacterial counts as well as determine the difference between the types of meats in relation to bacterial counts. In addition, the unbalanced ANOVA was also used to determine the difference between the levels of coliforms in the three streets, the difference between the types of meat in relation to coliform counts. The unbalanced ANOVA was preferred for this purpose because it is suited for determining the difference in a continuous variable (coliform and bacteria counts) across a categorical variable with at least three groups (street and type of meat). All the statistical analysis performed in this study was done with the help of the Statistical Package for the Social Sciences (SPSS) version 20.0

4.8 Results

4.8.1 Total bacterial count

Bacterial count in prepared food and water is a key factor in assessing the microbial quality and safety of food. It also reveals the level of hygiene methods applied by food handlers during the preparation of such foods. Food and water, in particular, have been described as a vehicle for the transmission of microbial diseases, amongst which are those caused by coliforms (Nkere *et al.*, 2011). Results of bacterial count in samples collected for this study are summarised in Tables 4.1, 4.2, 4.3, 4.4, 4.5 (Appendix 2.) and Figure 4.3, 4.4, 4.5. 4.6 and 4.6

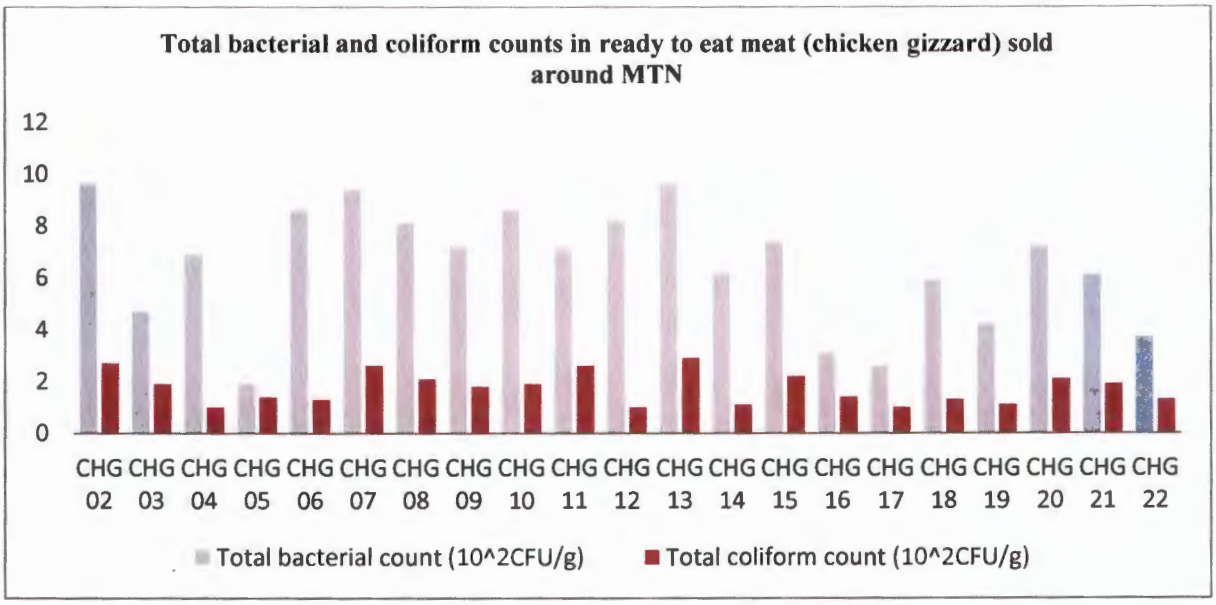

Figure 4.3 Total bacterial count and coliform count in chicken gizzard

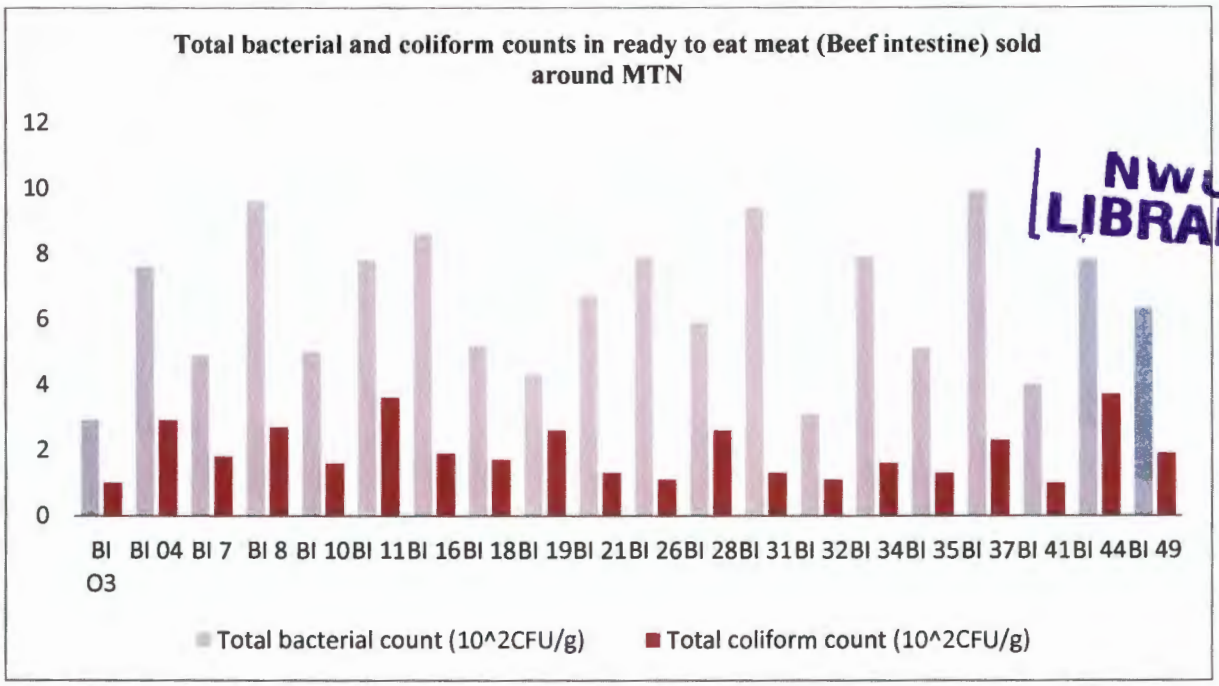

Figure 4.4 Total bacterial count and coliform count in beef intestine

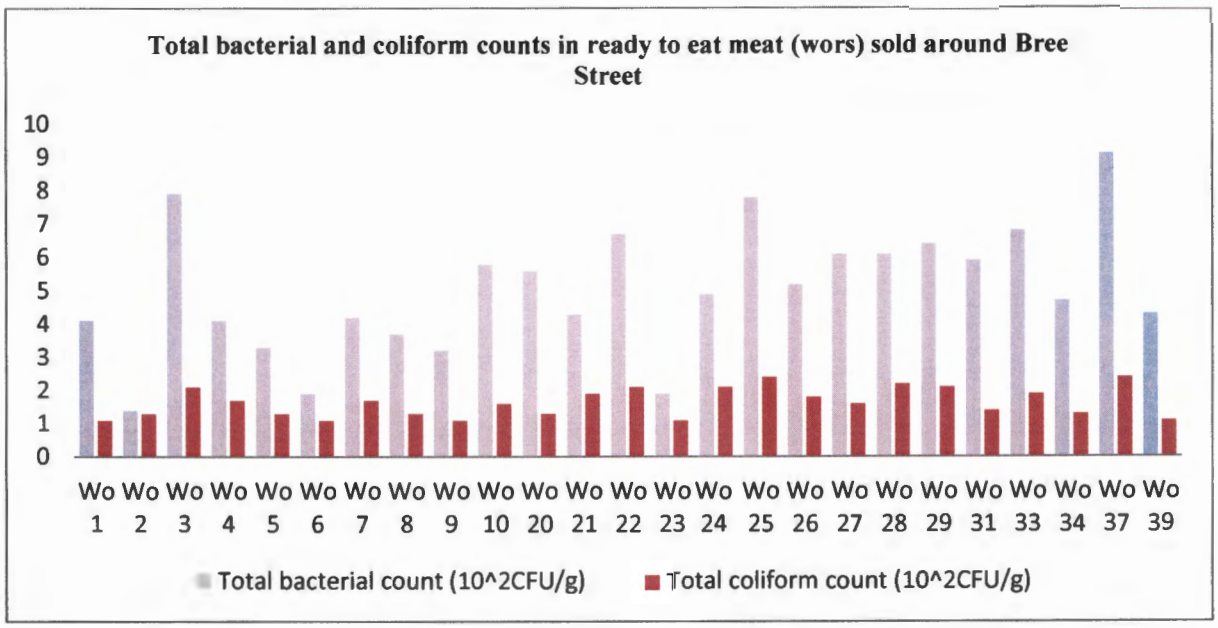

Figure 4.5 Total bacterial count and coliform count in wors

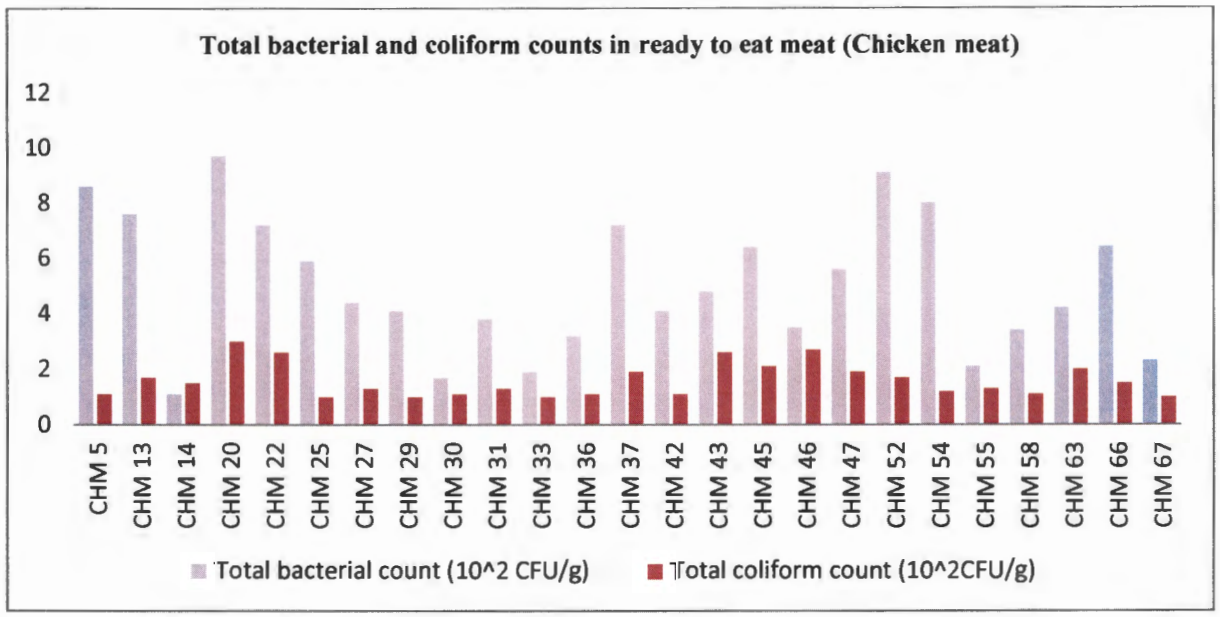

Figure 4.6 total bacterial count and coliform count in chicken meat

©

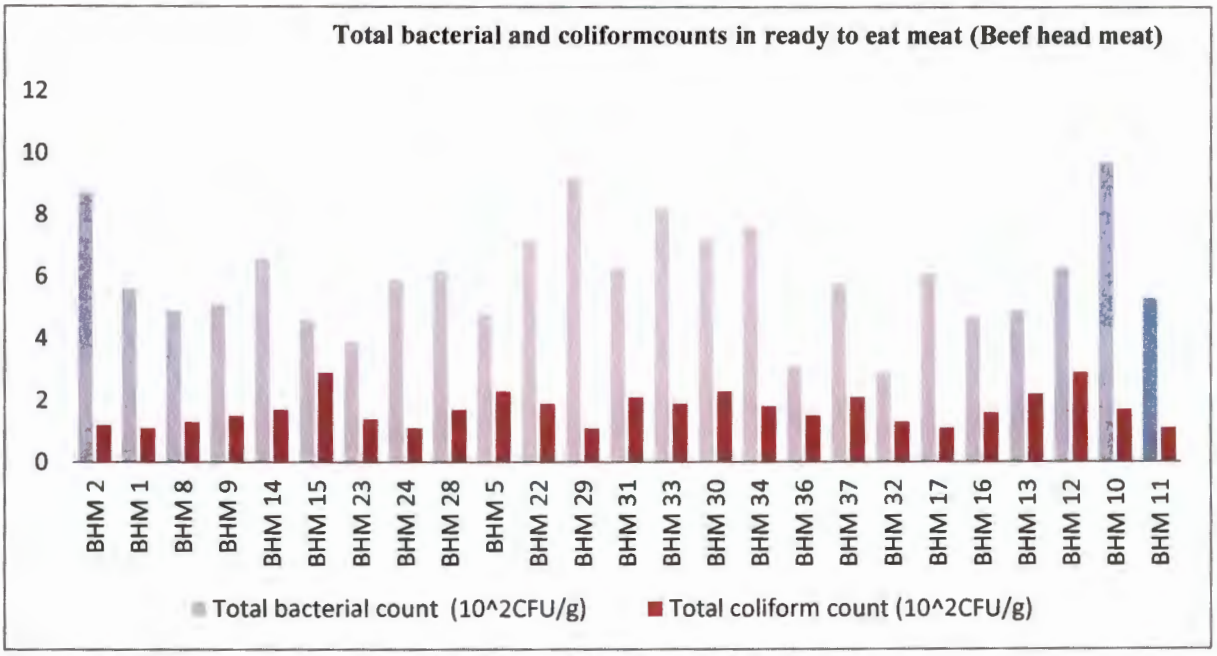

Figure 4.7 Total bacterial count and coliform counts in beef head meat

4.8.1.1 Summary of total bacterial and coliform counts per areas

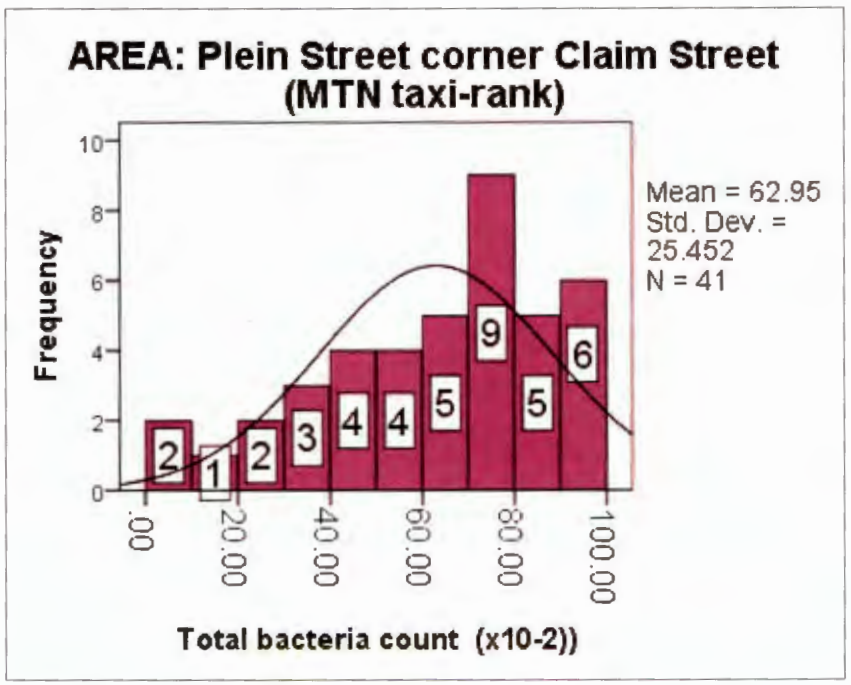

Figure 4.8 shows that the mean bacterial count at Plain Street (corner Claim Street) (MTN taxi-rank) is $62.95*10^2$ with n=41 and the distribution of bacterial count is slightly skewed to

the left, indicating that there are more upper counts of bacteria than lower counts. The modal class of bacteria count is $70*10^2$ to $<80*10^2$.

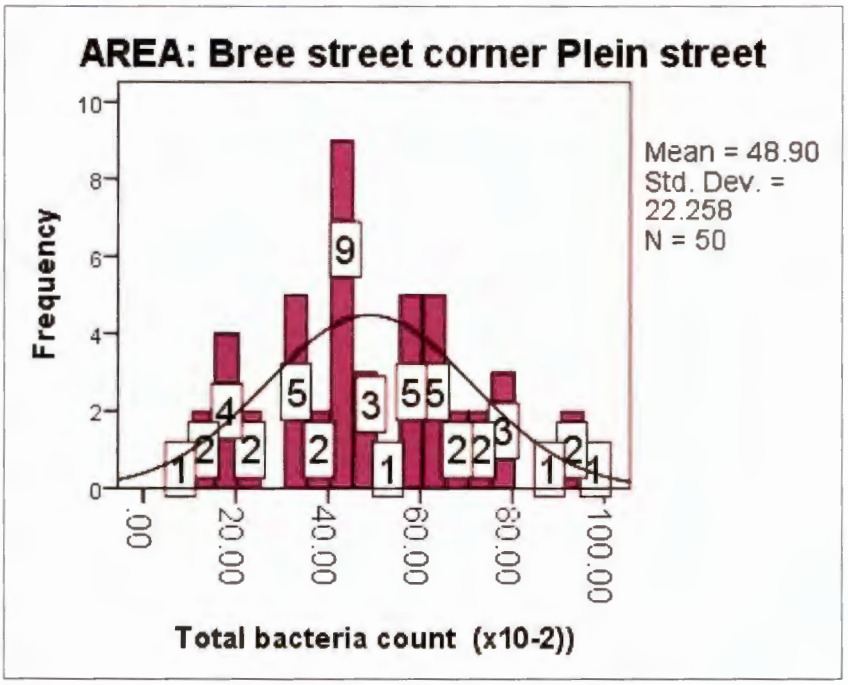

Figure 4.9 shows that the mean bacterial count at Bree Street (corner Plain Street) is $48.9*10^2$ with n=50 and the distribution of bacterial count is approximately normal, indicating that there are almost equal upper counts of bacteria and lower counts. The modal class of bacterial count is $40*10^2$ to $<45*10^2$.

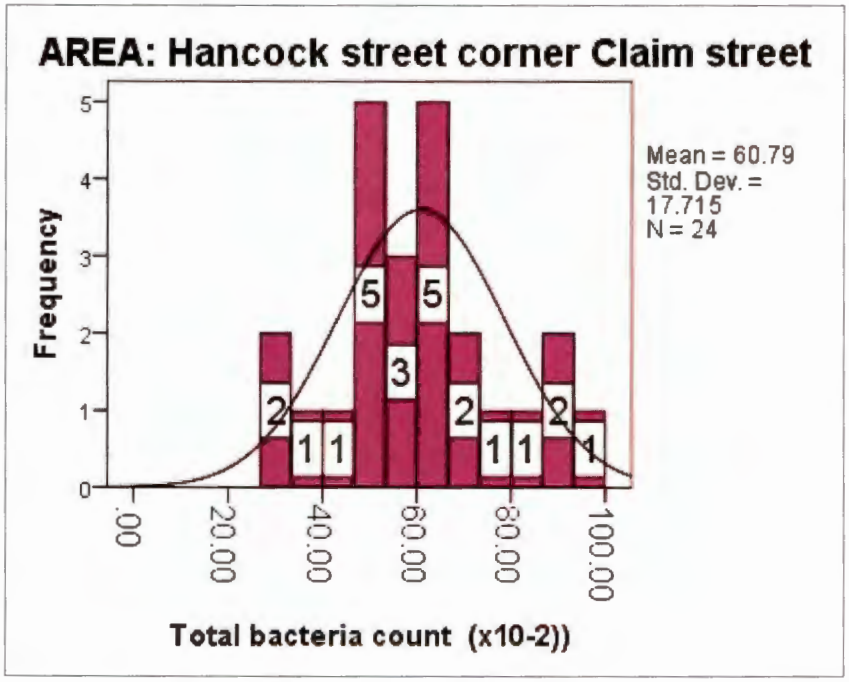

Figure 4.10 shows that the mean bacterial count at Hancock Street (corner Claim Street) is $60.79*10^2$ with n=24 and the distribution of bacterial count is approximately normal,

indicating that there are almost equal upper counts of bacteria and lower counts. The modal classes of bacterial counts are $46.7*10^2$ to $<53.3*10^2$ and $60*10^2$ to $<66.7*10^2$.

Coliform count by area

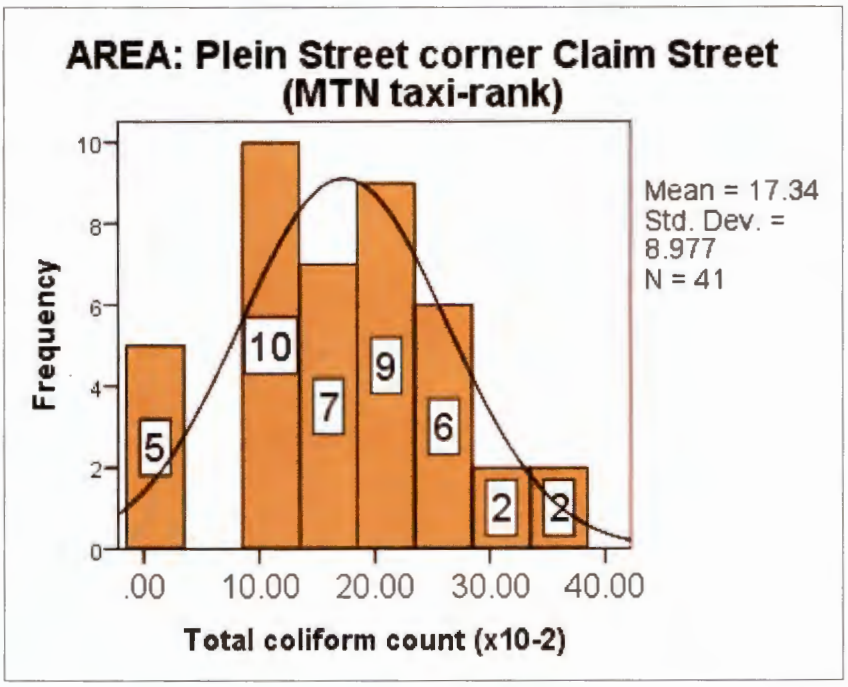

Figure 4.11 shows that the mean coliform count at Hancock Street (corner Claim Street) is $17.34*10^2$ with n=41 and the distribution of coliform count is approximately normal, indicating that there are almost equal upper counts of bacteria and lower counts. The modal class of coliform count is $10*10^2$ to $<15*10^2$.

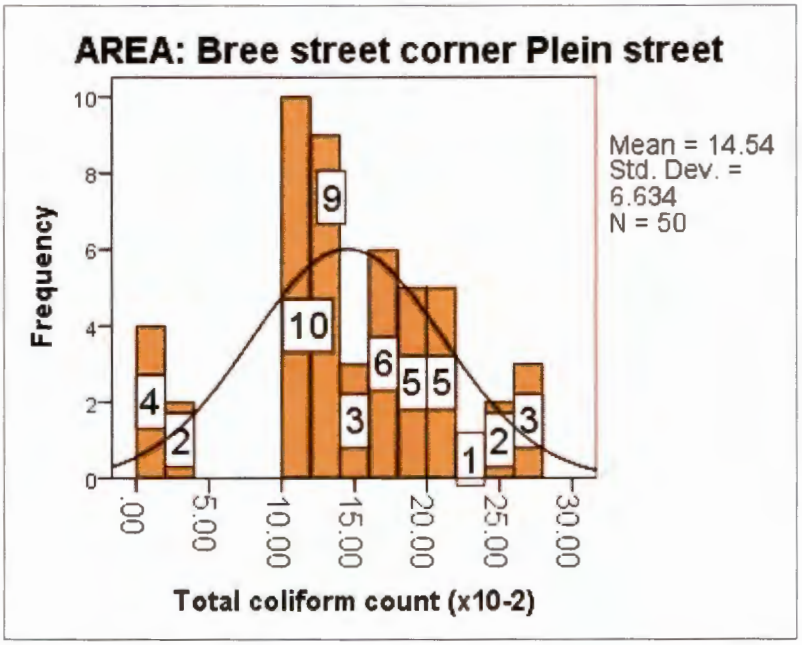

Figure 4.12 shows that the mean coliform count at Bree Street (corner Plain Street) is $14.54*10^{-2}$ with n=50 and the distribution of coliform count is approximately normal,

indicating that there are almost equal upper counts of coliform and lower counts. The modal class of bacterial count is $10*10^{-2}$ to $<12.5*10^{-2}$.

4.8.2: Comparison of bacterial counts between the three areas and coliform counts

The difference between the three streets in this study in relation to bacteria count showed that the mean bacterial count for samples collected from MTN taxi-rank and Hancock Street (corner Claim Street) did not have any statistical difference (Table 4.6). However, significant differences ($P<0.05$) were observed in bacterial counts for samples collected from Bree Street (corner Plein Street).

Table 4.6: Mean summary of bacterial count per Street

Descriptive statistics			
Dependent variable: total bacterial count ($x10^2$)			
area	Mean (10^2)	Std. deviation	n
MTN taxi-rank	62.95	25.45	41
Bree Street (corner Plein street)	48.90	22.25	50
Hancock Street (corner Claim Street)	60.79	17.71	24
Total	56.39	23.40	115

Table 4.7: Significance of total bacteria count between different areas of collection

Tests of between-subjects effects					
Dependent variable: total bacterial count ($x10^2$)					
Source	Type III sum of squares	Df	Mean square	F	Sig.
Corrected model	5035.031	2	2517.515	4.912	.009
Intercept	346347.155	1	346347.155	675.748	.000
AREA*[1]	5035.031	2	2517.515	4.912	.009
Error	57404.361	112	512.539		
Total	428137.000	115			
Corrected total	62439.391	114			
a. R squared = .081 (Adjusted R squared = .064)					

Table 4.6 shows that the sizes (n) are not equal; which implies that an unbalanced ANOVA was used to analyse the data. The mean bacteria count of at least one street was found to be statistically significant or different from one street to another and did not depend on food types sold by vendors (p-value <0.05).

Table 4.8: Summary of mean differences of bacterial count among different areas

Multiple comparisons						
Dependent variable: total bacterial count ($x10^2$) Tukey HSD						
(I) AREA	(J) AREA	Mean difference (I-J)	Std. error	Sig.	95% confidence interval	
					Lower bound	Upper bound
Plein Street (corner Claim Street) (MTN taxi-rank)	Bree Street (corner Plein Street)	14.05*[2]	4.76	.011	2.72	25.38
	Hancock Street (corner Claim Street)	2.15	5.81	.927	-11.66	15.98
Bree Street (corner Plein Street)	Plein Street (corner Claim Street) (MTN taxi-rank)	-14.05*	4.769	.011	-25.38	-2.72
	Hancock Street (corner Claim Street)	-11.89	5.62	.091	-25.24	1.46
Hancock Street (corner Claim Street)	Plein Street (corner Claim Street) (MTN taxi-rank)	-2.15	5.81	.927	-15.98	11.66
	Bree Street (corner Plein Street)	11.89	5.62	.091	-1.46	25.24

The mean bacterial count of at least one street was found to be statistically significant or different from one street to another and did not depend on food types sold by vendors (p-value <0.05). This is an indication that the bacterial count is dependent on the street. In this study, results of bacterial counts showed significant difference (P <0.05) between MTN taxi-rank and Bree street (corner Plein Street) (Table 4.8).

[2] *Significant at 5% level

Table 4.9: Determination of the difference between the types of meats in relation to bacterial count

Descriptive statistics			
Dependent variable: total bacterial count ($x10^2$)			
MEAT	Mean	Std. deviation	N
Chicken meat	47.6400	25.26506	25
Chicken gizzard	64.9048	23.35788	21
Beef head meat	60.7917	17.71509	24
Beef intestine	60.9000	27.94336	20
Wors	50.1600	19.23036	25
Total	56.3913	23.40329	115

Table 4.9 shows that the mean bacterial count is almost equal for the following types of meat: chicken gizzard; beef head meat; and beef intestine. However, the mean bacterial count is noticeably different between the types of meat and the following: chicken meat; and wors.

Table 4.10: Statistical comparison of bacterial count in different meat samples and their significance between areas of collection

Multiple comparisons						
Dependent variable: total bacterial count ($x10^2$) LSD						
(I) MEAT	(J) MEAT	Mean difference (I-J)	Std. error	Sig.	95% Confidence Interval	
					Lower bound	Upper bound
Chicken meat	Chicken gizzard	-17.2648*[3]	6.74764	.012	-30.6370	-3.8925
	Beef head meat	-13.1517*	6.51441	.046	-26.0617	-.2416
	Beef intestine	-13.2600	6.83870	.055	-26.8127	.2927
	Wors	-2.5200	6.44759	.697	-15.2976	10.2576
Chicken gizzard	Chicken meat	17.2648*	6.74764	.012	3.8925	30.6370
	Beef head meat	4.1131	6.81151	.547	-9.3857	17.6119
	Beef intestine	4.0048	7.12229	.575	-10.1099	18.1195
	Wors	14.7448*	6.74764	.031	1.3725	28.1170
Beef head meat	Chicken meat	13.1517*	6.51441	.046	.2416	26.0617
	Chicken gizzard	-4.1131	6.81151	.547	-17.6119	9.3857
	Beef intestine	-.1083	6.90173	.988	-13.7859	13.5693
	Wors	10.6317	6.51441	.106	-2.2784	23.5417
Beef intestine	Chicken meat	13.2600	6.83870	.055	-.2927	26.8127
	Chicken gizzard	-4.0048	7.12229	.575	-18.1195	10.1099
	Beef head meat	.1083	6.90173	.988	-13.5693	13.7859

[3]Significant at 5% level

	Wors	10.7400	6.83870	.119	-2.8127	24.2927
Wors	Chicken meat	2.5200	6.44759	.697	-10.2576	15.2976
	Chicken gizzard	-14.7448*	6.74764	.031	-28.1170	-1.3725
	Beef head meat	-10.6317	6.51441	.106	-23.5417	2.2784
	Beef intestine	-10.7400	6.83870	.119	-24.2927	2.8127

Results obtained in this study revealed that the mean bacterial count for at least one type of meat was statistically different from the other P <0.05 (Table 4.10). In terms of the results based on street food sampled between different areas in Johannesburg CBD, it was noted that there was a significant difference (P<0.05) in bacterial counts between chicken meat and beef head meat; chicken gizzard and chicken meat and chicken gizzard and wors as shown in Table 4.10.

Table 4.11: Summary of mean coliform count according to different collection sites

Descriptive statistics			
Dependent variable: total coliform count (x10-2)			
AREA	Mean	Std. deviation	N
Plein Street (corner Claim Street) (MTN taxi-rank)	17.3415	8.97666	41
Bree Street (corner Plein Street)	14.5400	6.63390	50
Hancock Street (corner Claim Street)	16.9583	4.68559	24
Total	16.0435	7.30704	115

The determination of the difference between the three areas under study in relation to coliform count showed no significant difference in the mean coliform between the three areas (P >0.05). Table 4.11

Table 4.12: Mean coliform count based on the type of meat

Descriptive statistics			
Dependent variable: total coliform count ($x10^2$)			
MEAT	Mean	Std. deviation	n
Chicken meat	12.6800	7.97245	25
Chicken gizzard	16.1429	8.26611	21
Beef head meat	16.9583	4.68559	24
Beef intestine	18.6000	9.71922	20
Wors	16.4000	4.36845	25
Total	16.0435	7.30704	115

The determination of the differences between the meat types in this study in relation to coliform count revealed no significant differences in mean coliform counts between the different types of meat analysed. Types of meat: chicken gizzard; beef head meat; and wors.

There was no statistically significant difference in the mean coliform count across the types of meat under study (p-value >0.05). as presented in Table 4.12

4.8.4 Overall bacterial isolates based on biochemical and morphological examination

The preliminary results obtained from morphology and biochemical tests (see appendix 3) showed that beef head meat samples were the most contaminated (27.50%) followed by chicken gizzard (23.20), beef intestine (17.40), wors (17.40%) and chicken meat (14.50%). In addition, the results show that *S. aureus* was the most contaminated in beef head meat (11.60%), followed by chicken gizzard (7.20%), while *Micrococcus* spp. were mostly found to be equal in all samples (2.90%), but with high concentration in wors (4.30%). *Enterococcus* spp., were found mostly in beef head meat at (7.20%) and *Enterococcus faecalis* were mostly found in chicken gizzard (5.80%). The overall results of bacterial identification using the biochemical test and morphological characterisation presented in Table 4.16 show that the most predominant isolates were *Staphylococcus aureus* (29%) followed by *Micrococcus caseolyticus* and *Bacillus* spp. 15.9% for each.

Table 4.16: Overall bacterial isolates based on biochemical and morphology tests

	Bacillus cereus	**Enterococcus faecalis**	**Macrococcus caseolyticus**	**Citrobacter sp**	**Staphylococcus aureus**	**Escherichia coli**	**Bacillus subtilis**	**Bacillus sp**	**Enterococcus sp**	**Staphylococcus sp**	**Enterococcus faecium**	**Enterobacter sp**	**Total**
Chicken gizzard	2.90 %	5.80%	2.90 %	1.40 %	7.20 %	1.40 %	1.40%	0.00%	0.00 %	0.00%	0.00 %	0.00 %	**23.20 %**
Beef intestine	1.40 %	0.00%	2.90 %	0.00 %	4.30 %	1.40 %	1.40%	4.30%	1.40 %	0.00%	0.00 %	0.00 %	**17.40 %**
Wors	2.90 %	0.00%	4.30 %	0.00 %	2.90 %	0.00 %	0.00%	5.80%	0.00 %	1.40%	0.00 %	0.00 %	**17.40 %**
Chicken meat	0.00 %	1.40%	2.90 %	1.40 %	2.90 %	0.00 %	0.00%	2.90%	0.00 %	1.40%	1.40 %	0.00 %	**14.50 %**
Beef head meat	2.90 %	0.00%	2.90 %	0.00 %	11.60%	0.00 %	0.00%	2.90%	0.00 %	0.00%	0.00 %	7.20 %	**27.50 %**
Total	**10.10 %**	**7.20%**	**15.90%**	**2.90 %**	**29.00%**	**2.90 %**	**2.90%**	**15.90 %**	**1.40 %**	**2.90 %**	**1.40 %**	**7.20 %**	**100.00 %**

4.8.5: Bacterial isolates based on Analytic Profile Index (API-Staph)

The results obtained using API-staph, as presented in Table 4.17, indicate that 100% of bacterial isolates analysed and subjected to API-staph were *Staphylococcus* species isolated from different meat samples. Among these samples, 7.40% were confirmed positive as *Staphylococcus aureus* from chicken meat and beef head meat samples; 11.11% were confirmed positive as *Staphylococcus haemolyticus*, from chicken gizzard and wors meat samples, while 40.74% were confirmed positive as *Staphylococcus* spp from different samples collected such as chicken meat, wors, beef intestine and chicken. 3.70% were confirmed positive with *Staphylococcus saprophiticus*, 14. 81% were confirmed positive with *Staphylococcus xylosus*, 3.70% were confirmed positive with *Staphylococcus cohnii* and 14.81 were confirmed positive with *Staphylococcus lentus* from different samples such as beef head meat, beef intestine, chicken gizzard and wors.

Table 4.17: Results obtained from API-Staph

Sample identification	**API –Staph results**
CH20M	*Staphylococcus sp*
CH D3L1	*Staphylococcus aureus*
GIZ 17D4	*Staphylococcus haemolyticus*
Wo 23 M	*Staphylococcus saprophiticus*
Wo 30D1	*Staphylococcus sp*
BI 19D4	*Staphylococcus sp*
Wo 32D2	*Staphylococcus haemolyticus*
CH5 D3	*Staphylococcus xylosus*
GIZ 2DL1	*Staphylococcus haemolyticus*
CH 22D2	*Staphylococcus sp*
Wo 5D4	*Staphylococcus cohnii*
BI 27D2	*Staphylococcus xylosus*
Wo 20D2	*Staphylococcus lentus*
GIZ 17D2	*Staphylococcus sp*
GIZ 9D3	*Staphylococcus lentus*
CH D3 18	*Staphylococcus sp*
GIZ 2D4 L1	*Staphylococcus sp*
Wo10 D4	*Staphylococcus sp*
CH 22 D2	*Staphylococcus sp*
Wo 32 D3 L1	*Staphylococcus xylosus*
BI 1D3	*Staphylococcus lentus*
CH 22D1	*Staphylococcus xylosus*
BHM12D4	*Staphylococcus lentus*
Wo 27	*Staphylococcus* spp
Wo 5D4	*Staphylococcus sp*
CH5D3	*Staphylococcus* spp
BHM 23 D3	*Staphylococcus aureus*

4.9 Analysis of Molecular identification

4.9.1 Genomic DNA of isolated bacteria

After running the gel, on automatic UV trans illuminator (UV tec, Sigma, Germany) was used to check the presence of bands of genomic DNA and photographed using Bio profile gel documentation system. The objective was to confirm the successful extraction of the DNA.

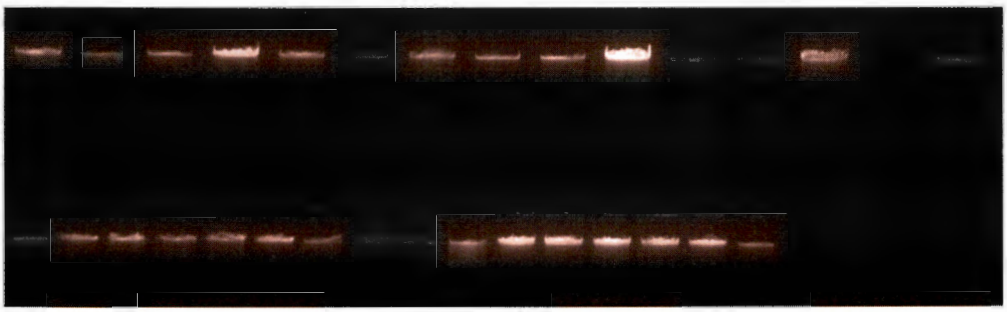

Figure 4.13 showing image of Agarose gel (1%w/v) of genomic DNA extracted from isolates bacteria.

4.9.1.1 Detection of 16S rDNA gene by PCR

Fifty (50) bacterial isolates were selected and subjected to PCR analysis. The results revealed a 1% (w/v) agarose gel representing 16S rDNA gene fragments (Figures 4.5, 4.6 and 4.7). The desired 1kb base pairs fragments were obtained after running the gel on electrophoresis for 65 minutes.

. M 1…2...3…4…5…6.7…8..9..10..11..12.13..14.15.16.17.18..M

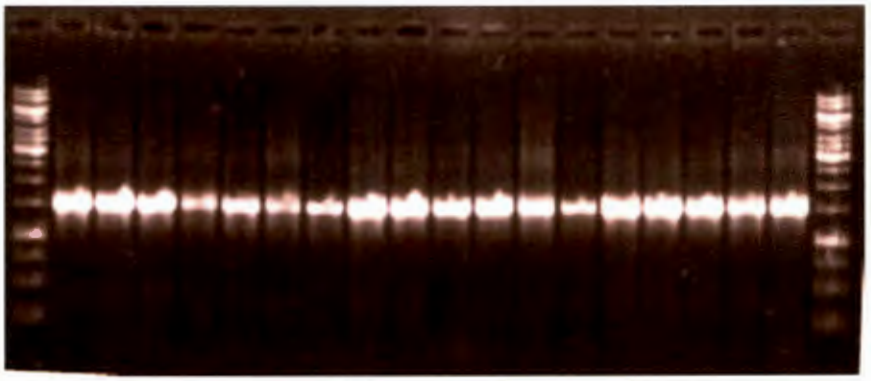

Figure 4.14 showing electrophoresis in (1%w/v) 16S rRNA gene fragments amplified from DNA extracted from isolated bacteria, Molecular weight marker (1.5 Kb DNA ladder laneM). Lanes 1-6 and 13 = *Staphylococcus aureus*, Lanes 7 = No similarity was found, Lanes 8 = *Bacillus thuringiens*, Lane 9 = *Planomicrobium glaciei*, Lane 10 = *Bacillus* sp, Lane 11-12 and = *Macrococcus caseolyticus*, Lane 14 = *Bacillus subtilis*, Lane 15-16 = *Bacillus cereus*, 18 = *Enterococcus faecalis*

M…1…2…3…4…5…6…7…8…9…10..11..12..13..14..15..16..17..18..M

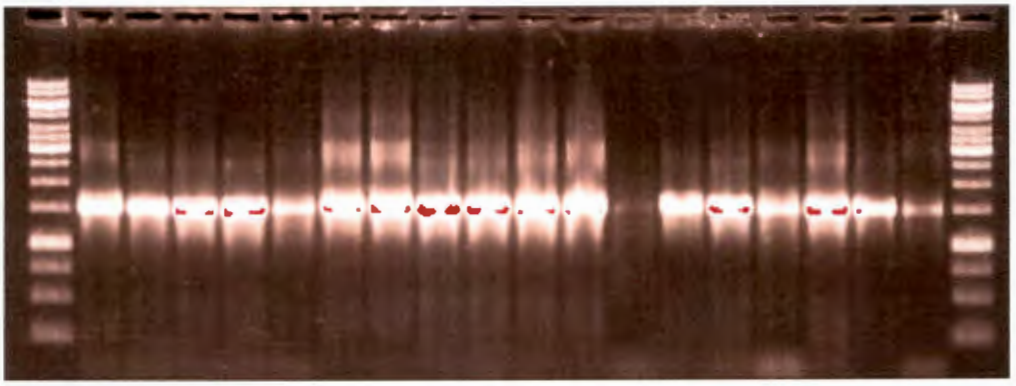

Figure 4.15: PCR products amplified from bacteria isolated from street-vended meat. Molecular weight marker (1Kb DNA ladder lane M)

Lane 1= *Planococcus antarcticus*, Lane 2 = *Staphylococcus aureus*, Lane = *Macrococcus caseolyticus*, Lane 4 = *Enterococcus faecium*, Lane 5 = *Staphylococcus aureus*, Lane 6 = no similarity was found, Lane 5-6 =, Lane 7 = *Staphylococcus equorum*, Lane 8-11 and 15 = *Macrococcus caseolyticus*, Lane 12 = no similarity was found, Lane 13 = *Staphylococcus equorum*, Lane 14 = *Bacillus* sp, Lane 16-17 = appears as uncultured bacteria, Lane 18 = no similarity was found.

M.....2...3...4...5...6...7...8...9..10...11..12..13.....M

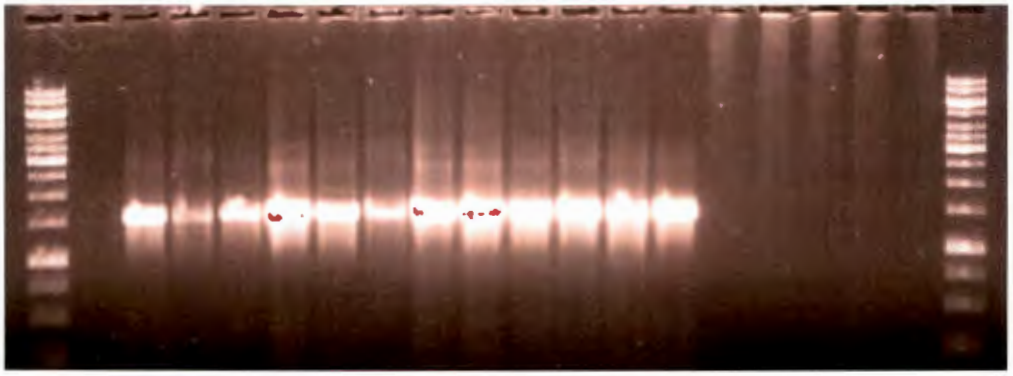

Figure 4.16: PCR products amplified from bacteria isolated from street-vended meat Lane 2 = *Lysinibacillus* sp, Lane 3 = no similarity was found, Lane 4 = *Kurthia* sp, Lane 5 = *Citrobacter* sp, Lane 6 = *Kurthia* sp, Lane 7 = uncultured bacteria, Lina 8 = *Lysinibacillus* sp, Lina 9 = *Bacillus cereus*, Lane 12 = *Staphylococcus vitulinus*, Lane 10 and 13 = no similarity was found, Lane 11 = *Enterococcus faecium* and lane 12 = *Staphylococcus vitulinus,* six isolated bacteria were found with very low concentrations of DNA and did not show up the bands of genomic DNA.

4.9.2 Confirmatory results of bacterial isolates based on PCR and sequencing

The PCR amplification method of 16S rRNA gave some identification results as a method, which was sensitive, fast and reliable. Amplification and sequencing of the 16S rDNA gene were performed on fifty selected bacterial isolates. The identity was observed around 94%–100% identity according to 16S rDNA BLAST sequence homology results. Similarity was not found for ten selected bacterial isolates among the fifty.

Table 4.18: Bacterial isolates based on PCR product and sequence analysis and their accession number

Sample identity	Bacterial identity	Accession number	Identity%
BI29D2 l1	*Staphylococcus aureus*	KX447584.1	94%
GIZ 20 D1	*Staphylococcus aureus*	KX447584.1	95%
BHM2 D2	*Staphylococcus aureus*	KX447584.1	99%
CH4 D3	*Staphylococcus aureus*	KX447584.1	95%
BHM 23M	*Staphylococcus aureus*	KX447584.1	98%
Wo D3	*Staphylococcus aureus*	KX447584.1	96%
Wo 8D1	*Bacillus thuringienis*	EU161995.1	94%
GIZ 15D1	*Planomicrobium glaciei*	FB03B07	95%
Wo 23 D3	*Bacillus sp*	KX453917.1	96%
CH 22 D1	*Macrococcus caseolyticus*	KP058399.1	97%
CH 22 D1	*Macrococcus caseolyticus*	KJ783380.1	99%
CH 22 D1	*Staphylococcus aureus*	KX447584.1	94%
BI 19D3	*Bacillus subtilis*	KU936340.1	99%
GIZ 3D3L1	*Bacillus cereus*	KU977289.1	78%
BI 04D2	*Bacillus cereus*	KP235535.1	81%
CH5 D3	*Macrococcus caseolyticus*	KC212015.1	95%
CH 11D2	*Enterococcus faecalis*	KJ571205.1	96%
GIZ 30DM L1	*Planococcus antarcticus*	KF318398.1	98%
Wo 32D3 l1	*Staphylococcus aureus*	KX447584.1	97%
BHM 1D3	*Macrococcus caseolyticus*	KC212015.1	95%
BHM 23D3	*Enterococcus faecium*	KR858813.1	95%
BHM 23 D3	*Staphylococcus aureus*	BX571856.1	88%
BHM 17D3	*Macrococcus caseolyticus*	KJ783380.1	97%
CH 3M	*Staphylococcus equorum*	DQ232735.1	96%
CH22 D2	*Macrococcus caseolyticus*	KP181626.1	94%
CH5 D3	*Macrococcus caseolyticus*	KP058399.1	97%
Wo5 D4	*Macrococcus caseolyticus*	KJ78380.1	95%
Wo10 D4	*Macrococcus caseolyticus*	KX062008.1	96%
CH25 ML1	*Planomicrobium glaciei*	JF411320.1	95%
BI3 D1	*Staphylococcus aureus*	KX447584.1	97%
BHM1 D4	*Bacillus sp*	JF701942.1	95%
Wo17 D1	*Macrococcus caseolyticus*	KP058399.1	95%
Wo3 D1	*Lysinibacillus sp*	LN483073.1	98%
CH20 M	*Kurthia sp*	CP013217.1	98%
BHM29 D4L1	*Citrobacter sp*	KT150059.1	83%

CH20 M	*Kurthia sp*	CP013217.1	97%
GIZ3 D1L1	*Lysinibacillus*	KT029127.1	95%
CH0 D4	*Bacillus cereus*	KP235535.1	82%
GIZ16 D4	*Enterococcus faecium*	FJ915708.1	94%
Wo12 D4	*Staphylococcus vitulinus*	JQ684238.1	97%

4.9.3 Phylogenetic tree of isolated bacteria

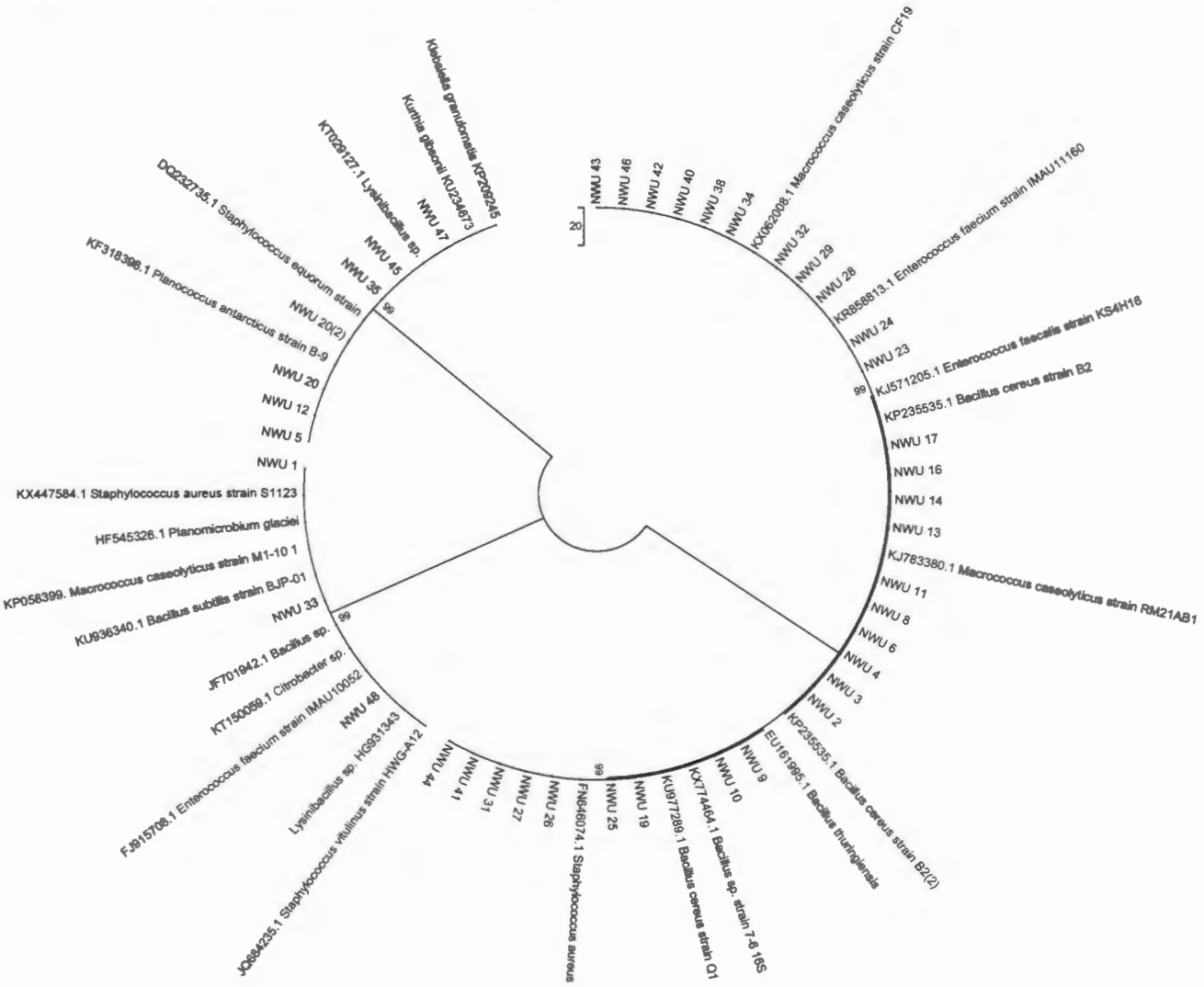

Figure 4.17 shows the Phylogenetic tree compared with 16S rDNA constructed using 40 isolates.

The tree shows the closest similarity with other microorganisms extracted from the gene bank.

4.9.3 Prevalence of isolated bacteria

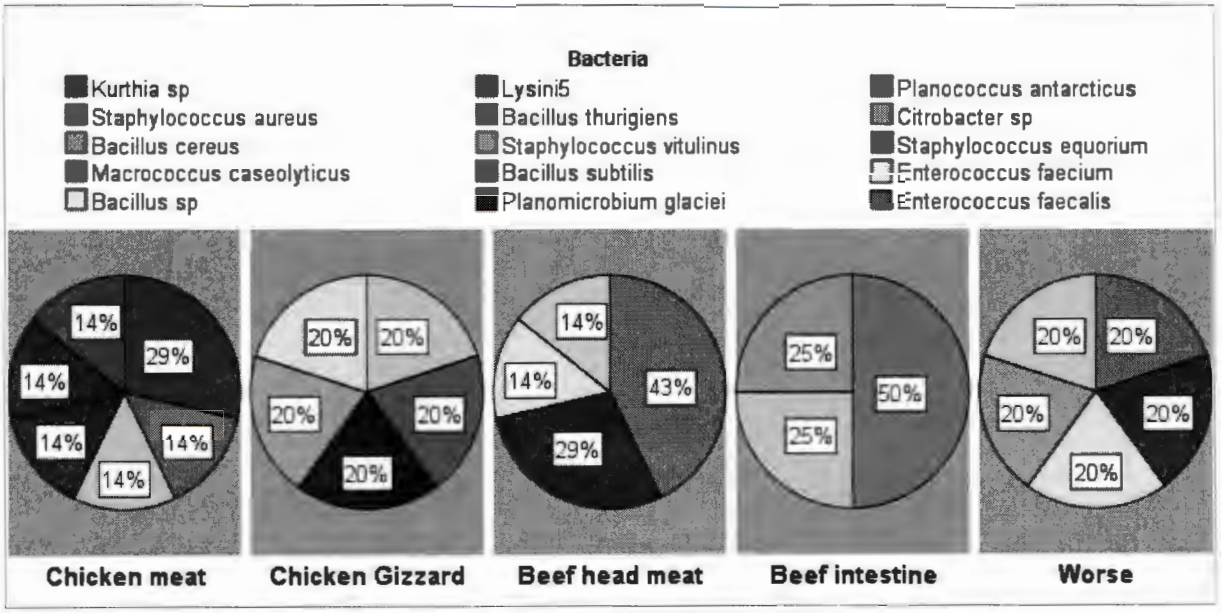

Figure 4.18: Percentage of bacterial isolates in each type of meat

The result revealed that *Staphylococcus aureus* was present in food at different levels (14% in chicken meat, 43% in beef head meat, 50% in beef intestine and 20% in wors). The majority of bacteria in chicken meat were *Kurthia* sp (representing 29%).

Key: Lysini5 = Lysinibacillus

4.9.4 Overall prevalence of bacteria

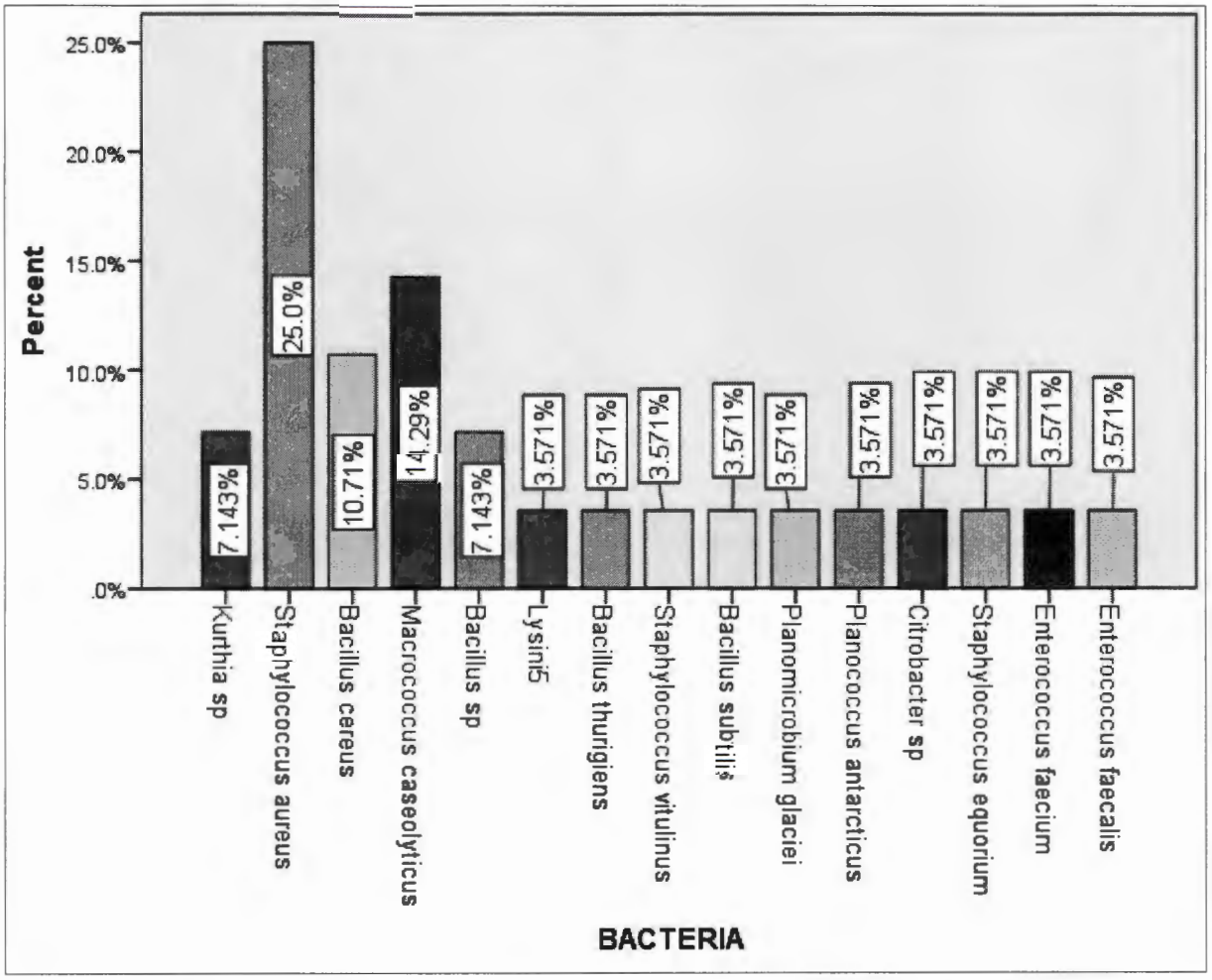

Figure 4.19: Prevalence of bacterial isolates around the three streets

The results obtained from PCR and sequencing summarised in Table 4.18 and Figures 4.8 and 4.10 shows that a quarter of bacteria detected in meat samples were: *Staphylococcus aureus* (25%); *Macrococcus caseolyticus* (14.29%); and *Bacillus cereus* (10.71%). *Kurthia* sp and *Bacillus* sp each comprised of 7.143% of the total bacteria detected in meat samples. The other ten bacteria were each prevalent 3.57%.

4.10 Discussion

4.10.1 Analysis of bacterial and coliform counts

Meat basically contains all necessary nutrients for microbial growth and metabolism, making it susceptible to microbial contamination. In this study the values obtained for total bacterial count were ranged for the highest 9.9 $x10^2$ cfu/g to the lowest $1.1x10^2$ cfu/g, the coliform count isolated on MacConkey agar were ranged from $3.7x10^2$cfu/g to 1.0 x 10^2 cfu/g as presented in Table 4.1, 4.2, 4.3, 4.4 and 4.5. (Appendix 2)

The result of total bacterial count observed in this study differ and are lower than those reported in previous studies for cooked meat and chicken sold on streets in Nigeria (Ekanem, 1998; Madueke *et al.*, 2014). Furthermore, the total coliform count obtained in this study are lower compared to previous studies (Oranusi *et al.*, 2012). Results obtained in this study are also similar to those obtained by Ekkarat *et al.* (2013) and Clarence *et al.* (2009). In their study on chicken and meat products, they also found a low value count of bacteria of index 10^{-1} to 10^{-4}. The results of this study differ from those of a recent study conducted in Gaborone, Botswana, by different researchers who analysed ready-to-eat meat and meat products in their study and found a high number of bacterial counts (Matsheka *et al.*, 2014; Shale & Malebo, 2011). The results obtained for bacterial count across the three areas revealed that the mean bacterial count was significantly different from one area to another p-value <0.05 (Tables 4.6 and 4.7). The mean bacterial count was significantly different across MTN-taxi rank and Bree Street (corner Plein Street) $P < 0.05$) (Table 4.8). The mean bacterial count was also found to be significantly different between chicken meat and beef head meat, chicken gizzard and chicken meat, and chicken gizzard and wors $P < 0.05$ (Table 4.10).

According to the international commission for microbiological specification for foods (ICMSF, 1996) states that ready-to-eat foods with plate count between 0-10^3 is acceptable, between $10^4 \leq 10^5$ is tolerable and 10^6 and above is unacceptable, hence street meat provided by vendors in Johannesburg CBD may be considered as acceptable microbiological quality.

However, the significant difference ($P < 0.05$) observed in this study across MTN-taxi rank and Bree street and between chicken meat and beef head meat, chicken gizzard and chicken meat, and chicken gizzard and wors in the level of microbial contamination, could be

associated with inadequate handling practices by vendors or contamination caused by poor storage facilities, either poor hygiene practices.

Therefore, the total bacterial count of all the samples presented in Tables 4.1, 4.2, 4.3, 4.4 and 4.5 (Appendix 2) were within the range of 10^2, which is classified as acceptable for ready-to-eat meat and meat products, while the total coliform (an indicator of faecal contamination and hygiene status) were also within the range 10^2, an indication that the hygienic state of food handlers was less than 40cfu/ml in all the food analysed (Cardinale *et al.*, 2002).

4.10.2 Analysis of isolated bacteria in meat samples and their prevalence

The occurrence of bacteria in meat has been reported from different parts of the world (Kinsella *et al.*, 2008). Molecular analysis based on 16S rDNA revealed a high frequency of occurrence of *Staphylococcus aureus* (25.0%), followed by *Bacillus cereus* (10.71 %) and *Bacillus spp.* (7.143 %), and other bacteria (Figure 4.10). Results obtained in this study showed that *Staphylococcus aureus* was present in different meat samples at different level, chicken meat (14%), beef head meat (43%), beef intestine (50%) and worse (20%). *Kurthia* spp was the most dominant in chicken meat (29%) as shown in Figure 4.19.

In addition, the high occurrence of *Staphylococcus aureus* in this study, reveals an unacceptable state of poor hygienic and sanitary practices applied during the preparation of food, which can be linked with the results of the observation study performed before sampling (Figures 3.1, 3.2 and 3.3), hence The presence of *Staphylococcus aureus* in different types of meat has also been reported by different researchers (Mensah *et al.*, 2002; Sina *et al.*, 2011). The results of this study correlate with the findings of the study conducted in Taiwan and Philippine where hot chicken, cold cooked chicken, hot grilled pork and cold cooked pork sold in the streets of Taichung, Taiwan and Laguna Philippines were found to be contaminated with some pathogenic bacteria such as *Staphylococcus aureus* and *Bacillus cereus* (Manguiat & Fang, 2013). The presence of *Staphylococcus aureus* and *Bacillus cereus* was also reported by Shale and Malebo (2011), isolated from Biltong.

The results of this study are similar with the findings of the study conducted in both Gangtok and Nainital (India) to assess the microbiological quality of ethnic street foods of the Himalayas. The study revealed the occurrence of different microorganisms such as *Enterococcus faecium, Bacillus subtilis, Bacillus cereus* and *Staphylococcus aureus* (Kharel *et al.*, 2016).

The results of this study are also in agreement with the finding of Akusu *et al.* (2016) who isolated *Bacillus* spp and *Staphylococcus aureus* in street-vended foods in Port Harcourt metropolis, Rivers State Nigeria. However, several studies have demonstrated and reported the contamination of street food, such as chicken, beef, pork, salads, fruits and water by different types of bacteria (Akusu *et al.*, 2016; Al Mamun *et al.*, 2013; Ghosh *et al.*, 2007; Kaul & Agarwal, 1988; Manguiat & Fang, 2013; Mosupye & von Holy, 1999; Nguyen *et al.*, 2014; Nyenje *et al.*, 2012; Tambekar *et al.*, 2009; Tambekar *et al.*, 2011).

The results of this study are in line with the findings of (Clarence *et al.*, 2009; Mensah *et al.*, 2002; Sina *et al.*, 2011). In their study, they found that poor hygienic practices and poor food handling could result in the occurrence of *Staphylococcus aureus*. Cho *et al.* (2011) identified risks associated with consuming contaminated street-vended foods that have high levels of coliform bacteria and the presence of pathogenic bacteria, such as *Escherichia coli, Salmonella* spp, *Staphylococcus aureus and Bacillus cereus* (Cho *et al.*, 2011).

Other species of *Staphylococcus* were also isolated in this study such as *Staphylococcus vitulinus* isolated in wors (20%) and *Staphylococcus equorum* isolated in chicken meat (14%) (Figure 4.18). The overall count in all meat samples was 3.571% for both (Figure 4.19).

Staphylococcus vitulinus and *Staphylococcus equorum* were also reported in bovine, caprine and ovine, in milk and dairy products (Bockelmann & Hoppe-Seyler, 2001; Meugnier *et al.*, 1996; Vernozy-Rozand *et al.*, 2000). *Staphylococcus equorum*, subsp. equorum was also isolated from healthy horses (Schleifer *et al.*, 1984), and later, isolates were obtained from milk of a cow with mastitis and from healthy goats (Meugnier *et al.*, 1996).

However, *Staphylococcus equorum* subsp. equorum strains were found in relevant human clinical materials (Alcaráz *et al.*, 2003; Marsou *et al.*, 2001). Thus, among the three *Staphylococcus* species isolated in this study, *Staphylococcus aureus* is the major one implicated in food poisoning. The amount of toxin necessary to cause illness depends on the susceptibility of the person. Nevertheless, epidemiological studies have revealed that as little as 1µg of *Staphylococcus aureus* can cause food poisoning (Tarekgne *et al.*, 2015). The results of this study also concur with the findings of Matsheka *et al.* (2014), who isolated different species from the genus *Staphylococcus*, from Biltong produced in butcheries in Gaborone, Botswana. The occurrence of *Staphylococcus* spp., is similar to the results obtained by Ali and Anil (2013), who isolated this bacteria from poultry meat sold in Bangkok, Thailand (Akbar & Anal, 2013). Furthermore, the presence of different species of

Staphylococcus spp., in street meat as observed in this study such as *Staphylococcus vitulinus* and *Staphylococcus equorum* may be originated from the hands of vendors or due to the poor handling practices and lack of washing hands and utensils could be incriminated as observed during the observational study (Figure 3.1; 3.2 and 3.3). The high occurrence of *Staphylococcus aureus* may be considered as unacceptable state of poor hygienic and sanitary practices employed by streets vendors during the preparation or serving (Clarence *et al.*, 2009).

In addition, *Micrococcus caseolyticus* isolated in this study were in different meat samples and from different areas under investigation. The prevalence of *Macrococcus caseolyticus* was found to be 14% in chicken meat, 29% in beef head meat and 20% in wors (Figure 4.18). The overall *Macrococcus caseolyticus* isolated in this study was 14.29% (Figure 4.19). No *Micrococcus caseolyticus* was found in chicken gizzard and beef intestine (Figure 4.18).

Micrococcus species are well known to cause spoilage in food, moreover these organisms are known as indicator of faecal contamination which suggests poor handling and hygiene status Oudiz (2004), which could have occurred during food preparation. This microorganism was also isolated from a skin swab of chicken (Tsubakishita *et al.*, 2010), hence its presence in food is not a threat to consumers because *Micrococcus spp.* are generally regarded as harmless saprophytes that inhabit or contaminate the skin, mucosa, and perhaps also, the oropharynx. However, they can be opportunistic pathogens for the immunocompromised (Bannerman *et al.*, 2006). They have been associated with various infections, including bacteremia, continuous ambulatory peritoneal dialysis peritonitis, and infections associated with ventricular shunts and central venous catheters. Other findings have revealed that the presence of *Micrococcus spp.* in analysed samples can be associated with central venous catheter infections in patients with pulmonary hypertension (Bannerman *et al.*, 2006; Marr *et al.*, 2015). The source of the *Micrococcus spp.* was traced to be skin (particularly in exposed regions of the body) of humans and animals, dairy products, and various environmental sources, including soil, marine and fresh water, plants, dust and air (Bannerman *et al.*, 2006; Dworkin *et al.*, 2005). The result of this study link with the findings of Baba *et al.* (2009), who isolated *Micrococcus caseolyticus* from cow milk, bovine organs and food (Baba *et al.*, 2009b). The presence of *Macrococcus caseolyticus* in this study may be linked to the poor handling practices observed among vendors and exposure of food to dust as observed before sampling (Figures 3.1, 3.2 and 3.3).

In this study different species of *Bacillus* was isolated from the three areas under study at different level as presented in Figure 4.9 and Figure 4.10. Various *Bacillus* spp have been isolated from food products, *Bacillus cereus* is the most common one associated with food poisoning (Hui *et al.,* 1994). These *Bacillus* spp can cause food spoilage or food-borne illnesses (Cousin & Layfayette, 1989). Among *Bacillus* species isolated, *Bacillus cereus* is widespread in nature and readily found in soil, where it adopts a saprophytic life cycle; germinating, growing and sporulating in this environment (Vilain *et al.*, 2006). *Bacillus cereus* is capable of producing one of two enterotoxins, a diarrheal toxin or an emetic toxin, and has been implicated in numerous foodborne illness outbreaks (Odumeru *et al.,* 1997). Widespread occurrence of the organism, its heat-resistant spores, and the ability of the vegetative cells to grow at refrigeration temperatures render toxigenic *Bacillus cereus* strains of particular concern in foods. Endospores of *Bacillus* survive the cooking process, but significant numbers are found only where temperature has not been adequately controlled after cooking (Varnam and Evans. 1991). This result is in agreement with the findings of Murindamombe *et al.*, (2005) who isolated *Bacillus cereus* in street foods in Botswana. The presence of *Bacillus cereus* was also reported by Shale and Malebo (2013), isolated from Biltong.

The results of this study are similar with the findings of the survey carried out in Brisbane on 1,263 retail food products. Eglezos *et al.* (2010) reported a high prevalence of *Bacillus cereus* on unbaked pizza, ready-to-reheat, frozen cooked meat pies, on processed meats and on raw diced chicken. The result of this study also concur with the findings of (Wijnands *et al.*, 2006a; Wijnands *et al.*, 2006b), who conducted a study on the prevalence of potentially pathogenic strains of *Bacillus cereus* in retail food samples. The presence of *Bacillus cereus* has also been reported in many studies conducted on the microbial quality of street food (Büyükyörük *et al.*, 2014; Kharel *et al.*, 2016; Madueke *et al.*, 2014; Murindamombe *et al.*, 2005; Olawale *et al.*, 2014). However, *Bacillus cereus* is recognised worldwide as a food-borne bacterium causing infections which account for a significant proportion of reported food-borne diseases with known aetiology (Moussa-Boudjemaa *et al.*, 2006). Its presence in different food types emphasises the public health importance of this organism. This is further supported by the fact that the organisms do not only cause food poisoning, which is usually self-limiting, but in addition, cause no-gastrointestinal infections which are life-threatening, particularly in immunocompromised patients (Avashia *et al.*, 2007; Naranjo *et al.*, 2011). The presence of different species of *Bacillus* in this study might be explained by the fact that most

of the meat samples were exposed to dust and flies as observed during the observational study (Figures 3.1, 3.2 and 3.3). *Lysinibacillus* spp. was also isolated in this study from chicken gizzard (20%) (Figure 4.18) and the overall in meat samples was 3.571% (Figure 4.19).

The result of this study is also in agreement with the findings of different researchers who isolated this microorganism from different samples Ahmed *et al.*, (2007) isolated this bacteria in the soil and it has been also isolated from plant tissues (Melnick *et al.*, 2011) from fermented plant seed products (Parkouda *et al.*, 2010) and even from puffer fish liver specimens (Wang *et al.*, 2010).

Enterococcus faecalis (3.571%), *Enterococcus faecium* (3.571%) and *Citrobacter* spp (3.571%) were also isolated in this study (Figure 4.19 and Tableau 4.18). A study conducted to assess the presence of Enterobacteriaceae in raw meat and handlers in Egypt also revealed the occurrence of *Citrobacter* spp., (Gwida *et al.*, 2014). The presence of *Enterococcus faecalis* in food was also reported by Olawa *et al.* (2014). The authors revealed that *Enterococcus faecalis* is an emerging pathogen of public health concern that causes a variety of human infections. Its presence in meat samples analysed may be due to poor handling practices observed before sampling (Figures 3.1, 3.2 and 3.3).

Other bacteria of health importance were also isolated in this study from different meat samples and from different streets under investigation such as *Kurthia* sp (7.14%) isolated from chicken meats (Figures 4.18 and 4.19 and Table 4.18). *Kurthia* spp. have also been isolated from various clinical materials, most commonly, from the faeces of patients suffering from diarrhoea, there is no evidence of pathogenicity in authentic numbers of the genus (Guang-Can *et al.*, 2008; Kline, 1948; Vos *et al.*, 2011). It presence in street meat may be due to poor handling applied by vendors

Planococcus antarcticus (3.571%) was isolated from chicken gizzard and *Planomicrobium glaciei* (3.571%) isolated from chicken gizzard and chicken meat (Figures 4.18 and 4.19). Their presence in street food might be an indication that street food could be subject to a different kind of bacteria with high impact on consumers, especially low income and immunocompromised people. Not too much has been done for these species, such as *Planomicrobium glaciei* and *Planococcus antarcticus;* they are considered as novel strains of bacteria (Reddy *et al.*, 2002; Zhang *et al.*, 2009).

4.10.3 Conclusion

Food contamination by different micro-organisms isolated in this study could be due to poor hygienic conditions under which street vendors manipulate and prepare meat and also the quality of the surrounding environment in which food is sold. Although some of the micro-organisms isolated in this study are normal flora of different parts of human and animal body such as *Staphylococcus aureus*, *Enterococcus faecalis, Citrobacter* spp., and some are widely found in environment such as *Bacillus sp*, *Bacillus cereus,* some micro-organisms isolated in this study have been associated with food-borne diseases and many problems such as *Staphylococcus aureus*, *Bacillus cereus* and *Bacillus* sp. The presence of *Staphylococcus aureus* and *Enterococcus faecalis* in street food is an indication that contamination may result from inappropriate processing, incomplete heating or a secondary contamination with contaminated equipment or poor handling process.

CHAPTER 5

ANTIBIOTIC RESISTANCE PROFILE OF BACTERIAL ISOLATES

5.1 INTRODUCTION

The intense use and misuse of antibiotics in Animals farms to prevent diseases are undoubtedly the major forces associated with high numbers of resistant pathogenic and commensal bacteria worldwide (Petrovic *et al.*, 2010). This could lead to antimicrobial resistance among pathogenic and commensal bacteria in these animals (Petrovic *et al.*, 2010). Food contamination with multi-drug resistant (MDR) bacteria is a major problem for public health, as resistance traits located on mobile genetic elements can be transferred to other bacteria of clinical significance (Petrovic *et al.*, 2015; Van *et al.*, 2007).

5.2 Objectives of the study

The objective of this chapter was to determine the antibiotic resistance profile of bacterial isolates using the disc diffusion method.

5.3 Materials and methods

5.3.1 Antimicrobial test

Twenty eight bacterial isolates from meat samples collected in three streets of Johannesburg Central Business District (CBD) were tested for antibiotic resistance profiles.

5.4 METHODOLOGY

This test was performed as described by Kirby (Bauer *et al.*, 1966). A Pure colony of isolates from a fresh culture was inoculated in 5ml of nutrient broth and incubated for 24 hours at 37°C. After incubation period, aliquots of 100 µl from the suspensions were spread plated on Mueller Hinton agar using a sterile L-shape through the entire surface of Muller Hinton agar plates. After the inoculum was dried for about 5 minutes, three standard antibiotic disks each containing specific concentration of antibiotics was applied per plate. The disks were gently pressed onto the inoculated Mueller Hinton agar to ensure intimate contact with the surface and plates were incubated aerobically at 37 °C for 18 to 24 hours. After incubation, the antibiotic inhibition zone diameters (IZD) were measured (mm). The inhibition zone was measured in millimetres to interpret sensitive, intermediate or resistance by following the guidelines of the Clinical Laboratory Institute Standards (CLSI. 2011).

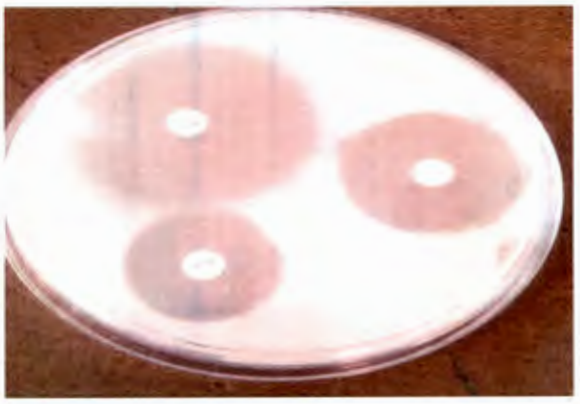

Figure 5.1: Antimicrobial test of *Staphylococcus aureus* against Ampicillin, Erythromycin and Ciprofloxacin

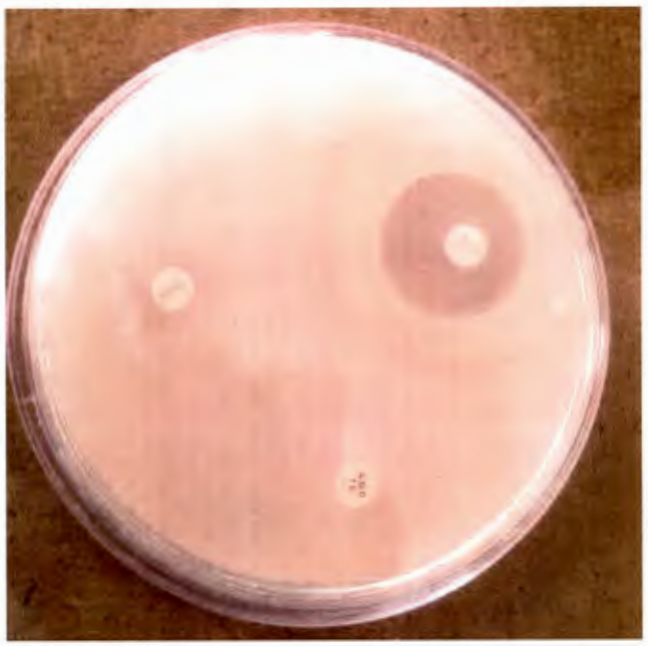

Figure 5.2: Antimicrobial test of *Staphylococcus aureus* against Ampicillin, Erythromycin and Ciprofloxacin

5.5 STATISTICAL ANALYSIS

Cross tabulation was used to summarise the result according to the profile categories namely: Resistant; Intermediate; and Susceptible. Chi-square was also used to determine the variations at 5% significance level among the isolates. All the statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 20.0.

Table 5.1: Guideline of antibiotic resistance according to the Clinical Laboratory Institute CLSI (2011)

Antibiotics	Abbreviation	Antibiotic concentration (Disc/ mg)	Zone diameter, Breakpoints Nearest whole Mm		
			Susceptible s	Intermediate I	Resistant R
Ampicillin	**AMP10**	(10 mg)	≥ 29	-	≤ 28
Gentamicin	**CN10**	(10 mg)	≥ 15	13-14	≤ 12
Tetracycline	**TE30**	(30 mg)	≥ 19	15-18	≤ 14
Sulphonamides	**S3 300**	(300 mg)	≥ 17	13-16	≤ 12
Streptomycin	**S 300**	(10 mg)	≥ 15	12-14	≤ 11
Ciprofloxacin	**CIP5**	(5 mg)	≥ 21	16-20	≤ 15
Chloramphenicol	**C30**	(30 mg)	≥ 18	13-17	≤ 12
Erythromycin	**E5**	(5 mg)	≥ 23	14-22	≤ 13

5.6 RESULTS OF ANTIMICROBIAL TEST

The antibiotic resistance profile of the bacteria isolates obtained from the samples collected at MTN-taxi rank (Figure 5.3). B. cereus had the strongest resistance (29%) to ampicillin when compared with other isolates (P. glaciei, Lysinibacillus, bacillus subtilis, P. antarcticus, and P. glaciei) with 14% resistance. All the isolates had 17% resistance to tetracycline, while P. glaciei, Lysinibacillus and S. aureus had 33% resistance to sulphonamides. In addition, it was discovered that P. glaciei had 100% resistance ability to chloramphenicol, while other isolates had no resistance to the antibiotic. B. subtilis, B cereus, P. antarcticus and P. glaciei had 25% resistance each to erythromycin.

The results obtained for the samples collected around Bree Street (Figure 5.4), revealed that most of the bacteria isolates were resistant to ampicillin with varying degree of resistance (Figure 5.4). *Kurthia* sp and *M. caseolyticus* had the strongest resistance (18%) while the remaining bacteria exhibited equal resistance ability of 9%. Resistance to tetracycline was observed on six bacteria, *Kurthia* sp had 29% resistance while the remaining five isolates had 14% resistance each. Only two (*S. aureus* and *M. caseolyticus*) out of all the isolates were resistance to sulphonamides with 50% resistance each, while *E. faecalis* exhibited 100% resistance to ciprofloxacin. Furthermore, four bacterial isolates (*Bacillus* sp, *Kurthia* sp, *S*

Vitulinus and *S. eqorium*) had equal resistance of 25% each to chloramphenicol while resistance to erythromycin, was exhibited by five bacterial isolates (*Bacillus* sp *M. caseolyticus B. thurigiens S. aureus* and *E. faecalis*) with 20% resistance each.

The results of antimicrobial test for the samples collected around Hancock Street (corner Claim) presented in Figure 5.5. The results revealed that *M. caseolyticus* exhibited 40% resistance to ampicillin while *S. aureus*, *Citrobacte*r sp, and *Bacillus* spp had 20%, while *S. aureus* showed 50% resistance to tetracycline followed by *M. caseolyticus* (33%) and *Bacillus* sp (17%). It was also found that *Bacillus* sp and *S. aureus* had 50% resistance each to sulphonamides. *S. aureus* had 100% resistance to streptomycin, while *S. aureus, Bacillus* sp and *M. caseolyticus* had 50, 25 and 25% resistance to chloramphenicol respectively. *Citrobacter* spp and S. *aureus* shared equal resistance ability of 50% each to erythromycin. The bacteria isolates showed different level of phenotypic resistance to different antibiotics tested. Most of these bacterial isolates showed resistance to more than two antibiotics tested. *S. aureus* showed resistance to ampicillin, tetracycline, sulphonamides, streptomycin, chloramphenicol and erythromycin. *Bacillus cereus* showed resistance to ampicillin, tetracycline and erythromycin. *Bacillus* spp showed resistance to ampicillin, tetracycline, sulphonamides, chloramphenicol and erythromycin as shown in appendix 4.

Results (see Appendix 4) were used to classify isolates as being resistant, intermediate or susceptible to a particular antibiotic using standard reference in Table 5.1 recommended by Clinical Laboratory Institute standards (Wayne, 2009; Wikler, 2006; Wikler, 2007).

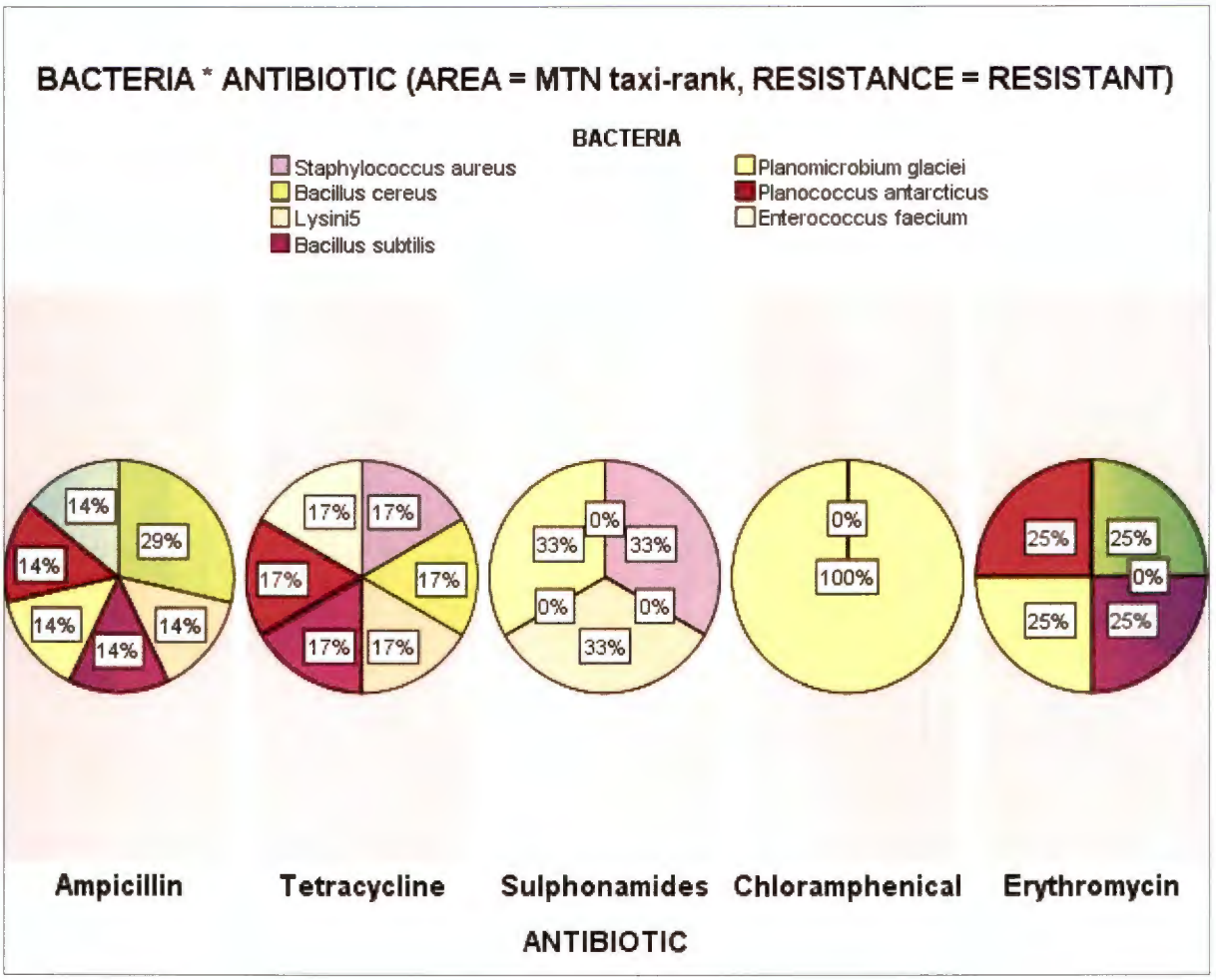

Figure 5.3: Summary of antibiotic resistance profile among the bacterial isolates according to the area of sampling.

The Figure shows that B. *cereus* represented 29%, while the other bacteria represented 14% and was resistant to Ampicillin. All the bacteria isolated (17%) at MTN taxi rank were found to be resistant to tetracycline. *P. glaciei, S. aureus* and *Lysinibacillus* (33%) were found to be resistant to sulphonamides, P. glaciei (100%) were found to be resistant to chloramphenicol, *B. subtilis*, *B. cereus, P. antarcticus and P. glaciei* (25%) were found to be resistant to erythromycin.

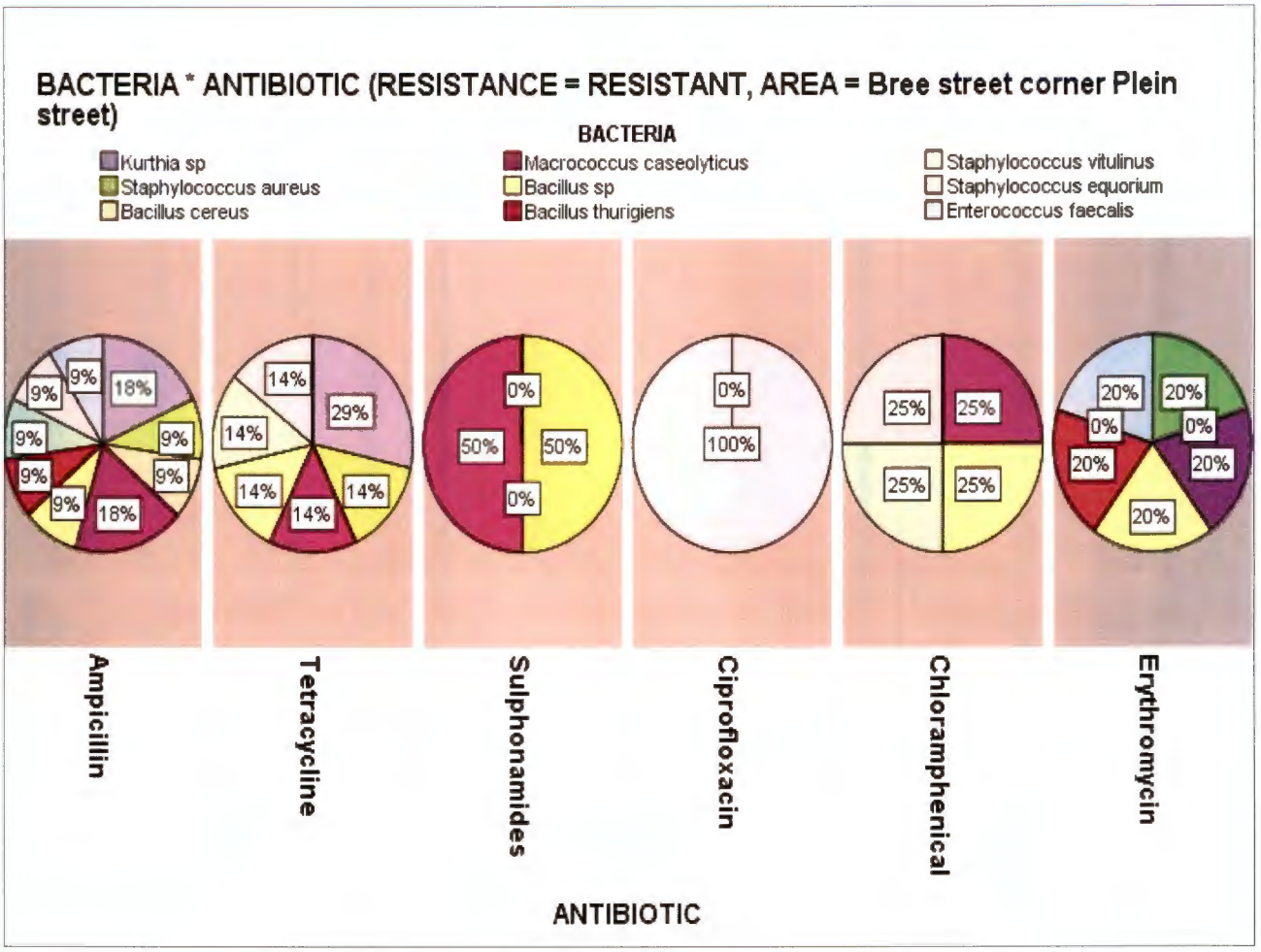

Figure 5.4: Summary of antibiotic resistance profile among the selected bacteria according to the Street of sampling

Figure 5.4 shows that all bacterial isolates were resistant to ampicillin, *Kurthia* sp (18%), *M. caseolyticus* (18%), and other bacteria at (9%). Resistance to tetracycline was observed on six bacteria *Kurthia* sp (29%), all five bacterial isolates in this Street respectively (14%), *S. aureus* (50%) and *M. caseolyticus* (50%), were resistant to sulphonamides, *E. faecalis* (100%) were resistant to ciprofloxacin, four bacterial isolates in this Street were resistant to chloramphenicol, resistance to erythromycin was observed for five bacterial isolates, *Bacillus* sp (20%), *M. caseolyticus* (20%), *B. thurigiens* (20%), *S. aureus* (20%) and *E. faecalis* (20%)

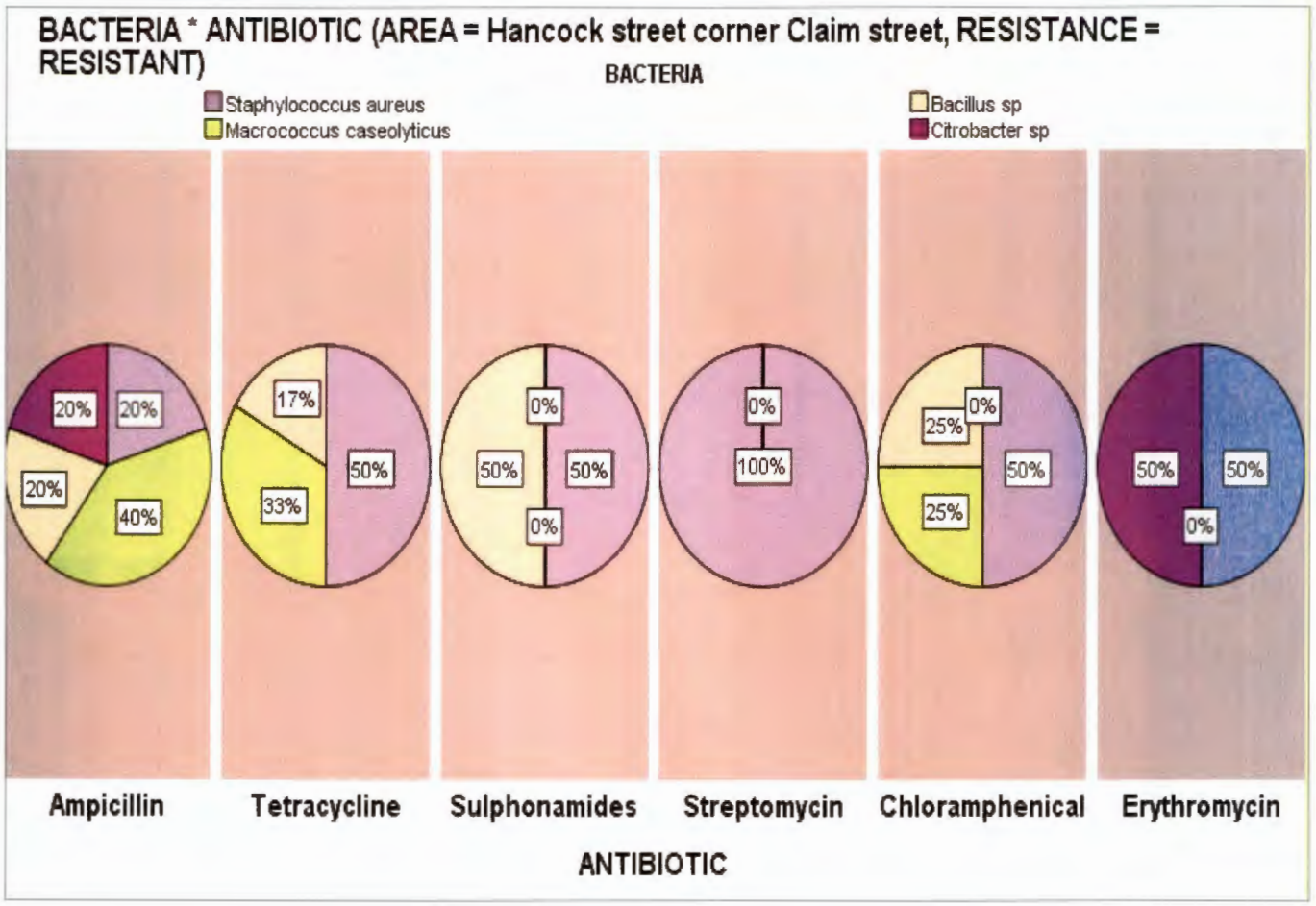

Figure 5.5: Summary of antibiotic resistance profile among the selected bacteria according to the street of sampling

Figure 5.5 shows that *S. aureus*, *Citrobacter* sp, *Bacillus* spp. (20% respectively), and *M. caseolyticus* 40%, were resistant to ampicillin, *S. aureus* (50%), *M. caseolyticus* (33%) and *Bacillus* sp (17%) were found to be resistant to tetracycline. It was also found that 50% of *Bacillus* sp and 50% of *S. aureus* (50%) were resistant to sulphonamides, 100% of *S. aureus* were resistant to streptomycin, *S. aureus* (50%), *Bacillus* sp (25%) and 25% of *M. caseolyticus* were resistant to chloramphenicol, 50% of Citrobacter and 50% of *S. aureus* were resistant to erythromycin (Figure 5.5).

Table 5.3: Resistance profile of different bacteria to different antibiotics

	BACTERIA														
	Kurthia sp	*Staphylococcus aureus*	*Bacillus cereus*	*Macrococcus caseolyticus*	*Bacillus* sp	*Lysinibacillus*	*Bacillus thurigiens*	*Staphylococcus vitulinus*	*Bacillus subtilis*	*Planomicrobium glaciei*	*Planococcus antarcticus*	*Citrobacter* sp	*Staphylococcus equorium*	*Enterococcus faecium*	*Enterococcus faecalis*
Ampicillin	2	2	3	4	2	1	1	1	1	1	1	1	1	1	1
Tetracycline	2	5	1	3	2	1	0	1	1	0	1	0	1	1	0
Sulphonamides	0	3	0	1	1	1	0	0	0	1	0	0	0	0	0
Streptomycin	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Ciproflo xacin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Chloramphenicol	0	2	0	2	2	0	0	1	0	1	0	0	1	0	0
Erythromycin	0	2	1	1	1	0	1	0	1	1	1	1	0	0	1

Table 5.3 shows Resistance profile of bacteria to different antibiotics: the counts differ because there were different types of bacteria and different areas (1, 2, 3, 4 and 5 represent the number of bacteria found to be resistant)

Table 5.4: Antimicrobial resistance profile for selected isolates

	BACTERIA							
	Kurthia sp	*Staphylococcus aureus*	*Bacillus cereus*	*Macrococcus caseolyticus*	*Bacillus sp*	*Lysinibacillus*	*Bacillus thurigiens*	*Staphylococcus vitulinus*
List of antibiotics that the bacteria are resistant to	Ampicillin Tetracycline	Ampicillin Tetracycline Sulphonamides Streptomycin Chloramphenicol Ciprofloxacin	Ampicillin Tetracycline Ciprofloxacin	Ampicillin Tetracycline Sulphonamides Chloramphenicol Ciprofloxacin	Ampicillin Tetracycline Sulphonamides Chloramphenicol Ciprofloxacin	Ampicillin Tetracycline Sulphonamides	Ampicillin Ciprofloxacin	Ampicillin Tetracycline Chloramphenicol

Table 5.4 shows that *Staphylococcus aureus* was resistant to six out of eight antibiotics, followed by *Macrococcus caseolyticus* and *Bacillus* sp (resistant to five out of eight antibiotics). *Kurthia* sp, *Citrobacter* sp, *Enterococcus faecium*, *Enterococcus faecalis*, *Citrobacter* sp, *Enterococcus faecium* and *Enterococcus faecalis* are resistant to relatively few antibiotics (resistant to only two out of eight antibiotics)

Table 5.5: Bacteria showing intermediate profile

	BACTERIA													
	Kurthia sp	*Staphylococcus aureus*	*Bacillus cereus*	*Macrococcus caseolyticus*	*Bacillus sp*	*Lysini5*	*Bacillus thuriginens*	*Staphylococcus vitulinus*	*Bacillus subtilis*	*Planococcus antarcticus*	*Citrobacter sp*	*Staphylococcus equorium*	*Enterococcus faecium*	*Enterococcus faecalis*
Ampicillin	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Gentamicin	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Tetracycline	0	0	2	1	0	0	1	0	0	0	1	0	0	1
Sulphonamides	0	1	0	0	1	0	0	0	0	1	0	1	0	1
Streptomycin	0	0	0	0	0	0	0	1	0	0	0	1	0	0
Ciprofloxacin	2	4	2	3	1	0	1	1	1	0	0	1	0	0
Chloramphenicol	0	3	1	0	0	0	1	0	1	1	1	0	0	0
Erythromycin	0	5	2	3	1	1	0	1	0	0	0	0	1	0

Table 5.5 presents the count of occurrences of instances where the bacteria had intermediate profile to antibiotics. The counts differ because there were different types of bacteria and because they were from different streets.

Table 5.6: Bacteria with intermediate profile

	BACTERIA							
	Kurthia sp	*Staphylococcus aureus*	*Bacillus cereus*	*Macrococcus caseolyticus*	*Bacillus* sp	*Lysinibacillus*	*Bacillus thurigiens*	*Staphylococcus vitulinus*
List of bacteria exhibited intermediate profile	Ciprofloxacin	Ampicillin Sulphonamides Ciprofloxacin Chloramphenicol Erythromycin	Tetracycline Ciprofloxacin Chloramphenicol Erythromycin	Tetracycline Ciprofloxacin Erythromycin	Sulphonamides Ciprofloxacin Erythromycin	Erythromycin	Tetracycline Ciprofloxacin Chloramphenicol	Streptomycin Ciprofloxacin Erythromycin

Table 5.6: Overall bacteria with intermediate profile (Continued)

BACTERIA						
	Bacillus subtilis	*Planococcus antarcticus*	*Citrobacter* sp	*Staphylococcus equorium*	*Enterococcus faecium*	*Enterococcus faecalis*
List of bacteria exhibited intermediate profile	Ciprofloxacin Chloramphenicol	Gentamicin Sulphonamides chloramphenicol	Tetracycline chloramphenicol	Sulphonamides Streptomycin Ciprofloxacin	Erythromycin	Tetracycline Sulphonamides

Table 5.6 show that *Staphylococcus aureus* had intermediate profile to most the antibiotics (five out of eight antibiotics), followed by *Bacillus cereus* (four out of eight antibiotics). *Kurthia* sp, *Lysinibacillus and Enterococcus faecium* had intermediate resistance to relatively few antibiotics (only one out of eight antibiotics).

Table 5.7: Summary of bacteria susceptible to antibiotics

	BACTERIA														
	Kurthia sp	*Staphylococcus aureus*	*Bacillus cereus*	*Macrococcus caseolyticus*	*Bacillus sp*	*Lysini5*	*Bacillus thurigiens*	*Staphylococcus vitulinus*	*Bacillus subtilis*	*Planomicrobium glaciei*	*Planococcus antarcticus*	*Citrobacter sp*	*Staphylococcus equoriu m*	*Enterococcus faeciu m*	*Enterococcus faecalis*
Ampicillin	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0
Gentamicin	2	7	3	4	2	1	1	1	1	1	0	1	1	1	1
Tetracycline	0	2	0	0	0	0	0	0	0	1	0	0	0	0	0
Sulphonamides	2	3	3	3	0	0	1	1	1	0	0	1	0	1	0
Streptomycin	2	6	3	4	2	1	1	0	1	1	1	1	0	1	1
Ciproflox acin	0	3	1	1	1	1	0	0	0	1	1	1	0	1	0
Chloramphenicol	2	2	2	2	0	1	0	0	0	0	0	0	0	1	1
Erythromycin	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0

Table 5.7 presents the count of occurrences of instances where the bacteria were susceptible to antibiotics.

Table 5.8: Summary of bacteria susceptible to different antibiotics

	BACTERIA							
	Kurthia sp	*Staphylococcus aureus*	*Bacillus cereus*	*Macrococcus caseolyticus*	*Bacillus* sp	*Lysinibacillus*	*Bacillus thurigiens*	Staphylococcus vitulinus
List of antibiotics that the bacteria are susceptible to	Gentamicin Sulphonamides Streptomycin Chloramphenicol Erythromycin	Ampicillin Gentamicin Tetracycline Sulphonamides Streptomycin Ciprofloxacin Chloramphenicol	Gentamicin Sulphonamides Streptomycin Ciprofloxacin Chloramphenicol	Gentamicin Sulphonamides Streptomycin Ciprofloxacin Chloramphenicol	Gentamicin Streptomycin Ciprofloxacin	Gentamicin Streptomycin Ciprofloxacin Chloramphenicol	Gentamicin Sulphonamides Streptomycin	Gentamicin Sulphonamides

Table 5.8: Summary of bacteria susceptible to different antibiotics (Continued)

	BACTERIA						
	Bacillus subtilis	*Planomicrobium glaciei*	*Planococcus antarcticus*	*Citrobacter* sp	*Staphylococcus equorium*	*Enterococcus faecium*	*Enterococcus faecalis*
List of antibiotics that the bacteria are susceptible to	Gentamicin Sulphonamides Streptomycin	Gentamicin Tetracycline Streptomycin Ciprofloxacin	Streptomycin Ciprofloxacin	Gentamicin Sulphonamides Streptomycin Ciprofloxacin	Gentamicin Erythromycin	Gentamicin Sulphonamides Streptomycin Ciprofloxacin Chloramphenicol	Gentamicin Streptomycin Chloramphenicol

Table 5.8 shows that *Staphylococcus aureus* was susceptible to most antibiotics (seven out of eight antibiotics), followed by *Kurthia* sp, *Bacillus cereus*, *Macrococcus caseolyticus* and *Enterococcus faecium* (five out of eight antibiotics). *Staphylococcus vitulinus* and *Planococcus antarcticus* were susceptible to relatively few antibiotics (only two out of eight antibiotics).

Table 5.9: Chi-Square test of association

Variables		Value	Df	Asymp. Sig. (2-sided)
Area	Bacteria	225.884	28.000	0.000
Meat	Bacteria	478.781	56.000	0.000

Table 5.9 shows that the type of bacteria is significantly associated with the area and the type of meat at 5% level of significance

Table 5.10: Difference in mean ranks between antibiotics, meat samples and bacteria

Variables			
Antibiotic resistance	Meat	Chi-Square	1.930
		Df	4.000
		Asymp. Sig.	0.749
Antibiotic resistance	Antibiotic	Chi-Square	98.618
		Df	7.000
		Asymp. Sig.	0.000
Antibiotic resistance	Bacteria	Chi-Square	8.402
		Df	14.000
		Asymp. Sig.	0.867

Table 5.10 shows that antibiotic resistance does not differ significantly across the types of meat at 5% level of significance. Also, on the other hand, antibiotic resistance does not differ significantly across the types of bacteria at 5% level of significance. Antibiotic resistances differ across the types of antibiotics at 5% level of significance.

5.7 DISCUSSION

5.8 ANALYSIS OF ANTIMICROBIAL RESISTANCE PROFILE OF BACTERIAL ISOLATES

The results obtained in this study revealed that most bacterial isolates were resistant to tetracycline and ampicillin, followed by chloramphenicol (Figures 5.3, 5.4 and 5.5; Tables 5.3 and 5.4). The results of this study are in agreement with the findings of the study by Darwish *et al.* (2013), who found high resistance of bacteria to tetracycline

The results of this study also revealed that most *S. aureus* isolated were resistant to tetracycline (Tables 5.3 and 5.4; Figures 5.4 and 5.5). These results correlate with the study conducted by Trzcinski *et al.* (2000), who tested sixty-six clinical isolates and found all of them resistant to tetracycline. After investigation, it was discovered that all isolates analysed carried at least one of the *tet* genes. Based on the results of the PCR, 24 isolates were classified as *tetM* genotype, 21 as *tetK* and 21 as *tetKM.* All MRSA analysed were *tetO-* and *tetL* (Trzcinski *et al.*, 2000). The resistance of *S. aureus* to tetracycline has also been reported by Schmitz *et al.* (2001). The researchers investigated the susceptibility of tetracycline 3052 *S. aureus* isolates from clinical samples.

In this study, *S. aureus* were found also to be resistant to ampicillin (Figures 5.4 and 5.5) and Chloramphenicol (Tables 1 and 2, Figure 3). This result correlates with the findings of Uwaezuoke and Aririatu (2004), who isolated *S. aureus* from clinical specimens and fount that *S. aureus was* resistant to Ampicillin, Tetracycline and Chloramphenicol. The result of this study is similar with a study conducted, on the prevalence and antibiotic resistance of *Staphylococcus* spp isolated from meat product sold in the street of Abidjan by Attien *et al.,* (2013), and found that *S. aureus* recovered from street meat were resistance to erythromycin and tetracycline. The result of this study correlate also with a study conducted by Achi & Madubuik (2007), on the prevalence and antimicrobial resistance of *Staphylococcus aureus* isolated from retail ready-to-eat foods in Nigeria, the researchers found that *Staphylococcus aureus* was exhibiting a resistance to more than 2 or 3 antibiotics, in their study it was observed high resistance to tetracycline, gentamicin, chloramphenicol and ciprofloxacin. Another study conducted in Cotonou, Benin by Sina *et al.,* (2011), on Characterisation of *Staphylococcus aureus* isolated from street foods: toxin profile and prevalence of antibiotic resistance revealed that *S.aureus* recovered from street food was exhibiting different level of resistance to different antibiotic.

The results of this study are consistent with the findings of Zou *et al.,* (2012). These researchers conducted a study on antimicrobial resistance and molecular epidemiological characteristics of clinical isolates of *S. aureus* in Changsha, China and found that *S. aureus* was resistant to ampicillin and erythromycin (Figure 5.5). *S. aureus* has been reported to be multi-drug resistant and the results of this study (Tables 5.3 and 5.4; Figures 5.3, 5.4 and 5.5) concur with the findings of several studies conducted on multidrug resistance of *S. aureus* isolated in meat and poultry (Abdalrahman *et al.*, 2015; Fan *et al.*, 2015; Schmitz *et al.*, 2001; Uwaezuoke & Aririatu, 2004; Waters *et al.*, 2011; Zou *et al.*, 2012). Resistance to Tetracycline, which has been identified in *S. aureus* may result from the acquisition of the *tetK* and *tetL* genes located on a plasmid, and ribosomal protection mediated by *tet* M or *tet*O (Trzcinski *et al.*, 2000).

Staphylococcus vitulinus from the second research site, isolated in wors meat sample, was found to be resistant to three antibiotics (ampicillin, tetracycline and chloramphenicol) (Figure 5.4) and intermediate to three antibiotics (erythromycin, ciprofloxacin and streptomycin) (Tables 5.5 and 5.6) and susceptible to two antibiotics (gentamicin and sulphonamides) (Tables 5.7 and 5.8). Resistance of *Staphylococcus vitulinus* to Chloramphenicol has also been reported by Fijalkowski *et al.* (2016). The researchers isolated *Staphylococcus vitulinus* and *Staphylococcus equorum* from ready-to-eat meat. These results differ from the findings of Fijalkowski *et al.* (2016). Resistance of *Staphylococcus vitulinus* to erythromycin and ciprofloxacin, which was found to be intermediate in this study (Appendix 4), and resistance to Gentamicin was not observed in this study (Fijałkowski *et al.*, 2016).

Staphylococcus equorum (from Street number two), isolated in chicken meat sample, was found to be resistant to two antibiotics (Ampicillin and Tetracycline) (Figure 5.4, Tables 5.3 and 5.4), intermediate to ciprofloxacin and erythromycin (Tables 5.5 and 5.6). Resistance of *Staphylococcus equorum* to Tetracycline was also reported by Fijalkowski *et al.* (2016), but resistance to erythromycin and chloramphenicol was not observed in this study, which reveals the difference in terms of findings with the study conducted by (Fijałkowski *et al.*, 2016).

In this study, three *B. cereus* isolated from different areas and meat samples were also tested for antibiotic resistance profile. The results revealed that *B. cereus* from Street number one, isolated from beef intestine, was found to be resistant to one antibiotic (ampicillin) (Figure 5.3), intermediate to tetracycline and ciprofloxacin, and susceptible to all other antibiotics tested (Table 5.8). Another *B.*

cereus from another area one, but isolated from chicken gizzard, was found to be resistant to three antibiotics out of eight tested (ampicillin, tetracycline and erythromycin) (Figure 5.3 and Table 5.4), intermediate to ciprofloxacin and chloramphenicol (Table 5.6) and susceptible to another antibiotic (Table 5.8).

The results of this study differ from the findings of Schlegelova (2003) who assessed whether foodstuffs contaminated with *Bacillus cereus* may concurrently be vectors of spreading resistance. In his study, *B. cereus* was found to be susceptible to ampicillin and resistant to streptomycin (Schlegelova *et al.*, 2003). In a study conducted in Nigeria to assess the incidence and antibiotic sensitivity of *B. cereus* isolated from ready-to-eat food sold in some markets, the findings correlate with related to gentamicin and streptomycin (Table 5.8), which was found susceptible, and differs regarding chloramphenicol, ciprofloxacin and erythromycin (Agwa *et al.*, 2012). The result of this study are also in line with the findings of Turnbull *et al.* (2004) who observed that *B. cereus* isolated from clinical and environment sources were resistant to tetracycline. However, the result obtained in this study differs from the findings of Fazlani *et al* (2011). The researchers evaluated antimicrobial susceptibility of bacterial species from mastitis milk samples of camel and found that tetracycline was highly sensitive.

Resistance of *B. cereus* isolated from meat or chicken to different antibiotics, such as ampicillin and tetracycline have been reported in several studies. The results of this study are in agreement with the findings of different researchers (Fazlani *et al.*, 2011; Jawad *et al.*, 2016; Kim *et al.*, 2013; Tahmasebi *et al.*, 2014; Turnbull *et al.*, 2004), who also reported the resistance of *B. cereus* to ampicillin and tetracycline.

The results revealed that *Bacillus* sp, from area number two, isolated in wors meat samples, was found to be resistant to four antibiotics out of eight (ampicillin, tetracycline, chloramphenicol and erythromycin) (Figure 5.4), intermediate to Sulphonamides (Table 5.6) and susceptible to other antibiotics (Table 5.8). It was also observed in this study that *Bacillus* sp from area number three, isolated in beef head meat sample, was also found to be resistant to four antibiotics (ampicillin, tetracycline, sulphonamides and chloramphenicol (Figure 5.5), intermediate to Ciprofloxacin (Table 5.6), and susceptible to all other antibiotics (Table 5.8).

These results correlate with the findings of the study conducted by Sadashiv & Kaliwal (2014) on the isolation, characterisation and antibiotic resistance of *Bacillus* species from bovine mastitis in the region

of north Karnataka, India. The study revealed that *Bacillus* spp. were resistant to ampicillin, erythromycin, tetracycline and chloramphenicol (which is a similar finding obtained in this study) (Sadashiv & Kaliwal, 2014). In the same study, *Bacillus* spp, was also found to be resistant to streptomycin and gentamicin, which did not match with the finding of this study. However, streptomycin and gentamicin were found to be susceptible to *Bacillus* spp. The result of this study is similar to the findings of Chauhan. (2013) who conducted a study on Prevalence and antibiotic resistance of *Bacillus* strains isolated from various food stuffs and found that different *Bacillus* spp were exhibiting different level of resistance to different antibiotic such as ampicillin, tetracycline, erythromycin and chloramphenicol.

The results of this study agree with the findings of the study conducted on the identification and antimicrobial resistance of Bacteria Isolated from Probiotic Products Used in Shrimp Culture which also revealed that *Bacillus* spp. were resistant to ampicillin, chloramphenicol and erythromycin (Uddin *et al.*, 2015).

Bacillus thurigiens isolated in wors meat samples from the second research site of this study, was found to be resistant to two antibiotics (ampicillin and erythromycin) (Figure 5.4), intermediate to three antibiotics (tetracycline, ciprofloxacin and chloramphenicol) (Table 5.6), and susceptible to three antibiotics (gentamicin, sulphonamides and streptomycin) (Table 5.8).

Bacillus subtilis, from the first research site, isolated in beef intestine, was found to be resistant to three antibiotics (ampicillin, tetracycline and erythromycin) (Figure 5.3). These results are in agreement with the findings of Sadashiv *et al.* (2014) who also obtained the same results in India on the isolation of *Bacillus* species from milk. The isolated *Bacillus* species were also found to be resistant to ampicillin, erythromycin and tetracycline as well as to other antibiotics (Table 5.6). However, in this study, the resistance of *Bacillus* species to gentamicin and ciprofloxacin were not observed (Sadashiv & Kaliwal, 2014). The results obtained in this study differ from the findings of Ayandiran *et al.*, (2014) who isolated *Bacillus* spp. from a polluted river in Nigeria and found that they were resistant to ciprofloxacin, chloramphenicol and streptomycin.

In this study, *M. caseolyticus* isolated from different sampling areas, mostly chicken meat and beef head meat samples, were found to be resistant to ampicillin and tetracycline (Figures 5.4 and 5.5). Resistance to chloramphenicol was also observed in one *M. caseolyticus* from the third research site isolated from

beef head meat (Figure 5.5). However, they presented intermediate profile to ciprofloxacin and erythromycin (Table 5.6) and were susceptible to other antibiotics used in this study (Table 5.8). *M. caseolyticus*, obtained from the second research site, and isolated in wors, were found to be resistant also to sulphonamides, chloramphenicol and erythromycin (Figure 5.4). These results are in line with those obtained by Enayat *et al.*, (2012) who found that *M. caseolyticus* was resistant to tetracycline. The results are also in line also with the findings of Baba *et al.*, (2009), who isolated *M. caseolyticus* from animal meat sold in supermarkets and found that *M. caseolyticus* was resistant to ampicillin, erythromycin and tetracycline (Table 5.3 and 5.4). However, these findings differ with the results of this study as gentamicin was not resistant (Baba *et al.*, 2009a).

Citrobacter spp. isolated from the third research site were mostly isolated in beef head meat and showed resistance mainly to two antibiotics: Ampicillin and Erythromycin (Figure 5.5). These results correlate with the findings of Metri *et al.*, (2013) who isolated *Citrobacter* spp. from urinary tract infection and observed their resistance to ampicillin. Resistance of *Citrobacter* spp. to ampicillin has also been reported by different researchers (Metri *et al.*, 2011; Moges *et al.*, 2014).

In this study, *Enterococcus faecium,* isolated in chicken gizzard collected from the first research site, were found to be resistant to Ampicillin and tetracycline (Figure 5.3). These results are in line with the findings of a study conducted in Brazil to assess the antimicrobial resistance profile of *E. faecium* and *faecalis* (Riboldi *et al.*, 2009). In addition, these results correlate with the findings of a study conducted in Turkey to assess the antibiotic resistance profile of *E. faecium* and *E. faecalis* isolated from broiler cloacal sample which showed that both *E. faecium* and *E. faecalis* were resistant to tetracycline and erythromycin.

Enterococcus faecalis, from the second research site, isolated in chicken meat sample, was found to be resistant to Ampicillin, Ciprofloxacin and Erythromycin (Figure 5.4 and Table 5.4). The results of this study correlate with the findings of another study conducted in China to investigate the multi-drug resistance of *E. faecalis.* It was found that the bacteria were resistant to Ciprofloxacin and Erythromycin (Liu *et al.*, 2014a). The results of this study concur with the findings of another study conducted in China, Xinjiang province to investigate the death of lambs. In the study, the researchers isolated *E. faecalis* from the brain of lambs and found that *E. faecalis* was resistant to ampicillin, erythromycin as well to other antibiotics (Xia *et al.*, 2013). The results of this study are also similar to the findings of another study conducted in Argentina to investigate the antimicrobial resistance profile of both *E.*

faecalis and *faecium* strains recovered from artisanal food of animal origin. The findings revealed that both *E. faecalis* and *faecium* were resistant to erythromycin, ampicillin, tetracycline and ciprofloxacin (Delpech *et al.*, 2012).

Kurthia sp, from the second research site, isolated in chicken meat samples, were found to be resistant to both Ampicillin and Tetracycline (Figure 5.4). *Lysinibacillus* spp., from the first research site, isolated in chicken gizzard, was found to be resistant to ampicillin, tetracycline and sulphonamides (Figure 5.3). These results are in line with the findings of Shanmuga *et al.*, (2016). The researchers conducted a study on the prevalence of multi-drug resistance among diverse bacteria communities in Bhavani river, isolated *Lysinibacillus* spp. from polluted water, and found that *Lysinibacillus* spp were resistant to ampicillin and tetracycline as well as to other antibiotics (Shanmuga *et al.*, 2016).

Planomicrobium glaciei, from the first research site, mostly isolated in chicken gizzard, were found to be resistant to ampicillin, sulphonamides, chloramphenicol and erythromycin (Figure 5.3), and susceptible to tetracycline, gentamicin, streptomycin and ciprofloxacin (Table 5.8).

In this study, *Planococcus antarcticus*, from the first sampling site, isolated in chicken gizzard, were found to be resistant to tetracycline, ampicillin and erythromycin (Figure 5.3), and intermediate to gentamicin, sulphonamides and chloramphenicol (Table 5.6). The results of this study differ from the findings of Yoon *et al.*, (2010), who isolated *Planococcus* spp. from sediments collected from a marine solar saltern at Byunsan on the west coast of Korea and found them to be susceptible to Streptomycin, chloramphenicol, ampicillin and gentamicin, resistance was observed for tetracycline (Yoon *et al.*, 2010).

The results of this study revealed that most of the bacteria isolated were resistant to ampicillin and tetracycline (Tables 5.3 and 5.4; Figures 5.3, 5.4 and 5.5). Resistance of bacteria to tetracycline have been reported in several studies (Chopra & Roberts, 2001; Michalova *et al.*, 2004; Speer *et al.*, 1992). The presence of tetracycline-resistant pathogens limits the use of these agents in the treatment of diseases. Tetracycline-resistance is often due to the acquisition of new genes, which code for energy-dependent efflux of Tetracycline or for a protein that protects bacterial ribosomes from the action of Tetracycline. Many of these genes are associated with mobile plasmids or transposons and can be distinguished from each other using molecular methods, including DNA-DNA hybridization with oligonucleotide probes and DNA sequencing (Chopra & Roberts, 2001). tetracycline-resistance

determinants can be found in the genomes of the physiological flora from humans, animals as well as from food and environmental sources (Michalova *et al.*, 2004). Additional mechanisms of tetracycline-resistance comprise of enzymatic inactivation of antibiotic, permeability barriers, mutations or multidrug transporter systems. Resistance of bacteria to ampicillin observed may be due to the acquisition of resistance gene (amp^r) codes for an enzyme (b-lactamase) secreted into the periplasmic space of the bacterium where it catalyzes hydrolysis of the b-lactam ring of the ampicillin. Thus, the gene product of the amp^r gene destroys the antibiotic and resistance to chloramphenicol may be due to the production of chloramphenicol acetyl-transferase (Ruiz *et al.*, 1999).

5.9 CONCLUSION

Bacterial isolates were subjected to antimicrobial resistance testing against eight antibiotics used in this study. The bacterial isolates showed a different level of phenotypic resistance to different antibiotics tested. Most of these bacterial isolates showed resistance to more than two antibiotics tested. *S. aureus* showed resistance to ampicillin, tetracycline, sulphonamides, streptomycin, chloramphenicol and erythromycin. *Bacillus cereus* showed resistance to ampicillin, tetracycline and erythromycin. *Bacillus* spp showed resistance to ampicillin, tetracycline, sulphonamides, chloramphenicol and erythromycin. These types of foods contaminated with multidrug resistance microorganisms can be potential vehicles for the transmission of foodborne infections among consumers and raise serious public health problems. The results showed that the quality of ready-to-eat meat sold around Johannesburg Central Business District is deteriorating and if the resistant pathogens are augmented in this kind of food, it could be a threat to consumers in the future. The relatively high, varying occurrence and multi-drug resistance in bacterial isolates requires the implementation of strong intervention measures such as monitoring and education to minimise cross-contamination at all stages of preparation of meat.

CHAPTER SIX

GENERAL CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

The main objective of this study was to use qualitative and quantitative approaches to inquiry in order to determine the molecular characterisation of foodborne pathogens and their antibiotic resistance profiles in ready-to-eat meat sold in streets around Johannesburg CBD. To achieve the main objectives of the

study, an observational study was conducted around Johannesburg CBD and meat samples such as chicken meat, chicken gizzard, beef intestine, beef head meat were collected around Johannesburg CBD. Considering the results of the observational study, there is still a need for food safety to be improved among street-vended food. Most of the food samples collected from the different streets was found to be contaminated at varying degrees. This situation may be due to the influence of bad hygienic practices and poor sanitation of vending areas as well as exposure of food to dust and flies (on the microbiological quality of street food analysed). Even the food handling practices by street vendors and the environment where street food vendors are operating raise serious concern, in an untidy environment, such conditions could lead to the transmission of foodborne pathogens.

Furthermore, the microbiological study revealed that ready-to-eat meat analysed were contaminated with different kinds of bacteria such as *Kurthia* sp, *Macrococcus caseolyticus, Lysinibacillus, Staphylococcus vitulinus, Bacillus subtilis, Planomicrobium glaciei, Planococcus antarcticus, Citrobacter* sp, *Staphylococcus equorum, Enterococcus faecium, Enterococcus faecalis, Staphylococcus aureus and Bacillus cereus.* This situation could be a potential public health danger among consumers. The study also revealed that there is a need for good hygiene practices, proper handling of foods, as well as a clean environment to ensure good quality and safe food.

Also, the presence of *Staphylococcus aureus* and *Enterococcus faecalis* isolated in this study is an indication that faecal contamination occurs during sales or unhygienic handling of street-vended meat. The presence of these organisms in cooked meat shows an unacceptable state of poor hygienic and sanitary practices applied to street-vended meat. Furthermore, the occurrence of *Bacillus* sp, *Bacillus cereus* and *Staphylococcus aureus* observed in this study are pathogenic bacteria and are a cause of foodborne diseases. Consequently, the implementation of good cooking methods and techniques, personal hygiene, good handling practices, as well as the sanitation of vending locations is necessary. It was also revealed that these types of ready-to-eat meat, contaminated with multi-antibiotic resistance microorganisms could be potential vehicles for the transmission of foodborne diseases. Resistant bacteria may be transmitted to the human food supply chain and increase the risk of treatment failures.

In this study, the identification of bacterial isolates was performed using conventional biochemical test, as well as molecular methods based on 16S rRNA, species specific gene amplification by PCR. It was revealed that PCR is a very useful tool for the detection of microorganisms. The method is a rapid and real approach for the detection of microorganisms.

Another objective of this study was to determine antibiotic resistance profile of isolates bacteria against different antibiotics commonly used in veterinary and human medicine. The results obtained in this study showed that many of the bacterial isolates were resistant to two or more than three antibiotics, and most of the isolates were resistant to Tetracycline, Ampicillin followed by Chloramphenicol as well as other antibiotics.

Moreover, the resistance of antibiotics observed in this study among bacteria might be explained as an exposure to low doses or over usage of these antibiotics on the animal in order to threat or prevent diseases. This might lead to resistance and the introduction of mutagenesis. The resistance of bacteria to antibiotic may be considered as an indication of antibiotic resistance and resistant genes among the public and the environment.

6.2 RECOMMENDATION

There is a need for further molecular studies on antibiotics among bacterial isolates. This could possibly assist in terms of determining the presence of antibiotic resistance genes and their spread in the environment. The government should establish inter-sectoral national strategies and action plan on antibiotic resistance, this approach could include food safety program and could promote the prudent use of antibiotics in all sectors. Otherwise the government could establish a platform with specific mechanism of interaction between the health ministry and other relevant ministries and authorities to address antibiotic resistance in the food chain. As significant numbers of unemployed from different areas of South Africa or different African countries earning income from street vending food, government should give attention to that, and provide the necessary infrastructure to the vendors in order to improve the safety of street vended food as well as the education.

6.3 LIMITATION OF THE STUDY

The study has for geographical scope the entire Johannesburg CBD. Even if the CBD is big and difficult to explore all the areas where ready to eat meat is sold, so to analyse only a reality of three streets in Johannesburg CBD cannot show the real picture of the situation as the street meat is increasing and growing day by day around Johannesburg CBD. In order to gain information on Microbial quality the three streets were chosen as samples of data collection. Morphological study was performed only on nutrient agar which gives some difficulty to differentiate the bacteria morphology clearly.

REFERENCE

Abdallah, M.S. & Mustafa, N.E.M. 2010. Bacteriological quality of street-vended Um-Jinger: a traditional Sudanese food. *Internet Journal of Food Safety,* 12 (1).

Abdalrahman, L.S., Stanley, A., Wells, H. & Fakhr, M.K. 2015. Isolation, virulence, and antimicrobial resistance of methicillin-resistant Staphylococcus aureus (MRSA) and methicillin sensitive Staphylococcus aureus (MSSA) strains from Oklahoma retail poultry meats. *International journal of environmental research and public health,* 12 (6):6148-6161.

Abdelgadir, A.M., Srivastava, K.K. & Reddy, P.G. 2009. Detection of *Listeria monocytogenes* in ready-to-eat meat products. *American Journal of Animal and Veterinary Sciences,* 4 (4):101-107.

Abdulgader, S., Shittu, A., Nicol, M. & Kaba, M. 2014. Molecular epidemiology of methicillin-resistant Staphylococcus aureus in Africa: A systematic review of the published literature. *International Journal of Infectious Diseases,* 21:107.

Achi, O., & Madubuik, K. 2007. Prevalence and antimicrobial resistance of Staphylococcus aureus isolated from retail ready to eat food inNigeria. Research Journal of Microbiology, 2 (6): 516-523.

Abdulgader, S.M., Shittu, A.O., Nicol, M.P. & Kaba, M. 2015. Molecular epidemiology of Methicillin-resistant Staphylococcus aureus in Africa: a systematic review. *Frontiers in microbiology,* 6:348.

Adam, I., Hiamey, S.E. & Afenyo, E.A. 2014. Students' food safety concerns and choice of eating place in Ghana. *Food Control,* 43:135-141.

Addis, M. & Sisay, D. 2015. A Review on Major Food Borne Bacterial Illnesses. *Journal of Tropical Diseases & Public Health,* 2015.

Adley, C., Dillon, C., Morris, C., Delappe, N. & Cormican, M. 2011. Prevalence of Salmonella in pig ear pet treats. *Food Research International,* 44 (1):193-197.

Adzitey, F., Rusul, G. & Huda, N. 2012. Prevalence and antibiotic resistance of Salmonella serovars in ducks, duck rearing and processing environments in Penang, Malaysia. *Food Research International,* 45 (2):947-952.

Agbodaze, D., Nmai, P., Robertson, F., Yeboah-Manu, D., Owusu-Darko, K. & Addo, K. 2005. Microbiological quality of "khebab" consumed in the Accra metropolis. *Ghana Medical Journal,* 39 (2):46-49.

Agwa, O., Uzoigwe, C. & Wokoma, E. 2012. Incidence and antibiotic sensitivity of Bacillus cereus isolated from ready to eat foods sold in some markets in Portharcourt, Rivers State, Nigeria. *Asian J. Microbiol. Biotechnol. Env. Sci,* 14 (1):13-18.

Ahmed, I., Yokota, A., Yamazoe, A. & Fujiwara, T. 2007. Proposal of Lysinibacillus boronitolerans gen. nov. sp. nov., and transfer of Bacillus fusiformis to Lysinibacillus fusiformis comb. nov. and Bacillus sphaericus to Lysinibacillus sphaericus comb. nov. *International Journal of Systematic and Evolutionary Microbiology,* 57 (5):1117-1125.

Akbar, A. & Anal, A.K. 2013. Prevalence and antibiogram study of Salmonella and Staphylococcus aureus in poultry meat. *Asian Pacific journal of tropical biomedicine,* 3 (2):163-168.

Akusu, O., Kiin-Kabari, D. & Wemedo, S. 2016. Microbiological quality of selected street vended foods in Port Harcourt metropolis, Rivers State, Nigeria.

Al-Masaudi, S., Day, M. & Russell, A. 1991. Antimicrobial resistance and gene transfer in Staphylococcus aureus. *Journal of Applied Bacteriology,* 70 (4):279-290.

Al Mamun, M., Rahman, S.M.M. & Turin, T.C. 2013. Microbiological quality of selected street food items vended by school-based street food vendors in Dhaka, Bangladesh. *International journal of food microbiology,* 166 (3):413-418.

Alcaráz, L.E., Satorres, S.E., Lucero, R.M. & Centorbi, O.N. 2003. Species identification, slime production and oxacillin susceptibility in coagulase-negative staphylococci isolated from nosocomial specimens. *Brazilian Journal of Microbiology,* 34 (1):45-51.

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., et al. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic acids research,* 25 (17):3389-3402.

Aluko, O.O., Ojeremi, T.T., Olaleke, D.A. & Ajidagba, E.B. 2014. Evaluation of food safety and sanitary practices among food vendors at car parks in Ile Ife, southwestern Nigeria. *Food Control,* 40:165-171.

Amissah, A. & Owusu, J. 2012. ASSESSING THE MICROBIOLOGICAL QUALITY OF FOOD SOLD ON AND AROUND KOFORIDUA POLYTECHNIC CAMPUS IN GHANA. *Ann. Food Sci. Tech,* 13 (1).

Andargie, G., Kassu, A., Moges, F., Tiruneh, M. & Huruy, K. 2008. Prevalence of bacteria and intestinal parasites among food-handlers in Gondar town, northwest Ethiopia. *Journal of Health, population and Nutrition*:451-455.

Annor, G.A. & Baiden, E.A. 2011. Evaluation of food hygiene knowledge attitudes and practices of food handlers in food businesses in Accra, Ghana. *Food and Nutrition sciences,* 2 (8):830.

Apha, A. 1998. Wpcf. *Standard methods for the examination of water and wastewater,* 20.

Asiegbu, C.V. 2015. The food safety knowledge and microbial hazards awareness of consumers of ready-to-eat street-vended foods and their exposure to microbiological hazard. p.

Asiegbu, C.V., Lebelo, S.L. & Tabit, F.T. 2016. The food safety knowledge and microbial hazards awareness of consumers of ready-to-eat street-vended food. *Food Control,* 60:422-429.

Attien, P., Sina, H., Moussaoui, W., Dadie, T., Djeni, T., Bankole, H., et al. 2013. Prevalence and antibiotic resistance of *Staphylococcus* strains isolated from meat products sold in Abidjan streets (Ivorycost). African Journal of Microbiology research, 7(26):3285-3293.

Avashia, S.B., Riggins, W., Lindley, C., Hoffmaster, A., Drumgoole, R., Nekomoto, T., et al. 2007. Fatal pneumonia among metalworkers due to inhalation exposure to Bacillus cereus containing Bacillus anthracis toxin genes. *Clinical infectious diseases,* 44 (3):414-416.

Ayaz, N.D. & Erol, I. 2010. Relation between serotype distribution and antibiotic resistance profiles of Listeria monocytogenes isolated from ground turkey. *Journal of Food Protection®,* 73 (5):967-972.

Baba, T., Kuwahara-Arai, K., Uchiyama, I., Takeuchi, F., Ito, T. & Hiramatsu, K. 2009a. Complete genome sequence of Macrococcus caseolyticus strain JCSC5402, reflecting the ancestral genome of the human-pathogenic staphylococci. *Journal of bacteriology,* 191 (10):3429.

Baba, T., Kuwahara-Arai, K., Uchiyama, I., Takeuchi, F., Ito, T. & Hiramatsu, K. 2009b. Complete genome sequence of Macrococcus caseolyticus strain JSCS5402, reflecting the ancestral genome of the human-pathogenic staphylococci. *Journal of bacteriology,* 191 (4):1180-1190.

Bannerman, T.L., Peacock, S., Murray, P., Baron, E., Jorgensen, J., Landry, M., et al. 2006. Staphylococcus, Micrococcus, and other catalase-positive cocci. *Manual of clinical microbiology: Volume 1,* (Ed. 9):390-411.

Barro, N., Bello, A.R., Savadogo, A., Ouattara, C.A.T., Ilboudo, A. & Traoré, A.S. 2006. Hygienic status assessment of dish washing waters, utensils, hands and pieces of money from street food processing sites in Ouagadougou (Burkina Faso). *African Journal of Biotechnology,* 5 (11).

Baş, M., Ersun, A.Ş. & Kıvanç, G. 2006. The evaluation of food hygiene knowledge, attitudes, and practices of food handlers' in food businesses in Turkey. *Food Control,* 17 (4):317-322.

Batista, J.E.C., Ferreira, E.L., Nascimento, D.C.d.O., Ventura, R.F., de Oliveira, W.L.M., Leal, N.C., et al. 2013. Antimicrobial Resistance and Detection of the mec A Gene Besides Enterotoxin-Encoding Genes Among Coagulase-Negative Staphylococci Isolated from Clam Meat of Anomalocardia brasiliana. *Foodborne pathogens and disease,* 10 (12):1044-1049.

Bauer, A., Kirby, W., Sherris, J.C. & Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disk method. *American journal of clinical pathology,* 45 (4):493.

Becker, K., Heilmann, C. & Peters, G. 2014. Coagulase-negative staphylococci. *Clinical microbiology reviews,* 27 (4):870-926.

Beerepoot, M.A., ter Riet, G., Nys, S., van der Wal, W.M., de Borgie, C.A., de Reijke, T.M., et al. 2011. Cranberries vs antibiotics to prevent urinary tract infections: a randomized double-blind noninferiority trial in premenopausal women. *Archives of internal medicine,* 171 (14):1270-1278.

Belluco, S., Losasso, C., Maggioletti, M., Alonzi, C.C., Paoletti, M.G. & Ricci, A. 2013. Edible insects in a food safety and nutritional perspective: a critical review. *Comprehensive Reviews in Food Science and Food Safety,* 12 (3):296-313.

Bertrand, S., Weill, F.-X., Cloeckaert, A., Vrints, M., Mairiaux, E., Praud, K., et al. 2006. Clonal emergence of extended-spectrum β-lactamase (CTX-M-2)-producing Salmonella enterica serovar Virchow isolates with reduced susceptibilities to ciprofloxacin among poultry and humans in Belgium and France (2000 to 2003). *Journal of Clinical Microbiology,* 44 (8):2897-2903.

Beutin, L. & Martin, A. 2012. Outbreak of Shiga toxin–producing Escherichia coli (STEC) O104: H4 infection in Germany causes a paradigm shift with regard to human pathogenicity of STEC strains. *Journal of Food Protection®,* 75 (2):408-418.

Bhattacharjya, H. & Reang, T. 2014. Safety of street foods in Agartala, North East India. *public health,* 128 (8):746-748.

Bizuye, A., Tewelde, S., Agimas, A., Asfaw, M., Tadele, E. & Mesfin, E. 2014. Bacteriological Quality of Street Vending Potato Chips In Gondar Town, North West Ethiopia. *International Journal of Bacteriology, Virology and Immunology,* 1 (2):014-019.

Bockelmann, W. & Hoppe-Seyler, T. 2001. The surface flora of bacterial smear-ripened cheeses from cow's and goat's milk. *International Dairy Journal,* 11 (4):307-314.

Bolton, D.J. 2011. Verocytotoxigenic (Shiga toxin–producing) Escherichia coli: virulence factors and pathogenicity in the farm to fork paradigm. *Foodborne pathogens and disease,* 8 (3):357-365.

Borgatta, B., Kmet-Lunaček, N. & Rello, J. 2012. E. coli O104: H4 outbreak and haemolytic–uraemic syndrome. *Medicina Intensiva (English Edition),* 36 (8):576-583.

Boucher, H.W. & Corey, G.R. 2008. Epidemiology of methicillin-resistant Staphylococcus aureus. *Clinical infectious diseases,* 46 (Supplement 5):S344-S349.

Bouchrif, B., Karraouan, B., Ennaji, M. & Timinouni, M. 2008. Émergence de la résistance aux quinolones chez Salmonella spp. à Casablanca–Maroc. *Médecine et maladies infectieuses,* 38 (11):615-616.

Bouchrif, B., Le Hello, S., Pardos, M., Karraouan, B., Perrier-Gros-Claude, J.-D., Ennaji, M.-M., et al. 2009a. Ceftazidime-resistant Salmonella enterica, Morocco. *Emerging infectious diseases,* 15 (10):1693.

Bouchrif, B., Paglietti, B., Murgia, M., Piana, A.F., Cohen, N., Ennaji, M.M., et al. 2009b. Prevalence and antibiotic-resistance of Salmonella isolated from food in Morocco. *The Journal of Infection in Developing Countries,* 3 (1):35-40.

Bryan, F.L., Michanie, S.C., Alvarez, P. & Paniagua, A. 1988. Critical control points of street-vended foods in the Dominican Republic. *Journal of Food Protection®,* 51 (5):373-383.

Bryan, F.L., Teufel, P., Riaz, S., Roohi, S., Qadar, F. & Malik, Z.-U.-R. 1992. Hazards and critical control points of vending operations at a railway station and a bus station in Pakistan. *Journal of Food Protection®,* 55 (7):534-541.

Bucknell, D., Gasser, R., Irving, A. & Whithear, K. 1997. Antimicrobial resistance in Salmonella and Escherichia coli isolated from horses. *Australian veterinary journal,* 75 (5):355-356.

Bukar, A., Uba, A. & Oyeyi, T. 2010. Occurrence of some enteropathogenic bacteria in some minimally and fully processed ready-to-eat foods in Kano metropolis, Nigeria. *African Journal of Food Science,* 4 (2):032-036.

Burnens, A., Boss, P., Ørskov, F., Ørskov, I., Schaad, U., Müller, F., et al. 1992. Occurrence and phenotypic properties of verotoxin producingEscherichia coli in sporadic cases of gastroenteritis. *European Journal of Clinical Microbiology and Infectious Diseases,* 11 (7):631-634.

Busani, L., Scavia, G., Luzzi, I. & Caprioli, A. 2005. Laboratory surveillance for prevention and control of foodborne zoonoses. *Annali dell'Istituto superiore di sanita,* 42 (4):401-404.

Büyükyörük, S., Beyaz, D., GÖKSOY, E.Ö., KÖK, F. & Kocak, P. 2014. Microbiological evaluation of ready-to-eat sandwiches served near hospitals and schools. *Academic Journal of Ankara Üniversitesi Veteriner Fakultesi Dergisi,* 61 (3):193-198.

Buzby, J.C. & Roberts, T. 1996. Economic costs and trade impacts of microbial foodborne illness. *World health statistics quarterly. Rapport trimestriel de statistiques sanitaires mondiales,* 50 (1-2):57-66.

Campbell, P.T. 2011. Assessing the knowledge, attitudes and practices of street food vendors in the city of Johannesburg regarding food hygiene and safety. University of the Western Capep.

Canet, C. & N'diaye, C. 1996. [Street food in Africa]. *Food, Nutrition and Agriculture (FAO).*

Cardinale, E., Gros-Claude, J.P., Tall, F., Gueye, E. & Salvat, G. 2005. Risk factors for contamination of ready-to-eat street-vended poultry dishes in Dakar, Senegal. *International journal of food microbiology,* 103 (2):157-165.

Carrasco, E., Morales-Rueda, A. & García-Gimeno, R.M. 2012. Cross-contamination and recontamination by Salmonella in foods: a review. *Food Research International,* 45 (2):545-556.

Casewell, M., Friis, C., Marco, E., McMullin, P. & Phillips, I. 2003. The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. *Journal of antimicrobial chemotherapy,* 52 (2):159-161.

Cattaneo, C., Quaresmini, G., Casari, S., Capucci, M., Micheletti, M., Borlenghi, E., et al. 2008. Recent changes in bacterial epidemiology and the emergence of fluoroquinolone-resistant Escherichia coli among patients with haematological malignancies: results of a prospective study on 823 patients at a single institution. *Journal of antimicrobial chemotherapy,* 61 (3):721-728.

Chajęcka-Wierzchowska, W., Zadernowska, A., Nalepa, B., Sierpińska, M. & Łaniewska-Trokenheim, Ł. 2014. Retail ready-to-eat food as a potential vehicle for Staphylococcus spp. harboring antibiotic resistance genes. *Journal of Food Protection®,* 77 (6):993-998.

Chajęcka-Wierzchowska, W., Zadernowska, A., Nalepa, B., Sierpińska, M. & Łaniewska-Trokenheim, Ł. 2015. Coagulase-negative staphylococci (CoNS) isolated from ready-to-eat food of animal origin–phenotypic and genotypic antibiotic resistance. *Food microbiology,* 46:222-226.

Chandran, A., Hatha, A.M., Varghese, S. & Sheeja, K.M. 2008. Prevalence of multiple drug resistant Escherichia coli serotypes in a tropical estuary, India. *Microbes and environments,* 23 (2):153-158.

Chang, M., Groseclose, S., Zaidi, A. & Braden, C. 2009. An ecological analysis of sociodemographic factors associated with the incidence of salmonellosis, shigellosis, and *E. coli* O157: H7 infections in US counties. *Epidemiology and Infection,* 137 (06):810-820.

Chauhan, A. 2013. Prevalence and antibiotic resistance of Bacillus strains isolated from various food stuffs. Journal of Biomedical and Pharmaceutical Research, 2 (3).

Cheesbrough, M. 2006. District laboratory practice in tropical countries. Cambridge university press.

Chemburu, S., Wilkins, E. & Abdel-Hamid, I. 2005. Detection of pathogenic bacteria in food samples using highly-dispersed carbon particles. *Biosensors and Bioelectronics,* 21 (3):491-499.

Cho, I.-H., Bhunia, A. & Irudayaraj, J. 2015. Rapid pathogen detection by lateral-flow immunochromatographic assay with gold nanoparticle-assisted enzyme signal amplification. *International journal of food microbiology,* 206:60-66.

Cho, J.I., Cheung, C.Y., lee, S.M., ko, S.I., kim, K.H., hwang, I.S., et al. 2011. assessment of microbial contamination levels of street-vended foods in korea. *Journal of Food Safety,* 31 (1):41-47.

Cho, S., Kim, J., Oh, K.-H., Hu, J., Seo, J., Oh, S., et al. 2014. Outbreak of enterotoxigenic Escherichia coli O169 enteritis in schoolchildren associated with consumption of kimchi, Republic of Korea, 2012. *Epidemiology and infection,* 142 (03):616-623.

Choma, C., Guinebretiere, M., Carlin, F., Schmitt, P., Velge, P., Granum, P., et al. 2000. Prevalence, characterization and growth of Bacillus cereus in commercial cooked chilled foods containing vegetables. *Journal of applied microbiology,* 88 (4):617-625.

Chopra, I. & Roberts, M. 2001. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiology and molecular biology reviews,* 65 (2):232-260.

Choudhury, M., Mahanta, L.B., Goswami, J.S. & Mazumder, M.D. 2011. Will capacity building training interventions given to street food vendors give us safer food?: A cross-sectional study from India. *Food Control,* 22 (8):1233-1239.

Chukuezi, C. 2010a. Entrepreneurs of the streets: Socio-economic features of street food vending in Owerri, Nigeria. *European Journal of Social Sciences,* 14 (2):183-188.

Chukuezi, C.O. 2010b. Food safety and hyienic practices of street food vendors in Owerri, Nigeria. *Studies in Sociology of Science,* 1 (1):50.

Clarence, S.Y., Obinna, C.N. & Shalom, N.C. 2009. Assessment of bacteriological quality of ready to eat food (Meat pie) in Benin City metropolis, Nigeria. *African Journal of Microbiology Research,* 3 (7):390-395.

Coburn, B., Grassl, G.A. & Finlay, B. 2007. Salmonella, the host and disease: a brief review. *Immunology and cell biology,* 85 (2):112-118.

Collard, J.-M., Place, S., Denis, O., Rodriguez-Villalobos, H., Vrints, M., Weill, F.-X., et al. 2007. Travel-acquired salmonellosis due to Salmonella Kentucky resistant to ciprofloxacin, ceftriaxone and co-trimoxazole and associated with treatment failure. *Journal of antimicrobial chemotherapy,* 60 (1):190-192.

Collignon, P.C., Conly, J.M., Andremont, A., McEwen, S.A., Aidara-Kane, A., Agerso, Y., et al. 2016. World Health Organization Ranking of Antimicrobials According to Their Importance in Human Medicine: A Critical Step for Developing Risk Management Strategies to Control Antimicrobial Resistance From Food Animal Production. *Clinical infectious diseases*:ciw475.

Cortes, J.A., Leal, A.L., Montañez, A.M., Buitrago, G., Castillo, J.S., Guzman, L., et al. 2013. Frequency of microorganisms isolated in patients with bacteremia in intensive care units in Colombia and their resistance profiles. *The Brazilian Journal of Infectious Diseases,* 17 (3):346-352.

Cortese, R.D.M., Veiros, M.B., Feldman, C. & Cavalli, S.B. 2016. Food safety and hygiene practices of vendors during the chain of street food production in Florianopolis, Brazil: A cross-sectional study. *Food Control,* 62:178-186.

Cousin, M. & Layfayette, W. 1989. Spore forming bacteria in foods. *Student research projects in food science, food technology and nutrition.*

Cuprasitrut, T., Srisorrachatr, S. & Malai, D. 2011. Food safety knowledge, attitude and practice of food handlers and microbiological and chemical food quality assessment of food for making merit for monks in Ratchathewi district, Bangkok. *Asia Journal of Public Health,* 2 (1):27-34.

Curtis, V., Cairncross, S. & Yonli, R. 2000. Review: Domestic hygiene and diarrhoea–pinpointing the problem. *Tropical medicine & international health,* 5 (1):22-32.

da Silva, S.A., Cardoso, R.d.C.V., Góes, J.Â.W., Santos, J.N., Ramos, F.P., de Jesus, R.B., et al. 2014. Street food on the coast of Salvador, Bahia, Brazil: A study from the socioeconomic and food safety perspectives. *Food Control,* 40:78-84.

Dagnew, M., Tiruneh, M., Moges, F. & Tekeste, Z. 2012. Survey of nasal carriage of Staphylococcus aureus and intestinal parasites among food handlers working at Gondar University, Northwest Ethiopia. *BMC Public Health,* 12 (1):1.

Dardano, E. 2003. Report of FAO, PAHO, and BNSI.

Das, A., Nagananda, G., Bhattacharya, S. & Bhardwaj, S. 2010. Microbiological quality of street-vended indian chaats sold in bangalore. *Journal of Biological Sciences,* 10 (3):255-260.

de Noordhout, C.M., Devleesschauwer, B., Angulo, F.J., Verbeke, G., Haagsma, J., Kirk, M., et al. 2014. The global burden of listeriosis: a systematic review and meta-analysis. *The Lancet infectious diseases,* 14 (11):1073-1082.

Delpech, G., Pourcel, G., Schell, C., De Luca, M., Basualdo, J., Bernstein, J., et al. 2012. Antimicrobial resistance profiles of Enterococcus faecalis and Enterococcus faecium isolated from artisanal food of animal origin in Argentina. *Foodborne pathogens and disease,* 9 (10):939-944.

den Heijer, C.D., Beerepoot, M.A., Prins, J.M., Geerlings, S.E. & Stobberingh, E.E. 2012. Determinants of antimicrobial resistance in Escherichia coli strains isolated from faeces and urine of women with recurrent urinary tract infections. *PloS one,* 7 (11):e49909.

Deng, X., Ran, L., Wu, S., Ke, B., He, D., Yang, X., et al. 2012. Laboratory-based surveillance of non-typhoidal Salmonella infections in Guangdong Province, China. *Foodborne pathogens and disease,* 9 (4):305-312.

Derbew, G., Sahle, S. & Endris, M. 2013. Bacteriological assessment of some street vended foods in Gondar, Ethiopia. *International Journal of Food Safety,* 15:33-38.

DeWaal, C.S. & Robert, N. 2005. Global & Local: Food Safety Around the World. Center for Science in the Public Interest.

DeWaal, C.S., Roberts, C. & Catella, C. 2012. Outbreak Alert! 1999-2008. *Center for Science in the Public Interest, Washington, DC.*

Dinges, M.M., Orwin, P.M. & Schlievert, P.M. 2000. Exotoxins of Staphylococcus aureus. *Clinical microbiology reviews,* 13 (1):16-34.

Donate-Correa, J., León-Barrios, M. & Pérez-Galdona, R. 2005. Screening for plant growth-promoting rhizobacteria in Chamaecytisus proliferus (tagasaste), a forage tree-shrub legume endemic to the Canary Islands. *Plant and Soil,* 266 (1-2):261-272.

Duarte, A., Santos, A., Manageiro, V., Martins, A., Fraqueza, M.J., Caniça, M., et al. 2014. Human, food and animal Campylobacter spp. isolated in Portugal: high genetic diversity and antibiotic resistance rates. *International journal of antimicrobial agents,* 44 (4):306-313.

Duff, S.B., Scott, E.A., Mafilios, M.S., Todd, E.C., Krilov, L.R., Geddes, A.M., et al. 2003. Cost-effectiveness of a targeted disinfection program in household kitchens to prevent foodborne illnesses in the United States, Canada, and the United Kingdom. *Journal of Food Protection®,* 66 (11):2103-2115.

Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K. & Stacebrandt, E. 2005. The Prokaryotes, vol. 3, Archaea and Bacteria: Firmicutes. *Actinomycetes*:998.

Eglezos, S., Huang, B., Dykes, G.A. & Fegan, N. 2010. The prevalence and concentration of Bacillus cereus in retail food products in Brisbane, Australia. *Foodborne pathogens and disease,* 7 (7):867-870.

Egorova, S., Kaftyreva, L., Grimont, P.A. & Weill, F.-X. 2007. Prevalence and characterization of extended-spectrum cephalosporin-resistant nontyphoidal Salmonella isolates in adults in Saint Petersburg, Russia (2002-2005). *Microbial Drug Resistance,* 13 (2):102-107.

Ekanem, E.O. 1998. The street food trade in Africa: safety and socio-environmental issues. *Food Control,* 9 (4):211-215.

El-Shenawy, M., El-Shenawy, M., Mañes, J. & Soriano, J.M. 2011. Listeria spp. in street-vended ready-to-eat foods. *Interdisciplinary perspectives on infectious diseases,* 2011.

Elobeid, T., Aziz, H., Mousa, R. & Alzahiri, A. 2014. Survey on the Microbial Quality of Traditional Foods Sold by Street Vendors in Qatar.

Espié, E., De Valk, H., Vaillant, V., Quelquejeu, N., Le Querrec, F. & Weill, F. 2005. An outbreak of multidrug-resistant Salmonella enterica serotype Newport infections linked to the consumption of imported horse meat in France. *Epidemiology and infection,* 133 (02):373-376.

Evans, M.R., Roberts, R., Ribeiro, C., Gardner, D. & Kembrey, D. 1996. A milk-borne Campylobacter outbreak following an educational farm visit. *Epidemiology and infection,* 117 (03):457-462.

Fakruddin, M. & Mannan, K. 2013. Methods for analyzing diversity of microbial communities in natural environments. *Ceylon Journal of Science (Biological Sciences),* 42 (1).

Fan, Y., Li, S., Deng, B. & Zhao, Y. 2015. Prevalence and relevance analysis of multidrug-resistant Staphylococcus aureus of meat, poultry and human origin. *Indian Journal of Animal Research,* 49 (1):86-90.

Fawole, M. & Oso, B. 2004. Characterization of bacteria. *Laboratory manual of microbiology. Spectrum Books Limited, Nigeria*:22-33.

Fazlani, S., Khan, S., Faraz, S. & Awan, M. 2011. Antimicrobial susceptibility of bacterial species identified from mastitic milk samples of camel. *African Journal of Biotechnology,* 10 (15):2959-2964.

Feglo, P. & Sakyi, K. 2012. Bacterial contamination of street vending food in Kumasi, Ghana. *Journal of Medical and Biomedical Sciences,* 1 (1):1-8.

Fellows, P. & Hilmi, M. 2011. Selling street and snack foods. FAO.

Fernandes, A.M., Balasegaram, S., Willis, C., Wimalarathna, H.M., Maiden, M.C. & McCarthy, N.D. 2015. Partial failure of milk pasteurization as a risk for the transmission of Campylobacter from cattle to humans. *Clinical infectious diseases*:civ431.

Fijałkowski, K., Peitler, D. & Karakulska, J. 2016. Staphylococci isolated from ready-to-eat meat–Identification, antibiotic resistance and toxin gene profile. *International journal of food microbiology,* 238:113-120.

Fitch, W. 1986. The estimate of total nucleotide substitutions from pairwise differences is biased. *Philosophical Transactions of the Royal Society of London B: Biological Sciences,* 312 (1154):317-324.

Fleckenstein, J.M., Hardwidge, P.R., Munson, G.P., Rasko, D.A., Sommerfelt, H. & Steinsland, H. 2010. Molecular mechanisms of enterotoxigenic Escherichia coli infection. *Microbes and Infection,* 12 (2):89-98.

Fletcher, S.M., McLaws, M.-L. & Ellis, J.T. 2013. Prevalence of gastrointestinal pathogens in developed and developing countries: systematic review and meta-analysis. *Journal of public health research,* 2 (1):9.

Fleury, M.D., Stratton, J., Tinga, C., Charron, D.F. & Aramini, J. 2008. A descriptive analysis of hospitalization due to acute gastrointestinal illness in Canada, 1995-2004. *Canadian Journal of Public Health/Revue Canadienne de Sante'e Publique*:489-493.

Food & Nations, A.O.o.t.U. 1990. Street Foods-Report of an FAO Expert Consultation Yogyakarta, Indonesia, 5-9 December 1988.

Forshell, L.P. & Wierup, M. 2006. Salmonella contamination: a significant challenge to the global marketing of animal food products. *Rev. sci. tech. Off. int. Epiz,* 25 (2):541-554.

Fowoyo, P. & Ogunbanwo, S. 2016. Virulence and toxigenicity of coagulase-negative staphylococci in Nigerian traditional fermented foods. *Canadian journal of microbiology,* 62 (999):1-7.

Frank, C., Werber, D., Cramer, J.P., Askar, M., Faber, M., an der Heiden, M., et al. 2011. Epidemic profile of Shiga-toxin–producing Escherichia coli O104: H4 outbreak in Germany. *New England Journal of Medicine,* 365 (19):1771-1780.

Gadaga, T., Zulu, R., Graffham, A. & Chibanda, D. 2005. Improving the safety of informally vended foods in Southern Africa–Training of Environmental Health Officers (EHO) in Lusaka and Harare.

Gadaga, T.H., Samende, B.K., Musuna, C. & Chibanda, D. 2008. The microbiological quality of informally vended foods in Harare, Zimbabwe. *Food Control,* 19 (8):829-832.

Gadaga, T., Ntsike, M. & Ntuli, V. 2014. Socio-economic and hygienic aspects of street food vending in Maseru City, Lesotho. UNISWA Journal of Agriculture, 15: 28-39.

Gao, J., Ferreri, M., Yu, F., Liu, X., Chen, L., Su, J., et al. 2012. Molecular types and antibiotic resistance of Staphylococcus aureus isolates from bovine mastitis in a single herd in China. *The Veterinary Journal,* 192 (3):550-552.

Garin, B., Aidara, A., Spiegel, A., Arrive, P., Bastaraud, A., Cartel, J.-L., et al. 2002. Multicenter study of street foods in 13 towns on four continents by the food and environmental hygiene study group of the international network of pasteur and associated institutes. *Journal of Food Protection®,* 65 (1):146-152.

Ghosh, M., Wahi, S., Kumar, M. & Ganguli, A. 2007. Prevalence of enterotoxigenic Staphylococcus aureus and Shigella spp. in some raw street vended Indian foods. *International journal of environmental health research,* 17 (2):151-156.

Gitahi, M., Wangoh, J. & Njage, P. 2012. Microbial safety of street foods in industrial area, Nairobi. *Research Journal of Microbiology,* 7 (6):297-308.

Gonzales-Siles, L. & Sjöling, Å. 2016. The different ecological niches of enterotoxigenic Escherichia coli. *Environmental microbiology.*

Guang-Can, T., Shu-Jun, T., Miao-Ying, C. & Guang-Hui, X. 2008. Phosphate-solubilizing and-mineralizing abilities of bacteria isolated from soils. *Pedosphere,* 18 (4):515-523.

Guibourdenche, M., Roggentin, P., Mikoleit, M., Fields, P.I., Bockemühl, J., Grimont, P.A., et al. 2010. Supplement 2003–2007 (No. 47) to the white-Kauffmann-Le minor scheme. *Research in microbiology,* 161 (1):26-29.

Gundogan, N., Citak, S., Yucel, N. & Devren, A. 2005. A note on the incidence and antibiotic resistance of Staphylococcus aureus isolated from meat and chicken samples. *Meat science,* 69 (4):807-810.

Guran, H.S. & Kahya, S. 2015. Species Diversity and Pheno-and Genotypic Antibiotic Resistance Patterns of Staphylococci Isolated from Retail Ground Meats. *Journal of food science,* 80 (6):M1291-M1298.

Guven, K., Mutlu, M.B., Gulbandilar, A. & Cakir, P. 2010. Occurrence and characterization of *Staphylococcus aureus* isolated from meat and dairy products consumed in Turkey. *Journal of Food Safety,* 30 (1):196-212.

Gwida, M., Hotzel, H., Geue, L. & Tomaso, H. 2014. Occurrence of Enterobacteriaceae in raw meat and in human samples from Egyptian retail sellers. *International Scholarly Research Notices,* 2014.

Hakim, A.S., Abuelnaga, A.S., Ezz-Eldeen, A.M., Bakry, M.A. & Ismail, S.A. 2015. Prevalence of some food poisoning bacteria in local and imported retail pork by-products in Egyptian markets. *African Journal of Microbiology Research,* 9 (22):1492-1498.

Han, F., Lestari, S.I., Pu, S. & Ge, B. 2009. Prevalence and antimicrobial resistance among Campylobacter spp. in Louisiana retail chickens after the enrofloxacin ban. *Foodborne pathogens and disease,* 6 (2):163-171.

Hanashiro, A., Morita, M., Matté, G.R., Matté, M.H. & Torres, E.A. 2005. Microbiological quality of selected street foods from a restricted area of São Paulo city, Brazil. *Food Control,* 16 (5):439-444.

Harakeh, S., Yassine, H., Gharios, M., Barbour, E., Hajjar, S., El-Fadel, M., et al. 2005. Isolation, molecular characterization and antimicrobial resistance patterns of *Salmonella* and *Escherichia coli* isolates from meat-based fast food in Lebanon. *Science of the Total Environment,* 341 (1):33-44.

Havelaar, A.H., Cawthorne, A., Angulo, F., Bellinger, D., Corrigan, T., Cravioto, A., et al. 2013. WHO initiative to estimate the global burden of foodborne diseases. *The Lancet,* 381:S59.

Havelaar, A.H., Kirk, M.D., Torgerson, P.R., Gibb, H.J., Hald, T., Lake, R.J., et al. 2015. World Health Organization Global estimates and regional comparisons of the burden of foodborne disease in 2010. *PLoS Med,* 12 (12):e1001923.

Hazariwala, A., Sanders, Q., Hudson, C.R., Hofacre, C., Thayer, S.G. & Maurer, J.J. 2002. Distribution of staphylococcal enterotoxin genes among Staphylococcus aureus isolates from poultry and humans with invasive staphylococcal disease. *Avian diseases,* 46 (1):132-136.

Helms, M., Evans, S., Vastrup, P. & Gerner-Smidt, P. 2003. Short and long term mortality associated with foodborne bacterial gastrointestinal infections: registry based studyCommentary: matched cohorts can be useful. *Bmj,* 326 (7385):357.

Hennekinne, J.-A., De Buyser, M.-L. & Dragacci, S. 2012. Staphylococcus aureus and its food poisoning toxins: characterization and outbreak investigation. *FEMS microbiology reviews,* 36 (4):815-836.

Hill, J. 2016. The development of a street-food vending model that offers healthy foods for sale.

Hiramatsu, K., Katayama, Y., Matsuo, M., Sasaki, T., Morimoto, Y., Sekiguchi, A., et al. 2014. Multi-drug-resistant Staphylococcus aureus and future chemotherapy. *Journal of Infection and Chemotherapy,* 20 (10):593-601.

Hird, S., Stein, C., Kuchenmüller, T. & Green, R. 2009. Meeting report: second annual meeting of the World Health Organization initiative to estimate the global burden of foodborne diseases. *International Journal of Food Microbiology,* 133 (1):210-212.

Hoffman, R.E., Greenblatt, J., Matyas, B.T., Sharp, D.J., Esteban, E., Hodge, K., et al. 2005. Capacity of state and territorial health agencies to prevent foodborne illness. *Emerg Infect Dis,* 11 (1):11-16.

Humphrey, S., Chaloner, G., Kemmett, K., Davidson, N., Williams, N., Kipar, A., et al. 2014. Campylobacter jejuni is not merely a commensal in commercial broiler chickens and affects bird welfare. *MBio,* 5 (4):e01364-01314.

Hui, Y.H., J.R., Gorhamm, K.D. Murrel & D.O. Cliver. 1994. Foodborne diseases handbook: diseases caused by bacteria. Marcel Dekker, Inc., New York.

Husain, S., Yasmin, S. & Islam, M.S. 2015. Assessment of the Socioeconomic Aspects of Street Vendors in Dhaka City: Evidence from Bangladesh. *Asian Social Science,* 11 (26):1.

Ifediora, A., Nkere, C. & Iroegbu, C. 2006. Weaning food preparations consumed in Umuahia, Nigeria: evaluation of the bacteriological quality. *J Food Technol,* 4:101-105.

Indikova, I., Humphrey, T.J. & Hilbert, F. 2015. Survival with a helping hand: Campylobacter and microbiota. *Frontiers in microbiology,* 6.

Iovine, N.M. 2013. Resistance mechanisms in Campylobacter jejuni. *Virulence,* 4 (3):230-240.

Isara, A., Isah, E., Lofor, P. & Ojide, C. 2010. Food contamination in fast food restaurants in Benin City, Edo State, Nigeria: Implications for food hygiene and safety. *public health,* 124 (8):467-471.

Issack, M.I., Hendriksen, R.S., Lun, P.L.K., Lutchun, R.K. & Aarestrup, F.M. 2009. Salmonella enterica serovar Typhimurium in Mauritius linked to consumption of marlin mousse. *Foodborne pathogens and disease,* 6 (6):739-741.

Jahan, S. 2012. Epidemiology of foodborne illness. INTECH Open Access Publisher.

Jami, M., Ghanbari, M., Zunabovic, M., Domig, K.J. & Kneifel, W. 2014. Listeria monocytogenes in aquatic food products—a review. *Comprehensive Reviews in Food Science and Food Safety,* 13 (5):798-813.

Jawad, N., Mutalib, S.A. & Abdullah, A. 2016. Antimicrobial resistance pattern of Bacillus cereus Strains Isolated from fried rice samples. *International Journal of ChemTech Research,* 9 (1):160-167.

Jooste, P., Jordan, K., Leong, D., Alvarez-Ord & oñez, A. 2016. Listeria monocytogenes in food: Control by monitoring the food processing environment. *African Journal of Microbiology Research,* 10 (1):1-14.

Jørgensen, H., Mørk, T., Caugant, D., Kearns, A. & Rørvik, L. 2005. Genetic variation among Staphylococcus aureus strains from Norwegian bulk milk. *Applied and environmental microbiology,* 71 (12):8352-8361.

Kaul, M. & Agarwal, G. 1988. Microbial load of common chat products. *Indian J Nutr Diet,* 25:197-199.

Kersters, K. & Vancanneyt, M. 2005. Bergey's manual of systematic bacteriology: Springer Verlag.

Kharel, N., Palni, U. & Tamang, J.P. 2016. Microbiological assessment of ethnic street foods of the Himalayas. *Journal of Ethnic Foods,* 3 (3):235-241.

Kinsella,K.J.,Prendergast, D.M., McCann, M.S., Blair, I.S., McDowell D.A & Sheridan, J.J. 2008. The survival of *Salmonella* enterica serovar Typhimurium DT 104 and total viable counts on beef surfaces at different relative humidities and temperatures. Journal of applied Microbiology, 106:171-180

Kim, M., Kwon, T.-H., Jung, S.-M., Cho, S.-H., Jin, S.Y., Park, N.-H., et al. 2013. Antibiotic resistance of bacteria isolated from the internal organs of edible snow crabs. *PloS one,* 8 (8):e70837.

Kirk, J.L., Beaudette, L.A., Hart, M., Moutoglis, P., Klironomos, J.N., Lee, H., et al. 2004. Methods of studying soil microbial diversity. *Journal of microbiological methods,* 58 (2):169-188.

Klein, E. 2007. Hospitalizations and Deaths Caused by Methicillin-Resistant Staphylococcus aureus, United States, 1999–2005-Volume 13, Number 12—December 2007-Emerging Infectious Disease journal-CDC.

Kline, E. 1948. Bergey's manual of determinative bacteriology. *American Journal of Public Health and the Nations Health,* 38 (12):1700.

Kubheka, L., Mosupye, F. & Von Holy, A. 2001. Microbiological survey of street-vended salad and gravy in Johannesburg city, South Africa. *Food Control,* 12 (2):127-131.

Kuchenmüller, T., Abela-Ridder, B., Corrigan, T. & Tritscher, A. 2013. World Health Organization initiative to estimate the global burden of foodborne diseases. *Revue scientifique et technique (International Office of Epizootics),* 32 (2):459-467.

Kuchenmüller, T., Hird, S., Stein, C., Kramarz, P., Nanda, A. & Havelaar, A. 2009. Diseases. *Surveillance and outbreak reports,* 14:18-19.

Kuo, H.-W., Kasper, S., Jelovcan, S., Höger, G., Lederer, I., König, C., et al. 2009. A food-borne outbreak of Shigella sonnei gastroenteritis, Austria, 2008. *Wiener Klinische Wochenschrift,* 121 (3-4):157-163.

Lamberti, L.M., Bourgeois, A.L., Walker, C.L.F., Black, R.E. & Sack, D. 2014. Estimating diarrheal illness and deaths attributable to Shigellae and enterotoxigenic Escherichia coli among older children, adolescents, and adults in South Asia and Africa. *PLoS Negl Trop Dis,* 8 (2):e2705.

Landers, T.F., Cohen, B., Wittum, T.E. & Larson, E.L. 2012. A review of antibiotic use in food animals: perspective, policy, and potential. *Public health reports*:4-22.

Latham, M.C. 1965. *Human nutrition in tropical Africa*: FAO.

Lawrynowicz-Paciorek, M., Kochman, M., Piekarska, K., Grochowska, A. & Windyga, B. 2007. The distribution of enterotoxin and enterotoxin-like genes in Staphylococcus aureus strains isolated from nasal carriers and food samples. *International journal of food microbiology,* 117 (3):319-323.

Le Loir, Y., Baron, F. & Gautier, M. 2003. Staphylococcus aureus and food poisoning. *Genet Mol Res,* 2 (1):63-76.

Liu, L., Johnson, H.L., Cousens, S., Perin, J., Scott, S., Lawn, J.E., et al. 2012. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. *The Lancet,* 379 (9832):2151-2161.

Liu, S., Liu, Z., Zhang, H., Lu, L., Liang, J. & Huang, Q. 2015. Knowledge, attitude and practices of food safety amongst food handlers in the coastal resort of Guangdong, China. *Food Control,* 47:457-461.

Liu, Y., Wang, Y., Schwarz, S., Wang, S., Chen, L., Wu, C., et al. 2014a. Investigation of a multiresistance gene cfr that fails to mediate resistance to phenicols and oxazolidinones in Enterococcus faecalis. *Journal of antimicrobial chemotherapy,* 69 (4):892-898.

Liu, Z., Zhang, G. & Zhang, X. 2014b. Urban street foods in Shijiazhuang city, China: Current status, safety practices and risk mitigating strategies. *Food Control,* 41:212-218.

Losasso, C., Cibin, V., Cappa, V., Roccato, A., Vanzo, A., Andrighetto, I., et al. 2012. Food safety and nutrition: improving consumer behaviour. *Food Control,* 26 (2):252-258.

Low, J. & Donachie, W. 1991. Clinical and serum antibody responses of lambs to infection by Listeria monocytogenes. *Research in veterinary science,* 51 (2):185-192.

Lowy, F.D. 1998. Staphylococcus aureus infections. *New England Journal of Medicine,* 339 (8):520-532.

Lucca, A. & da Silva Torres, E.A.F. 2006. Street-food: the hygiene conditions of hot-dogs sold in São Paulo, Brazil. *Food Control,* 17 (4):312-316.

Lues, J.F., Rasephei, M.R., Venter, P. & Theron, M.M. 2006. Assessing food safety and associated food handling practices in street food vending. *International journal of environmental health research,* 16 (5):319-328.

Madueke, S., Awe, S. & Jonah, A. 2014. Microbiological analysis of street foods along Lokoja-Abuja Express Way, Lokoja. *American Journal of Research Communication,* 2 (1):196-211.

Mafune, T.S., Takalani, T.K., Anyasi, T.A. & Ramashia, S.E. 2016. Microbial Safety of Street Vended Foods Sold in Thohoyandou, South Africa. *J Hum Ecol,* 53 (3):205-212.

Mahmoud, B.S. 2011. Salmonella: A Dangerous Foodborne Pathogen. InTech.

Majowicz, S.E., Musto, J., Scallan, E., Angulo, F.J., Kirk, M., O'Brien, S.J., et al. 2010. The global burden of nontyphoidal Salmonella gastroenteritis. *Clinical infectious diseases,* 50 (6):882-889.

Manga, B. & Oyeleke, S. 2008. Essentials of Laboratory Practical's in Microbiology: Tobes publishers.

Manguiat, L.S. & Fang, T.J. 2013. Microbiological quality of chicken-and pork-based street-vended foods from Taichung, Taiwan, and Laguna, Philippines. *Food microbiology,* 36 (1):57-62.

Mankee, A., Ali, S., Chin, A.-L., Indalsingh, R., Khan, R., Mohammed, F., et al. 2005. Microbial quality of "doubles" sold in Trinidad. *Food microbiology,* 22 (6):601-607.

Marr, C.R., McSweeney, J.E., Mullen, M.P. & Kulik, T.J. 2015. Central venous line complications with chronic ambulatory infusion of prostacyclin analogues in pediatric patients with pulmonary arterial hypertension. *Pulmonary circulation,* 5 (2):322.

Marsou, R., Bes, M., Brun, Y., Boudouma, M., Idrissi, L., Meugnier, H., et al. 2001. Molecular techniques open up new vistas for typing of coagulase-negative staphylococci. *Pathologie Biologie,* 49 (3):205-215.

Martínez-Tomé, M., Vera, A.M. & Murcia, M.A. 2000. Improving the control of food production in catering establishments with particular reference to the safety of salads. *Food Control,* 11 (6):437-445.

Martins, J. 2006. Socio-economic and hygiene features of street food vending in Gauteng. *South African Journal of Clinical Nutrition,* 19 (1):18-25.

Matsheka, M.I., Mpuchane, S., Gashe, B.A., Allotey, J., Khonga, E.B., Coetzee, S.H., et al. 2014. Microbial quality assessment and predominant microorganism of biltong produced in butcheries in Gaborone, Botswana. *Food and Nutrition sciences,* 5 (17):1668.

McCabe-Sellers, B.J. & Beattie, S.E. 2004. Food safety: emerging trends in foodborne illness surveillance and prevention. *Journal of the American Dietetic Association,* 104 (11):1708-1717.

Mead, P.S., Slutsker, L., Dietz, V., McCaig, L.F., Bresee, J.S., Shapiro, C., et al. 1999. Food-related illness and death in the United States. *Emerging infectious diseases,* 5 (5):607.

Mellon, M.G., Benbrook, C. & Benbrook, K.L. 2001. Hogging it: estimates of antimicrobial abuse in livestock. Union of Concerned Scientists.

Melnick, R.L., Suárez, C., Bailey, B.A. & Backman, P.A. 2011. Isolation of endophytic endospore-forming bacteria from Theobroma cacao as potential biological control agents of cacao diseases. *Biological Control,* 57 (3):236-245.

Mensah, P., Owusu-Darko, K., Yeboah-Manu, D., Ablordey, A., Nkrumah, F. & Kamiya, H. 1997. The role of street vended foods in the transmission of enteric pathogens. *Ghana medical journal,* 33:19-29.

Mensah, P., Yeboah-Manu, D., Owusu-Darko, K. & Ablordey, A. 2002. Street foods in Accra, Ghana: how safe are they? *Bulletin of the World Health Organization,* 80 (7):546-554.

Metri, B., Jyothi, P. & Peerapur, B. 2011. Anti-microbial resistance profile of Citrobacter species in a tertiary care hospital of southern India. *Indian journal of medical sciences,* 65 (10):429.

Meugnier, H., Bes, M., Vernozy-Rozand, C., Mazuy, C., Brun, Y., Freney, J., et al. 1996. Identification and ribotyping of Staphylococcus xylosus and Staphylococcus equorum strains isolated from goat milk and cheese. *International journal of food microbiology,* 31 (1):325-331.

Michalova, E., Novotna, P. & Schlegelova, J. 2004. Tetracyclines in veterinary medicine and bacterial resistance to them. *VETERINARNI MEDICINA-PRAHA-,* 49 (3):79-100.

Miladi, H., Ben Slama, R., Mili, D., Zouari, S., Bakhrouf, A. & Ammar, E. 2013. Chemical composition and cytotoxic and antioxidant activities of Satureja montana L. essential oil and its antibacterial potential against Salmonella spp. strains. *Journal of Chemistry,* 2013.

Moges, F., Endris, M., Belyhun, Y. & Worku, W. 2014. Isolation and characterization of multiple drug resistance bacterial pathogens from waste water in hospital and non-hospital environments, Northwest Ethiopia. *BMC research notes,* 7 (1):215.

Molla, B., Berhanu, A., Muckle, A., Cole, L., Wilkie, E., Kleer, J., et al. 2006. Multidrug resistance and distribution of Salmonella serovars in slaughtered pigs. *Journal of Veterinary Medicine, Series B,* 53 (1):28-33.

Monney, I., Agyei, D. & Owusu, W. 2013. Hygienic practices among food vendors in educational institutions in Ghana: the case of Konongo. *Foods,* 2 (3):282-294.

Montso, K.P. & Ateba, C.N. 2014. Molecular Detection of Clostridium Species in Beef Obtained from Retail Shops in North West Province, South Africa. *Journal of Food and Nutrition Research,* 2 (5):236-243.

Mosupye, F.M. & von Holy, A. 1999. Microbiological quality and safety of ready-to-eat street-vended foods in Johannesburg, South Africa. *Journal of Food Protection®,* 62 (11):1278-1284.

Mosupye, F.M. & Von Holy, A. 2000. Microbiological hazard identification and exposure assessment of street food vending in Johannesburg, South Africa. *International journal of food microbiology,* 61 (2):137-145.

Moussa-Boudjemaa, B., Gonzalez, J. & Lopez, M. 2006. Heat resistance of Bacillus cereus spores in carrot extract acidified with different acidulants. *Food Control,* 17 (10):819-824.

Muinde, O. & Kuria, E. 2005. Hygienic and sanitary practices of vendors of street foods in Nairobi, Kenya. *African Journal of Food, Agriculture, Nutrition and Development,* 5 (1).

Murindamombe, G.Y., Collison, E.K., Mpuchane, S.F. & Gashe, B.A. 2005. Presence of Bacillus cereus in street foods in Gaborone, Botswana. *Journal of Food Protection®,* 68 (2):342-346.

Mutalib, A., Azira, N., Amin Nordin, S., Sakai, K. & Shirai, Y. 2015. An overview of foodborne illness and food safety in Malaysia. *International Food Research Journal,* 22 (3):896-901.

Muyanja, C., Nayiga, L., Brenda, N. & Nasinyama, G. 2011. Practices, knowledge and risk factors of street food vendors in Uganda. *Food Control,* 22 (10):1551-1558.

Muzaffar, A.T., Huq, I. & Mallik, B.A. 2009. Entrepreneurs of the streets: an analytical work on the street food vendors of Dhaka city. *International Journal of Business and Management,* 4 (2):80.

Nakari, U.-M., Rantala, L., Pihlajasaari, A., Toikkanen, S., Johansson, T., Hellsten, C., et al. 2014. Investigation of increased listeriosis revealed two fishery production plants with persistent Listeria contamination in Finland in 2010. *Epidemiology and infection,* 142 (11):2261-2269.

Naranjo, M., Denayer, S., Botteldoorn, N., Delbrassinne, L., Veys, J., Waegenaere, J., et al. 2011. Sudden death of a young adult associated with Bacillus cereus food poisoning. *Journal of clinical microbiology,* 49 (12):4379-4381.

Newell, D.G., Koopmans, M., Verhoef, L., Duizer, E., Aidara-Kane, A., Sprong, H., et al. 2010. Food-borne diseases—the challenges of 20years ago still persist while new ones continue to emerge. *International Journal of Food Microbiology,* 139:S3-S15.

Ngoma, L., Esau, B. & Babalola, O.O. 2013. Isolation and characterization of beneficial indigenous endophytic bacteria for plant growth promoting activity in Molelwane Farm, Mafikeng, South Africa. *African Journal of Biotechnology,* 12 (26).

Ngoma, L., Mogatlanyane, K. & Babalola, O.O. 2014. Screening of endophytic bacteria towards the development of cottage industry: an in vitro study. *J Hum Ecol,* 47 (1):45-63.

Nguyen, V.D., Sreenivasan, N., Lam, E., Ayers, T., Kargbo, D., Dafae, F., et al. 2014. Cholera Epidemic Associated with Consumption of Unsafe Drinking Water and Street-Vended Water—Eastern Freetown, Sierra Leone, 2012. *The American journal of tropical medicine and hygiene,* 90 (3):518-523.

Nicolas, B., Razack, B.A., Yollande, I., Aly, S., Tidiane, O.C.A., Philippe, N.A., et al. 2007. Street-vended foods improvement: Contamination mechanisms and application of Food Safety Objective Strategy: Critical review. *Pakistan Journal of Nutrition,* 6 (1):1-10.

Niehaus, A.J., Apalata, T., Coovadia, Y.M., Smith, A.M. & Moodley, P. 2011. An outbreak of foodborne salmonellosis in rural KwaZulu-Natal, South Africa. *Foodborne pathogens and disease,* 8 (6):693-697.

Njaya, T. 2014. Operations of street food vendors and their impact on sustainable urban life in high density suburbs of Harare, in Zimbabwe.

Nkere, C.K., Ibe, N.I & Iroegbu, C.U. 2011. Bacteriological quality of foods and water sold by vendors and in restaurants in Nsukka, Enugu State, Nigeria: A comparative study of three microbiological methods. *Journal of health, population, and nutrition,* 29 (6):560.

Nsoesie, E.O., Kluberg, S.A. & Brownstein, J.S. 2014. Online reports of foodborne illness capture foods implicated in official foodborne outbreak reports. *Preventive medicine,* 67:264-269.

Nunes, B.N., Cruz, A.G., Faria, J.A., Sant, A.S., Silva, R. & Moura, M.R. 2010. A survey on the sanitary condition of commercial foods of plant origin sold in Brazil. *Food Control,* 21 (1):50-54.

Nwachukwu, E., Ezeama, C. & Ezeanya, B. 2008. Microbiology of polyethylene-packaged sliced watermelon (Citrullus lanatus) sold by street vendors in Nigeria. *African Journal of Microbiology Research,* 2 (8):192-195.

Nyenje, M.E., Odjadjare, C.E., Tanih, N.F., Green, E. & Ndip, R.N. 2012. Foodborne pathogens recovered from ready-to-eat foods from roadside cafeterias and retail outlets in Alice, Eastern Cape Province, South Africa: public health implications. *International journal of environmental research and public health,* 9 (8):2608-2619.

Odumeru, J.A., Toner, A.K., Muckle, C.N., Griffiths, M.W. & Lynch, J.A. 1997. Detection of Bacillus cereus diarrhoel enterotoxin in raw and pasteurised milk. Journal of Applied Microbiology, 60:1391-1393.

Oguttu, J.W., McCrindle, C.M., Makita, K. & Grace, D. 2014. Investigation of the food value chain of ready-to-eat chicken and the associated risk for staphylococcal food poisoning in Tshwane Metropole, South Africa. *Food Control,* 45:87-94.

Ohiokpehai, O. 2003. Promoting the nutritional goodness of traditional food products. *Pakistan Journal of Nutrition,* 2 (4):267-270.

Okeke, I.N., Aboderin, O.A., Byarugaba, D.K., Ojo, K.K. & Opintan, J.A. 2007. Growing problem of multidrug-resistant enteric pathogens in Africa.

Okeke, I.N., Klugman, K.P., Bhutta, Z.A., Duse, A.G., Jenkins, P., O'Brien, T.F., et al. 2005. Antimicrobial resistance in developing countries. Part II: strategies for containment. *The Lancet infectious diseases,* 5 (9):568-580.

Okojie, P. & Isah, E. 2014. Sanitary conditions of food vending sites and food handling practices of street food vendors in Benin City, Nigeria: implication for food hygiene and safety. *Journal of Environmental and Public Health,* 2014.

Olawale, A., Onasanya, A., Oyelakin, O., David, O. & Famurewa, O. 2014. Enterococcus faecalis isolates of food origin and detection of their virulence determinant factors and genes in Osun State, Nigeria. *Microbiology Research International,* 2 (2):18-27.

Oliver, S., Jayarao, B. & Almeida, R. 2005a. Foodborne pathogens, mastitis, milk quality, and dairy food safety. (*In* NMC Annual Meeting Proceedings organised by

Oliver, S.P., Jayarao, B.M. & Almeida, R.A. 2005b. Foodborne pathogens in milk and the dairy farm environment: food safety and public health implications. *Foodbourne Pathogens & Disease,* 2 (2):115-129.

Omari, R., Jongerden, J., Essegbey, G., Frempong, G. & Ruivenkamp, G. 2013. Fast food in the Greater Accra Region of Ghana: Characteristics, availability and the cuisine concept. *Food Studies,* 1 (4):29-44.

Omemu, A. & Aderoju, S. 2008. Food safety knowledge and practices of street food vendors in the city of Abeokuta, Nigeria. *Food Control,* 19 (4):396-402.

Ommi, D., Hemmatinezhad, B., Hafshejani, T.T. & Khamesipour, F. 2016. Incidence and Antimicrobial Resistance of Campylobacter and Salmonella from Houseflies (Musca Domestica) in Kitchens, Farms, Hospitals and Slaughter Houses. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*:1-7.

Onyeneho, S.N. & Hedberg, C.W. 2013. An assessment of food safety needs of restaurants in Owerri, Imo State, Nigeria. *International Journal of Environmental Research and Public Health,* 10 (8):3296-3309.

Oranusi, S., Braide, W. & Nezianya, H. 2012. Microbiological and chemical quality assessment of some commercially packed fruit juices sold in Nigeria. *Greener Journal of Biological Sciences,* 2 (1):001-006.

Oranusi, S., Oguoma, O. & Agusi, E. 2013. Microbiological quality assessment of foods sold in student's cafeterias. *Global Research Journal of Microbiology,* 3 (1):1-7.

Organization, W.H. 2001. WHO global strategy for containment of antimicrobial resistance.

Organization, W.H. 2002. The world health report 2002: reducing risks, promoting healthy life. World Health Organization.

Organization, W.H. 2007. Initiative to estimate the global burden of foodborne diseases. (*In* First formal meeting of the Foodborne Disease Burden Epidemiology Reference Group. Geneva organised by

Organization, W.H. 2008. Foodborne disease outbreaks: guidelines for investigation and control. World Health Organization.

Organization, W.H. 2011a. The International Food Safety Authorities Network (INFOSAN) progress report 2004-2010. (*In* The International Food Safety Authorities Network (INFOSAN) progress report 2004-2010. p.).

Organization, W.H. 2011b. Microbial fact sheets. *World Health Organization Guidelines for Drinking-Water Quality (WHO GDWQ),* 4:271-273.

Organization, W.H. 2014. Safety evaluation of certain food additives and contaminants. Vol. 68: World Health Organization.

Organization, W.H. 2016. WHO estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2007-2015.

Osaili, T.M., Jamous, D.O.A., Obeidat, B.A., Bawadi, H.A., Tayyem, R.F. & Subih, H.S. 2013. Food safety knowledge among food workers in restaurants in Jordan. *Food Control,* 31 (1):145-150.

Osman, K., Badr, J., Al-Maary, K.S., Moussa, I.M., Hessain, A.M., Girah, Z.M.A., et al. 2016. Prevalence of the antibiotic resistance genes in coagulase-positive-and negative-Staphylococcus in chicken meat retailed to consumers. *Frontiers in microbiology,* 7.

Osorio Ospina, N. 2012. Oferta y consumo de alimentos de venta callejera en el Parque Nacional en Bogotá DC.

Oueslati, W., Rjeibi, M.R., Mhadhbi, M., Jbeli, M., Zrelli, S. & Ettriqui, A. 2016. Prevalence, virulence and antibiotic susceptibility of Salmonella spp. strains, isolated from beef in Greater Tunis (Tunisia). *Meat science,* 119:154-159.

Oudiz, R.J. 2004. Micrococcus associated Central venous catheter infection in patients with pulmonary arerial hypertension. Chest, 126: 90-94.

Ozgumus, O.B., Sandalli, C., Sevim, A., Celik-Sevim, E. & Sivri, N. 2009. Class 1 and class 2 integrons and plasmid-mediated antibiotic resistance in coliforms isolated from ten rivers in northern Turkey. *The Journal of Microbiology,* 47 (1):19-27.

Page, R. 1996. TREEVIEW, tree drawing software for Apple Macintosh and Microsoft Windows. *Division of Environmental and Evolutionary Biology, Instituteof Biomedical and Life Sciences, University of Glasgow. Glasgow, Scotland, UK.*

Palmer, S., Gully, P., White, J., Pearson, A., Suckling, W., Jones, D., et al. 1983. Water-borne outbreak of Campylobacter gastroenteritis. *The Lancet,* 321 (8319):287-290.

Pang, F. & See Toh, P. 2008. Hawker food industry: food safety/public health strategies in Malaysia. *Nutrition & Food Science,* 38 (1):41-51.

Parkouda, C., Thorsen, L., Compaoré, C.S., Nielsen, D.S., Tano-Debrah, K., Jensen, J.S., et al. 2010. Microorganisms associated with maari, a baobab seed fermented product. *International journal of food microbiology,* 142 (3):292-301.

Parry, S.M. & Salmon, R.L. 1998. Sporadic STEC O157 infection: secondary household transmission in Wales. *Emerging infectious diseases,* 4 (4):657.

Patricia V. Azanza, C.F.G., Melba P. Ortega, Ma. 2000. Food safety knowledge and practices of streetfood vendors in a Philippines university campus. *International Journal of Food sciences and Nutrition,* 51 (4):235-246.

Peterson, L.R. 2009. Bad bugs, no drugs: no ESCAPE revisited. *Clinical infectious diseases,* 49 (6):992-993.

Petrovic, J., Babic, J., Stojanov, I. & Velhner, M. 2015. The change in antimicrobial resistance profile of meat chain-associated Salmonella in Serbia. *Procedia Food Science,* 5:231-234.

Petrovic, J., Plavsa, N., Milanov, D. & Kapetanov, M. 2010. Antimicrobial resistance of foodborne pathogenes as a food safety issue. *LUCRARI stiintifice,* 43 (2):197-203.

Pett, M.A. 2015. Nonparametric statistics for health care research: Statistics for small samples and unusual distributions. Sage Publications.

Pichler, J., Much, P., Kasper, S., Fretz, R., Auer, B., Kathan, J., et al. 2009. An outbreak of febrile gastroenteritis associated with jellied pork contaminated with Listeria monocytogenes. *Wiener Klinische Wochenschrift,* 121 (3-4):149-156.

Pitout, J.D. & Laupland, K.B. 2008. Extended-spectrum β-lactamase-producing Enterobacteriaceae: an emerging public-health concern. *The Lancet infectious diseases,* 8 (3):159-166.

Pradel, N., Boukhors, K., Bertin, Y., Forestier, C., Martin, C. & Livrelli, V. 2001. Heterogeneity of Shiga toxin-producing Escherichia coli strains isolated from hemolytic-uremic syndrome patients, cattle, and food samples in central France. *Applied and environmental microbiology,* 67 (6):2460-2468.

Proietti, I., Frazzoli, C. & Mantovani, A. 2014. Identification and management of toxicological hazards of street foods in developing countries. *Food and chemical toxicology,* 63:143-152.

Proietti, I., Mantovani, A., Mouquet-Rivier, C. & Guyot, J.P. 2013. Modulation of chelating factors, trace minerals and their estimated bioavailability in Italian and African sorghum (Sorghum bicolor (L.) Moench) porridges. *International Journal of Food Science & Technology,* 48 (7):1526-1532.

Pswarayi, F., Mutukumira, A.N., Chipurura, B., Gabi, B. & Jukes, D.J. 2014. Food control in Zimbabwe: A situational analysis. *Food Control,* 46:143-151.

Purkayastha, J., Sugla, T., Paul, A., Solleti, S., Mazumdar, P., Basu, A., et al. 2010. Efficient in vitro plant regeneration from shoot apices and gene transfer by particle bombardment in Jatropha curcas. *Biologia Plantarum,* 54 (1):13-20.

Qadri, F., Svennerholm, A.-M., Faruque, A. & Sack, R.B. 2005. Enterotoxigenic Escherichia coli in developing countries: epidemiology, microbiology, clinical features, treatment, and prevention. *Clinical microbiology reviews,* 18 (3):465-483.

Quinlan, J.J. 2013. Foodborne illness incidence rates and food safety risks for populations of low socioeconomic status and minority race/ethnicity: a review of the literature. *International Journal of Environmental Research and Public Health,* 10 (8):3634-3652.

Rane, S. 2011. Street vended food in developing world: hazard analyses. *Indian Journal of Microbiology,* 51 (1):100-106.

Rask, K.J. & Rask, N. 2011. Economic development and food production–consumption balance: a growing global challenge. *Food Policy,* 36 (2):186-196.

Reddy, G., Prakash, J., Vairamani, M., Prabhakar, S., Matsumoto, G. & Shivaji, S. 2002. Planococcus antarcticus and Planococcus psychrophilus spp. nov. isolated from cyanobacterial mat samples collected from ponds in Antarctica. *Extremophiles,* 6 (3):253-261.

Redmond, E.C. & Griffith, C.J. 2003. A comparison and evaluation of research methods used in consumer food safety studies. *International Journal of Consumer Studies,* 27 (1):17-33.

Riboldi, G.P., Frazzon, J., d'Azevedo, P.A. & Frazzon, A.P.G. 2009. Antimicrobial resistance profile of Enterococcus spp isolated from food in Southern Brazil. *Brazilian Journal of Microbiology,* 40 (1):125-128.

Riet, H. 2002. The role of street foods in the diet of low-income urban residents, the case of Nairobi. Wageningen Univ.

Rivera, F.P., Medina, A.M., Aldasoro, E., Sangil, A., Gascon, J., Ochoa, T.J., et al. 2013. Genotypic characterization of enterotoxigenic Escherichia coli strains causing traveler's diarrhea. *Journal of clinical microbiology,* 51 (2):633-635.

Rocourt, J., Moy, G., Vierk, K. & Schlundt, J. 2003. The present state of foodborne disease in OECD countries. *Geneva: WHO,* 1.

Rodrigues, C.S., Sá, C.V.G.C.d. & Melo, C.B.d. 2017. An overview of Listeria monocytogenes contamination in ready to eat meat, dairy and fishery foods. *Ciência Rural,* 47 (2).

Ruiz, J., Capitano, L., Nuñez, L., Castro, D., Sierra, J.M., Hatha, M., et al. 1999. Mechanisms of resistance to ampicillin, chloramphenicol and quinolones in multiresistant Salmonella typhimurium strains isolated from fish. *Journal of antimicrobial chemotherapy,* 43 (5):699-702.

Rzhetsky, A. & Nei, M. 1992. A simple method for estimating and testing minimum-evolution trees. *Mol Biol Evol,* 9 (5):945-967.

Sadashiv, S. & Kaliwal, B. 2014. Isolation, characterization and antibiotic resistance of Bacillus sps. from bovine mastitis in the region of north Karnataka, India. *Int. J. Curr. Microbiol. App. Sci,* 3 (4):360-373.

Saitou, N. & Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular biology and evolution,* 4 (4):406-425.

Samapundo, S., Climat, R., Xhaferi, R. & Devlieghere, F. 2015. Food safety knowledge, attitudes and practices of street food vendors and consumers in Port-au-Prince, Haiti. *Food Control,* 50:457-466.

Samapundo, S., Thanh, T.C., Xhaferi, R. & Devlieghere, F. 2016. Food safety knowledge, attitudes and practices of street food vendors and consumers in Ho Chi Minh city, Vietnam. *Food Control,* 70:79-89.

Samuel, O.O. 2013. Infant Mortality in Nigeria: Assessing Knowledge of Predisposing Risk Factors among Mothers and Bacteriological Profile of the Weaning Foods. *American Journal of Food and Nutrition,* 1 (3):22-26.

Santos, M.-J., Nogueira, J.R., Patarata, L. & Mayan, O. 2008. Knowledge levels of food handlers in Portuguese school canteens and their self-reported behaviour towards food safety. *International Journal of Environmental Health Research,* 18 (6):387-401.

Sarmah, A.K., Meyer, M.T. & Boxall, A.B. 2006. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. *Chemosphere,* 65 (5):725-759.

Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M.-A., Roy, S.L., et al. 2011. Foodborne illness acquired in the United States—major pathogens. *Emerg Infect Dis,* 17 (1).

Schlech, W.F. & Acheson, D. 2000. Foodborne listeriosis. *Clinical infectious diseases,* 31 (3):770-775.

Schlecht, L.M., Peters, B.M., Krom, B.P., Freiberg, J.A., Hänsch, G.M., Filler, S.G., et al. 2015. Systemic Staphylococcus aureus infection mediated by Candida albicans hyphal invasion of mucosal tissue. *Microbiology,* 161 (1):168-181.

Schlegelova, J., Babak, V., Brychta, J., Klimova, E. & Napravnikova, E. 2003. The prevalence of and resistance to antimicrobial agents of Bacillus cereus isolates from foodstuffs. *Veterinarni Medicina-UZPI (Czech Republic).*

Schleifer, K., Kilpper-Bälz, R. & Devriese, L. 1984. Staphylococcus arlettae sp. nov., S. equorum sp. nov. and S. kloosii sp. nov.: three new coagulase-negative, novobiocin-resistant species from animals. *Systematic and applied microbiology,* 5 (4):501-509.

Schlundt, J. 2002. New directions in foodborne disease prevention. *International Journal of Food microbiology,* 78 (1):3-17.

Schmitz, F.-J., Krey, A., Sadurski, R., Verhoef, J., Milatovic, D. & Fluit, A.C. 2001. Resistance to tetracycline and distribution of tetracycline resistance genes in European Staphylococcus aureus isolates. *Journal of antimicrobial chemotherapy,* 47 (2):239-240.

Scott, W., Scott, H., Lake, R. & Baker, M. 2000. Economic cost to New Zealand of foodborne infectious disease. *The New Zealand Medical Journal,* 113 (1113):281-284.

Sezgin, A.C. & Şanlıer, N. 2016. Street food consumption in terms of the food safety and health. *Journal of Human Sciences,* 13 (3):4072-4083.

Shale, K. & Malebo, N. 2011. Quantification and antibiotic susceptibility profiles of Staphylococcus aureus and Bacillus cereus strains isolated from Biltong. *Journal of Food Safety,* 31 (4):559-569.

Shao, D., Shi, Z., Wei, J. & Ma, Z. 2011. A brief review of foodborne zoonoses in China. *Epidemiology and infection,* 139 (10):1497-1504.

Shiferaw, B., Shallow, S., Marcus, R., Segler, S., Soderlund, D., Hardnett, F.P., et al. 2004. Trends in population-based active surveillance for shigellosis and demographic variability in FoodNet sites, 1996–1999. *Clinical infectious diseases,* 38 (Supplement 3):S175-S180.

Silbergeld, E.K., Graham, J. & Price, L.B. 2008. Industrial food animal production, antimicrobial resistance, and human health. *Annu. Rev. Public Health,* 29:151-169.

Silva, F.F.P.d., Horvath, M.B., Silveira, J.G., Pieta, L. & Tondo, E.C. 2014. Occurrence of Salmonella spp. and generic Escherichia coli on beef carcasses sampled at a brazilian slaughterhouse. *Brazilian Journal of Microbiology,* 45 (1):17-24.

Silva, J., Leite, D., Fernandes, M., Mena, C., Gibbs, P.A. & Teixeira, P. 2011. Campylobacter spp. as a foodborne pathogen: a review. *Frontiers in microbiology,* 2.

Simpson, E., Wittet, S., Bonilla, J., Gamazina, K., Cooley, L. & Winkler, J.L. 2007. Use of formative research in developing a knowledge translation approach to rotavirus vaccine introduction in developing countries. *BMC Public Health,* 7 (1):1.

Sina, H., Baba-Moussa, F., Kayodé, A., Noumavo, P., Sezan, A., Hounhouigan, J., et al. 2011. Characterization of Staphylococcus aureus isolated from street foods: Toxin profile and prevalence of antibiotic resistance. *Journal of Applied Biosciences,* 46:3133-3143.

Singh, S., Yadav, A.S., Singh, S.M. & Bharti, P. 2010. Prevalence of Salmonella in chicken eggs collected from poultry farms and marketing channels and their antimicrobial resistance. *Food Research International,* 43 (8):2027-2030.

Sjöling, Å., von Mentzer, A. & Svennerholm, A.-M. 2015. Implications of enterotoxigenic Escherichia coli genomics for vaccine development. *Expert review of vaccines,* 14 (4):551-560.

Soares, L.S., Almeida, R.C., Cerqueira, E.S., Carvalho, J.S. & Nunes, I.L. 2012. Knowledge, attitudes and practices in food safety and the presence of coagulase-positive staphylococci on hands of food handlers in the schools of Camaçari, Brazil. *Food Control,* 27 (1):206-213.

Sockett, P. 1991. The economic implications of human salmonella infection. *Journal of Applied Bacteriology,* 71 (4):289-295.

Sockett, P. & Roberts, J. 1991. The social and economic impact of salmonellosis: a report of a national survey in England and Wales of laboratory-confirmed Salmonella infections. *Epidemiology and infection,* 107 (02):335-347.

Sodagari, H.R., Mashak, Z. & Ghadimianazar, A. 2015. Prevalence and antimicrobial resistance of Salmonella serotypes isolated from retail chicken meat and giblets in Iran. *The Journal of Infection in Developing Countries,* 9 (05):463-469.

Soon, J., Singh, H. & Baines, R. 2011. Foodborne diseases in Malaysia: A review. *Food Control,* 22 (6):823-830.

Speer, B.S., Shoemaker, N.B. & Salyers, A.A. 1992. Bacterial resistance to tetracycline: mechanisms, transfer, and clinical significance. *Clinical microbiology reviews,* 5 (4):387-399.

Stenutz, R., Weintraub, A. & Widmalm, G. 2006. The structures of Escherichia coli O-polysaccharide antigens. *FEMS microbiology reviews,* 30 (3):382-403.

Su, C. & Brandt, L.J. 1995. Escherichia coli O157: H7 infection in humans. *Annals of Internal Medicine,* 123 (9):698-707.

Su, L.-H., Chiu, C.-H., Chu, C. & Ou, J.T. 2004. Antimicrobial resistance in nontyphoid Salmonella serotypes: a global challenge. *Clinical infectious diseases,* 39 (4):546-551.

Subratty, A., Beeharry, P. & Chan Sun, M. 2004. A survey of hygiene practices among food vendors in rural areas in Mauritius. *Nutrition & Food Science,* 34 (5):203-205.

Suresh, T., Hatha, A., Sreenivasan, D., Sangeetha, N. & Lashmanaperumalsamy, P. 2006. Prevalence and antimicrobial resistance of Salmonella enteritidis and other salmonellas in the eggs and egg-storing trays from retails markets of Coimbatore, South India. *Food microbiology,* 23 (3):294-299.

Tadesse, D.A., Zhao, S., Tong, E., Ayers, S., Singh, A., Bartholomew, M.J., et al. 2012. Antimicrobial drug resistance in Escherichia coli from humans and food animals, United States, 1950–2002. *Emerg Infect Dis,* 18 (5):741-749.

Tahmasebi, H., Talebi, R. & Zarif, B.R. 2014. Isolated of Bacillus Cereus in Chicken Meat and Investigation β-Lactamase Antibiotic-Resistant in Bacillus Cereus from Chicken Meat. *Advances in Life Sciences,* 4 (4):200-206.

Tambekar, D., Jaiswal, V., Dhanorkar, D., Gulhane, P. & Dudhane, M. 2008. Identification of microbiological hazards and safety of ready-to-eat food vended in streets of Amravati City, India. *Journal of Appl. Bioscience,* 7:195-201.

Tambekar, D., Jaiswal, V., Dhanorkar, D., Gulhane, P. & Dudhane, M. 2009. Microbial quality and safety of street vended fruit juices: a case study of Amravati city. *Internet Journal of Food Safety,* 10 (7).

Tambekar, D., Kulkarni, R., Shirsat, S. & Bhadange, D. 2011. Bacteriological quality of street vended food Panipuri: A case study of Amravati city (MS) India. *Bioscience discovery,* 2 (3):350-354.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular biology and evolution,* 28 (10):2731-2739.

Tarekgne, E., Skeie, S., Rudi, K., Skjerdal, T. & Narvhus, J.A. 2015. Staphylococcus aureus and other Staphylococcus species in milk and milk products from Tigray region, Northern Ethiopia. *African Journal of Food Science,* 9 (12):567-576.

Tauxe, R.V., Doyle, M.P., Kuchenmüller, T., Schlundt, J. & Stein, C. 2010. Evolving public health approaches to the global challenge of foodborne infections. *International journal of food microbiology,* 139:S16-S28.

Teisl, M.F. & Roe, B.E. 2010. Consumer willingness-to-pay to reduce the probability of retail foodborne pathogen contamination. *Food Policy,* 35 (6):521-530.

Tent, H. 1999. Research on food safety in the 21st century. *Food Control,* 10 (4):239-241.

Thomas, A. 2016. Listeria monocytogenes in Fresh Fruits and Vegetables. Virginia Techp.

Thompson, J.D., Higgins, D.G. & Gibson, T.J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic acids research,* 22 (22):4673-4680.

Tinker, I. 1997. Street foods: Urban food and employment in developing countries. Oxford University Press.

Toh, P.S. & Birchenough, A. 2000. Food safety knowledge and attitudes: culture and environment impact on hawkers in Malaysia.: Knowledge and attitudes are key attributes of concern in hawker foodhandling practices and outbreaks of food poisoning and their prevention. *Food Control,* 11 (6):447-452.

Tong, S.Y., Davis, J.S., Eichenberger, E., Holland, T.L. & Fowler, V.G. 2015. Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clinical microbiology reviews,* 28 (3):603-661.

Trzcinski, K., Cooper, B.S., Hryniewicz, W. & Dowson, C.G. 2000. Expression of resistance to tetracyclines in strains of methicillin-resistant Staphylococcus aureus. *Journal of antimicrobial chemotherapy,* 45 (6):763-770.

Tsubakishita, S., Kuwahara-Arai, K., Baba, T. & Hiramatsu, K. 2010. Staphylococcal cassette chromosome mec-like element in Macrococcus caseolyticus. *Antimicrobial agents and chemotherapy,* 54 (4):1469-1475.

Turnbull, P.C., Sirianni, N.M., LeBron, C.I., Samaan, M.N., Sutton, F.N., Reyes, A.E., et al. 2004. MICs of selected antibiotics for Bacillus anthracis, Bacillus cereus, Bacillus thuringiensis, and Bacillus mycoides from a range of clinical and environmental sources as determined by the Etest. *Journal of clinical microbiology,* 42 (8):3626-3634.

Uddin, G.M.N., Larsen, M.H., Christensen, H., Aarestrup, F.M., Phu, T.M. & Dalsgaard, A. 2015. Identification and antimicrobial resistance of bacteria isolated from probiotic products used in shrimp culture. *PloS one,* 10 (7):e0132338.

Umoh, V. & Odoba, M. 1999. Safety and quality evaluation of street foods sold in Zaria, Nigeria. *Food Control,* 10 (1):9-14.

Unnevehr, L.J. 2000. Food safety issues and fresh food product exports from LDCs. *Agricultural Economics,* 23 (3):231-240.

Uwaezuoke, J. & Aririatu, L. 2004. A survey of antibiotic resistant Staphylococcus aureus strains from clinical sources in Owerri.

Vacher, S., Menard, A., Bernard, E., Santos, A. & Megraud, F. 2005. Detection of mutations associated with macrolide resistance in thermophilic Campylobacter spp. by real-time PCR. *Microbial Drug Resistance,* 11 (1):40-47.

Van, T.T.H., Moutafis, G., Tran, L.T. & Coloe, P.J. 2007. Antibiotic resistance in food-borne bacterial contaminants in Vietnam. *Applied and Environmental microbiology,* 73 (24):7906-7911.

Vandenesch, F., Naimi, T., Enright, M.C., Lina, G., Nimmo, G.R., Heffernan, H., et al. 2003. Community-acquired methicillin-resistant Staphylococcus aureus carrying Panton-Valentine leukocidin genes: worldwide emergence. *Emerging infectious diseases,* 9 (8):978-984.

Varnam, A.H. & Evans M.G. 1991. Food borne pathogens: An illustrated text. Wolfe Publishing, London

Vasconcelos, N., Pereira, V., Araújo Júnior, J. & da Cunha, M. 2011. Molecular detection of enterotoxins E, G, H and I in Staphylococcus aureus and coagulase-negative staphylococci isolated from clinical samples of newborns in Brazil. *Journal of applied microbiology,* 111 (3):749-762.

Vasconcelos, N.G. & de Souza, M.d.L.R. 2010. Staphylococcal enterotoxins: molecular aspects and detection methods. *Journal of Public Health and Epidemiology,* 2 (3):29-42.

Vázquez-Boland, J.A., Kuhn, M., Berche, P., Chakraborty, T., Domínguez-Bernal, G., Goebel, W., et al. 2001. Listeria pathogenesis and molecular virulence determinants. *Clinical microbiology reviews,* 14 (3):584-640.

Vernozy-Rozand, C., Mazuy, C., Meugnier, H., Bes, M., Lasne, Y., Fiedler, F., et al. 2000. Staphylococcus fleurettii sp. nov., isolated from goat's milk cheeses. *International Journal of Systematic and Evolutionary Microbiology,* 50 (4):1521-1527.

Vilain, S., Luo, Y., Hildreth, M.B. & Brözel, V.S. 2006. Analysis of the life cycle of the soil saprophyte Bacillus cereus in liquid soil extract and in soil. *Applied and environmental microbiology,* 72 (7):4970-4977.

Von Holy, A. & Makhoane, F. 2006. Improving street food vending in South Africa: Achievements and lessons learned. *International Journal of food microbiology,* 111 (2):89-92.

Vongkamjan, K., Roof, S., Stasiewicz, M.J. & Wiedmann, M. 2013. Persistent Listeria monocytogenes subtypes isolated from a smoked fish processing facility included both phage susceptible and resistant isolates. *Food microbiology,* 35 (1):38-48.

Vos, P., Garrity, G., Jones, D., Krieg, N.R., Ludwig, W., Rainey, F.A., et al. 2011. Bergey's Manual of Systematic Bacteriology: Volume 3: The Firmicutes. Vol. 3: Springer Science & Business Media.

Vos, T., Flaxman, A.D., Naghavi, M., Lozano, R., Michaud, C., Ezzati, M., et al. 2013. Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. *The Lancet,* 380 (9859):2163-2196.

Vuong, C. & Otto, M. 2002. Staphylococcus epidermidis infections. *Microbes and Infection,* 4 (4):481-489.

Wada-Kura, A., Maxwell, R., Sadiq, H., Tijjani, M., Abdullahi, I., Aliyu, M., et al. 2009. Microbiological quality of some ready-to-eat foods and fomites in some cafeterias in Ahmadu Bello University, Zaria. *Biol. Environ. Sci. J. Trop,* 6 (1):6-9.

Walker, C.L.F., Sack, D. & Black, R.E. 2010. Etiology of diarrhea in older children, adolescents and adults: a systematic review. *PLoS Negl Trop Dis,* 4 (8):e768.

Walker, E., Pritchard, C. & Forsythe, S. 2003. Food handlers' hygiene knowledge in small food businesses. *Food Control,* 14 (5):339-343.

Wang, J., Fan, Y. & Yao, Z. 2010. Isolation of a Lysinibacillus fusiformis strain with tetrodotoxin-producing ability from puffer fish Fugu obscurus and the characterization of this strain. *Toxicon,* 56 (4):640-643.

Waters, A.E., Contente-Cuomo, T., Buchhagen, J., Liu, C.M., Watson, L., Pearce, K., et al. 2011. Multidrug-resistant Staphylococcus aureus in US meat and poultry. *Clinical infectious diseases,* 52 (10):1227-1230.

Wayne, P. 2009. Clinical and Laboratory Standards Institute (CLSI) performance standards for antimicrobial disk diffusion susceptibility tests 19th ed. approved standard. *CLSI document M100-S19,* 29.

Webb, M. & Morancie, A. 2015. Food safety knowledge of foodservice workers at a university campus by education level, experience, and food safety training. *Food Control,* 50:259-264.

Whichard, J.M., Gay, K., Stevenson, J.E., Joyce, K.J., Cooper, K.L., Omondi, M., et al. 2007. Human Salmonella and concurrent decreased susceptibility to quinolones and extended-spectrum cephalosporins. *Emerg Infect Dis,* 13 (11):1681-1688.

White, D.G., Zhao, S., Sudler, R., Ayers, S., Friedman, S., Chen, S., et al. 2001. The isolation of antibiotic-resistant Salmonella from retail ground meats. *New England Journal of Medicine,* 345 (16):1147-1154.

WHO, N.D. 2002. Food safety and foodborne illness. *biochimica clinica,* 26 (4):39.

Widdowson, M.-A., Sulka, A., Bulens, S.N., Beard, R.S., Chaves, S.S., Hammond, R., et al. 2005. Norovirus and foodborne disease, United States, 1991–2000. *Emergency Infection Diseases,* 11 (1):95-102.

Wieneke, A., Roberts, D. & Gilbert, R. 1993. Staphylococcal food poisoning in the United Kingdom, 1969–90. *Epidemiology and infection,* 110 (03):519-531.

Wijnands, L., Dufrenne, J., Rombouts, F. & Van Leusden, F. 2006a. Prevalence of potentially pathogenic Bacillus cereus in food commodities in the Netherlands. *Journal of Food Protection®,* 69 (11):2587-2594.

Wijnands, L., Dufrenne, J., Zwietering, M. & Van Leusden, F. 2006b. Spores from mesophilic Bacillus cereus strains germinate better and grow faster in simulated gastro-intestinal conditions than spores from psychrotrophic strains. *International journal of food microbiology,* 112 (2):120-128.

Wikler, M.A. 2006. Performance standards for antimicrobial susceptibility testing: Sixteenth informational supplement. Vol. 26: Clinical and Laboratory Standards Institute.

Wikler, M.A. 2007. Performance standards for antimicrobial susceptibility testing: Seventeenth informational supplement. Clinical and Laboratory Standards Institute.

Willford, J., Manley, W., Rebelein, T. & Goodridge, L.D. 2007. Pulsed field, ribotype and integron analysis of multidrug resistant isolates of Salmonella enterica serovar Newport. *Food Research International,* 40 (8):975-981.

Winarno, F. & Allain, A. 1991. Street foods in developing countries: lessons from Asia. *Food, Nutrition and Agriculture (FAO).*

Winters, C. & Gelband, H. 2011. Part I. The Global Antibiotic Resistance Partnership (GARP). *SAMJ: South African Medical Journal,* 101 (8):556-557.

Wise, R., Hart, T., Cars, O., Streulens, M., Helmuth, R., Huovinen, P., et al. 1998. Antimicrobial resistance is a major threat to public health. *British Medical Journal,* 317 (7159):609-611.

Wu, C.-J., Hsueh, P.-R. & Ko, W.-C. 2011. A new health threat in Europe: Shiga toxin–producing Escherichia coli O104: H4 infections. *Journal of microbiology, Immunology and Infection,* 44 (5):390-393.

www.wikipedia.org/wiki/Johannesburg#cite-ref 30: Johannesburg & Gauteng and climate. Retrieved 31 August 2017.

www.wikipedia.org/wiki/Johannesburg # cite_31: UV indexof Johannesburg. UVawareness. Archived from the original on 22 December 2015: Retrieved 31 august 2017.

Xia, Z., Xichu, W., Bingjiao, G. & Xiaolan, W. 2013. Isolation and identification of Enterococcus faecalis and detection of its virulence factor genes in lambs presenting with encephalitis in Xinjiang province, China. *African Journal of Microbiology Research,* 7 (20):2238-2244.

Xue, J. & Zhang, W. 2013. Understanding China's food safety problem: An analysis of 2387 incidents of acute foodborne illness. *Food Control,* 30 (1):311-317.

Yang, B., Xi, M., Cui, S., Zhang, X., Shen, J., Sheng, M., et al. 2012. Mutations in gyrase and topoisomerase genes associated with fluoroquinolone resistance in Salmonella serovars from retail meats. *Food Research International,* 45 (2):935-939.

Yang, X., Huang, J., Wu, Q., Zhang, J., Liu, S., Guo, W., et al. 2016. Prevalence, antimicrobial resistance and genetic diversity of Salmonella isolated from retail ready-to-eat foods in China. *Food Control,* 60:50-56.

Yildirim, Y., Gonulalan, Z., Pamuk, S. & Ertas, N. 2011. Incidence and antibiotic resistance of Salmonella spp. on raw chicken carcasses. *Food Research International,* 44 (3):725-728.

Yoon, J.-H., Kang, S.-J., Lee, S.-Y., Oh, K.-H. & Oh, T.-K. 2010. Planococcus salinarum sp. nov., isolated from a marine solar saltern, and emended description of the genus Planococcus. *International Journal of Systematic and Evolutionary Microbiology,* 60 (4):754-758.

Yu, Q., Zhai, L., Bie, X., Lu, Z., Zhang, C., Tao, T., et al. 2016. Survey of five food-borne pathogens in commercial cold food dishes and their detection by multiplex PCR. *Food Control,* 59:862-869.

YU, S.-m., CONG, X.-x., SU, Y. & HUANG, X.-l. 2010. Investigation and Anaysis on Food Poisoning Caused by Salmonella Contaminated Cold Dish [J]. *Occupation and Health,* 8:024.

Yuli, H., Robin, T., Lay, C.C., Lee, H.Y., Tang, S.Y. & Radu, S. 2008. Characterization of Enterobacter cloacae isolated from street foods. *ASEAN Food Journal,* 15 (1):57-64.

Zhang, D.-C., Liu, H.-C., Xin, Y.-H., Yu, Y., Zhou, P.-J. & Zhou, Y.-G. 2009. Planomicrobium glaciei sp. nov., a psychrotolerant bacterium isolated from a glacier. *International Journal of Systematic and Evolutionary Microbiology,* 59 (6):1387-1390.

Zou, M., Zhou, R., Wu, W., Zhang, N., Liu, W., Hu, F., et al. 2012. Antimicrobial resistance and molecular epidemiological characteristics of clinical isolates of Staphylococcus aureus in Changsha area. *Chinese medical journal,* 125 (13):2289-2294.

Zweifel, C. & Stephan, R. 2012. Spices and herbs as source of Salmonella-related foodborne diseases. *Food Research International,* 45 (2):765-769.

APPENDICES

Appendix 1: Survey on hygiene practices - observation form

Time of observation:

Starting time: …….. **Ending time……**

Date…… **Location……**

---

1 Hygiene of vending site

Presence of stagnant water ..... Yes

No

.....

Presence of salubrious at vending location.....Yes

No

.....

Presence of flies at vending location.....Yes

No

.....

2 General hygiene practices of street vendors

Washing of hands before handling food.....Yes

No

.....

Washing of hands after touching money.....Yes

No

.....

Washing of hands after handling waste foods.....Yes

No

.....

Handling money while serving food.....Yes

No

.....

Touching money with gloves.....Yes

No

.....

Washing of hands with cold water only.....Yes

No

.....

Washing of hands with cold water and soap.....Yes

No

.....

Presence of food debris on vendor's hands.....Yes

No

.....

Wearing of gloves during preparation.....Yes

No

.....

Touching money with gloves.....Yes

No

.....

3 Food hygiene

Exposure of foods to flies and dust.....Yes

No

.....

Storage of foods in a cooler box.....Yes

No

.....

Use of polythene bags in serving food.....Yes

No

.....

3. Hygiene of serving utensils

Cleaning dishes with clean water and soap..... Yes

No

.....

Cleaning dishes with the same water.....Yes

No

.....

Changing water to clean dishes.....Yes

No

.....

Cleaning tables with clean cloth.....Yes

No

.....

Cleaning table with the same cloth .....Yes

No

.....

Appendix 2: Bacterial count

Bacteria count by area

Table 4.1: Total bacterial and total coliform counts in ready-to-eat meat (chicken gizzard) sold at and around MTN taxi rank, Johannesburg CBD

Sample identification	Total bacterial count (CFU/g)	Total coliform count (CFU/g)
CHG 02	9.6×10^2	2.7×10^2
CHG 03	4.7×10^2	1.9×10^2
CHG 04	6.9×10^2	1.0×10^2
CHG 05	1.9×10^2	1.4×10^2
CHG 06	8.6×10^2	1.3×10^2
CHG 07	9.4×10^2	2.6×10^2
CHG 08	8.1×10^2	2.1×10^2
CHG 09	7.2×10^2	1.8×10^2
CHG 10	8.6×10^2	1.9×10^2
CHG 11	7.1×10^2	2.6×10^2
CHG 12	8.2×10^2	1.0×10^2
CHG 13	9.6×10^2	2.9×10^2
CHG 14	6.2×10^2	1.1×10^2
CHG 15	7.4×10^2	2.2×10^2
CHG 16	3.1×10^2	1.4×10^2
CHG 17	2.6×10^2	1.0×10^2
CHG 18	5.9×10^2	1.3×10^2
CHG 19	4.2×10^2	1.1×10^2
CHG 20	7.2×10^2	2.1×10^2
CHG 21	6.1×10^2	1.9×10^2
CHG 22	3.7×10^2	1.3×10^2

CHG (Chicken gizzard)

Table 4.2: Total bacterial and coliform counts in ready-to-eat meat (cooked beef intestine), sold around MTN taxi rank, Johannesburg CBD

Sample identity	Total bacterial count (CFU/g)	Total coliform count (CFU/g)
BI O3	2.9×10^2	1.0×10^2
BI 04	7.6×10^2	2.9×10^2
BI 7	4.9×10^2	1.8×10^2
BI 8	9.6×10^2	2.7×10^2
BI 10	5.0×10^2	1.6×10^2
BI 11	7.8×10^2	3.6×10^2
BI 16	8.6×10^2	1.9×10^2
BI 18	5.2×10^2	1.7×10^2
BI 19	4.3×10^2	2.6×10^2
BI 21	6.7×10^2	1.3×10^2
BI 26	7.9×10^2	1.1×10^2
BI 28	5.9×10^2	2.6×10^2
BI 31	9.4×10^2	1.3×10^2
BI 32	3.1×10^2	1.1×10^2
BI 34	7.9×10^2	1.6×10^2
BI 35	5.1×10^2	1.3×10^2
BI 37	9.9×10^2	2.3×10^2
BI 41	4.0×10^2	1.0×10^2
BI 44	7.8×10^2	3.7×10^2
BI 49	6.3×10^2	1.9×10^2

BI (Beef intestine)

Table 4.3: Total bacterial and coliform counts for ready-to-eat meat (wors) sold around Bree street (corner Claim Street), Johannesburg CBD

Sample identity	Total bacterial count (CFU/g)	Total coliform count (CFU/g)
Wo 1	4.1×10^2	1.1×10^2
Wo 2	1.4×10^2	1.3×10^2
Wo 3	7.9×10^2	2.1×10^2
Wo 4	4.1×10^2	1.7×10^2
Wo 5	3.3×10^2	1.3×10^2
Wo 6	1.9×10^2	1.1×10^2
Wo 7	4.2×10^2	1.7×10^2
Wo 8	3.7×10^2	1.3×10^2
Wo 9	3.2×10^2	1.1×10^2
Wo 10	5.8×10^2	1.6×10^2
Wo 20	5.6×10^2	1.3×10^2
Wo 21	4.3×10^2	1.9×10^2
Wo 22	6.7×10^2	2.1×10^2
Wo 23	1.9×10^2	1.1×10^2
Wo 24	4.9×10^2	2.1×10^2
Wo 25	7.8×10^2	2.4×10^2
Wo 26	5.2×10^2	1.8×10^2
Wo 27	6.1×10^2	1.6×10^2
Wo 28	6.1×10^2	2.2×10^2
Wo 29	6.4×10^2	2.1×10^2
Wo 31	5.9×10^2	1.4×10^2
Wo 33	6.8×10^2	1.9×10^2
Wo 34	4.7×10^2	1.3×10^2
Wo 37	9.1×10^2	2.4×10^2
Wo 39	4.3×10^2	1.1×10^2

Wo (Wors)

Table 4.4: Total bacterial and coliform counts in ready-to-eat meat (braai Chicken) sold in Bree Street (corner Claim Street) Johannesburg CBD

Sample identity	Total bacterial count (CFU/g)	Total coliform count (CFU/g)
CHM 5	8.6×10^2	1.1×10^2
CHM 13	7.6×10^2	1.7×10^2
CHM 14	1.1×10^2	1.5×10^2
CHM 20	9.7×10^2	3.0×10^2
CHM 22	7.2×10^2	2.6×10^2
CHM 25	5.9×10^2	1.0×10^2
CHM 27	4.4×10^2	1.3×10^2
CHM 29	4.1×10^2	1.0×10^2
CHM 30	1.7×10^2	1.1×10^2
CHM 31	3.8×10^2	1.3×10^2
CHM 33	1.9×10^2	1.0×10^2
CHM 36	3.2×10^2	1.1×10^2
CHM 37	7.2×10^2	1.9×10^2
CHM 42	4.1×10^2	1.1×10^2
CHM 43	4.8×10^2	2.6×10^2
CHM 45	6.4×10^2	2.1×10^2
CHM 46	3.5×10^2	2.7×10^2
CHM 47	5.6×10^2	1.9×10^2
CHM 52	9.1×10^2	1.7×10^2
CHM 54	8.0×10^2	1.2×10^2
CHM 55	2.1×10^2	1.3×10^2
CHM 58	3.4×10^2	1.1×10^2
CHM 63	4.2×10^2	2.0×10^2
CHM 66	6.4×10^2	1.5×10^2
CHM 67	2.3×10^2	1.0×10^2

CHM (Chicken meat)

Table 4.5: Total bacterial and coliform counts in cooked beef head meat sold around Hancock Street (corner Claim Street), Johannesburg CBD

Sample identity	Total bacterial count (CFU/g)	Total coliform count (CFU/g)
BHM 2	8.7×10^2	1.2×10^2
BHM 1	5.6×10^2	1.1×10^2
BHM 8	4.9×10^2	1.3×10^2
BHM 9	5.1×10^2	1.5×10^2
BHM 14	6.6×10^2	1.7×10^2
BHM 15	4.6×10^2	2.9×10^2
BHM 23	3.9×10^2	1.4×10^2
BHM 24	5.9×10^2	1.1×10^2
BHM 28	6.2×10^2	1.7×10^2
BHM 5	4.8×10^2	2.3×10^2
BHM 22	7.2×10^2	1.9×10^2
BHM 29	9.2×10^2	1.1×10^2
BHM 31	6.3×10^2	2.1×10^2
BHM 33	8.2×10^2	1.9×10^2
BHM 30	7.2×10^2	2.3×10^2
BHM 34	7.6×10^2	1.8×10^2
BHM 36	3.1×10^2	1.5×10^2
BHM 37	5.8×10^2	2.1×10^2
BHM 32	2.9×10^2	1.3×10^2
BHM 17	6.1×10^2	1.1×10^2
BHM 16	4.7×10^2	1.6×10^2
BHM 13	4.9×10^2	2.2×10^2
BHM 12	6.3×10^2	2.9×10^2
BHM 10	9.7×10^2	1.7×10^2
BHM 11	5.3×10^2	1.1×10^2

BHM (Beef head meat)

Appendix 3: Preliminary results based on microscopic examination and biochemical characteristics

Bacterial isolates from samples obtained from the three areas in this study were subjected to microscopic examinations, studies subsequent to preliminary biochemical tests such as Gram stain, oxidase test, catalase test, indole test and voges proskauer. The results are presented in Table 1. Preliminary results based on morphology and biochemical characterisation for samples collected around MTN-taxi rank (Table 2) showed the results of morphology and biochemical tests for the samples collected around Bree Street (corner Plein Street). Table 3 shows the morphology and biochemical characteristics of samples collected at Hancock Street (corner Claim Street).

Results of biochemical tests and microscopic examination revealed the occurrence of different strains of bacteria such as *Staphylococcus* spp, *Bacillus* spp, *Enterococcus* spp, *Citrobacter* spp. and *Macrococcus* spp which were tentatively identified before being subjected to molecular identification using PCR, for further investigation and confirmation.

Table 1: Preliminary results based on morphological and biochemical characteristics

Sample identification	Morphology	Gr	Ox	Cat	VP	IN	Probable organism
GIZ 3 D1L1	Rod, irregular, curled, raised, opaque, smooth	V^+	V^+	V^+	V^+	V^-	*Bacillus cereus*
GIZ 0 D4	Cocci, ovoid, edges, raised, white colony, smooth	V^+	V^-	V^-	V^+	V^-	*Enterococcus faecalis*
GIZ 16 D4	Cocci, irregular, curled, raised, white, smooth	V^+	V^-	V^-	V^+	V^-	*Enterococcus faecalis*
GIZ 12 D4	Cocci, circular, entire, convex, beige, yellow	V^+	V^+	V^+	V^+	V^-	*Macrococcus caseolyticus*
GIZ29D4 L1	R Rod, circular, entire, raised, white, smooth	V^-	V^-	V^+	V^+	V^+	Citrobacter spp
GIZ 3 D1 L1	Rod, irregular, curled, raised, opaque, smooth	V^+	V^+	V^+	V^+	V^-	*Bacillus cereus*
GIZ 3 D3 L1	Cocci, circular, entire, convex, rough, golden	V^+	V^-	V^+	V^+	V^-	*Staphylococcus aureus*
GIZ 8M	Cocci, circular, entire, convex, shiny, yellow	V^+	V^-	V^+	V^+	V^-	*Staphylococcus aureus*
GIZ 15 D1	Cocci, irregular, curled, raised, white	V^+	V^-	V^-	V^+	V^-	*Enterococcus faecalis*
GIZ 27 D4	Cocci, circular, entire, convex, rough, opaque	V^+	V^-	V^+	V^+	V^-	*Staphylococcus aureus*
GIZ 17 D2	Cocci, circular, entire, convex, beige, shiny	V^+	V^+	V^+	V^+	V^-	*Macrococcus caseolyticus*
GIZ 17 D4	Cocci, circular, entire, curled, raised, white, smooth	V^+	V^-	V^-	V^+	V^-	*Enterococcus faecalis*
GIZ 2 D4 L1	Cocci, circular, entire, convex, rough, yellow	V^+	V^-	V^+	V^+	V^-	*Staphylococcus aureus*
GIZ 9 D3	Rods, circular, entire, raised, shiny, colorless	V^-	V^-	V^+	V^-	V^+	*Escherichia coli*
GIZ30DML 1	Rods, irregular, flat, lobate, cream, smooth	V^+	V^+	V^+	V^+	V^-	*Bacillus subtilis*
GIZ 26 DM	Cocci, circular, entire, convex, rough, yellow	V^-	V^-	V^+	V^+	V^-	*Staphylococcus aureus*
B.I 19 D3	Rods, circular, flat, dry colony,	V^+	V^-	V^+	V^+	V^-	*Bacillus subtilis*

	cream, smooth						
B.I 29 D2 L1	Cocci, circular, entire, convex, rough, opaque	V^+	V-	V^+	V^+	V^-	*Staphylococcus aureus*
B.I 28 D1L1	Rods, circular, entire, raised, white, smooth	V^+	V^-	V^+	V^+	V^+	*Bacillus* spp
B.I 1 D4 L1	Rods, circular, entire, raised, white, smooth	V^+	V^+	V^+	V^+	V^-	*Bacillus* spp
B.I 3 D1	Cocci, circular, entire, convex, rough, opaque	V^+	V^-	V^+	V^+	V^-	*Staphylococcus aureus*
B.I 1 D3	Cocci, circular, entire, convex, rough, opaque	V^+	V^+	V^+	V^+	V^-	*Staphylococcus aureus*
B.I 19 D4	Cocci, regular, entire edge, flat, cream, smooth	V^+	V^-	V^-	V^-	V^-	*Enterococcus* spp
B.I 27 D2	Rods, circular, entire, raised, white, smooth	V^+	V^+	V^+	V^+	V^-	*Bacillus* spp
B.I 25 ML1	Rods, irregular undolutate , flat, opaque, smooth	V^+	V^+	V^+	V^+	V^-	*Bacillus cereus*
B.I 23 D3	Rods, circular, entire, raised, shiny, colorless	V^-	V^-	V^+	V^-	V^+	*Escherichia coli*
B.I 5 D3	Cocci, pinhead, entire, convex, yellow, smooth	V^+	V^+	V^+	V^+	V^+	*Macrococcus caseolyticus*
B.I 04 M	Cocci, circular, entire, convex, beige, shiny	V^+	V^+	V^+	V^+	V^+	*Macrococcus caseolyticus*

Positive = (V^+); Negative = (V^-); Gram stain = Gr; Oxidase test= (Ox); Catalase test= (Cat); VP = Voges proskauer test ; IN = Indole test

Table 2: Preliminary results based on morphological and biochemical characteristics

Sample identification	Morphology	Gr	Oxi	Cat	VP	IN	Presumptive bacteria
Wo 23 D3 L1	Rod, circular, entire, flat, white, dull	V^+	V^-	V^+	V^+	V^-	*Bacillus* spp
Wo 3D1	Rod, circular, entire, raised, cream, dull	V^+	V^+	V^+	V^+	V^-	*Bacillus* spp
Wo 32 D 3	Rod, circular, entire, raised, cream, dull	V^+	V^+	V^+	V^+	V^-	*Bacillus* spp
Wo 2 D2	Cocci, circular, entire, convex, rough, yellow	V^+	V^-	V^+	V^+	V^-	*Staphylococcus* spp
Wo 10 D4	Cocci, circular, entire,	V^+	V^+	V^+	V^+	V^-	Macrococcus

	convex, beige, shiny						caseolyticus
Wo 29 D3	Rods, irregular, undolutate, flat, opaque	V^+	V^-	V^+	V^+	V^-	*Bacillus cereus*
Wo 5 D4	Cocci, circular, entire, convex, beige, shiny	V^+	V^+	V^+	V^+	V^-	*Macrococcus caseolyticus*
Wo 12 D2	Rod, circular, entire, flat, cream, dull	V^+	V^-	V^+	V^+	V^-	*Bacillus* spp
Wo 8DM	Cocci, circular, entire, convex, rough, opaque	V^+	V^+	V^+	V^+	V^-	*Staphylococcus aureus*
Wo 12 D4	Cocci, circular, entire, convex, beige, shiny	V^+	V^+	V^+	V^+	V^-	*Macrococcus caseolyticus*
Wo 0 D3	Cocci, circular, entire, convex, rough, opaque	V^+	V^-	V^+	V^+	V^-	*Staphylococcus aureus*
Wo 5 D5	Rod, irregular, undolutate, flat, opaque	V^+	V^-	V^+	V^+	V^-	*Bacillus cereus*
CH 11 D2	Cocci, circular, entire, raised, smooth	V^+	V^-	V^-	V^+	V^-	*Enterococcus faecalis*
CH 5 D3	Cocci, circular, entire, convex, beige, shiny	V^+	V^+	V^+	V^+	V^-	*Macrococcus caseolyticus*
CH 4 D3 L1	Cocci, circular, entire, convex, dirty white, rough,	V^+	V^-	V^+	V^+	V^-	*Staphylococcus aureus*
CH 22 D1	Cocci, circular, entire, convex, dirty white, rough	V^+	V^-	V^+	V^+	V^-	Staphylococcus aureus
CH 25 ML1	Rod, circular, entire, flat, cream, dull	V^+	V^-	V^+	V^+	V^-	*Bacillus* spp
CH 3M	Cocci, circular, entire, convex, rough, yellow	V^+	V^-	V^+	V^+	V^-	*Staphylococcus* spp
CH 22 D2	Cocci, circular, entire, convex, beige, shiny	V^+	V^+	V^+	V^+	V^-	*Macrococcus caseolyticus*
CH 0 D4	Cocci, regular, entire, flat, cream, smooth	V^+	V^-	V^-	V^-	V^-	Enterococcus faecium
CH 20 M	Rod, circular, entire, flat, cream, dull	V^+	V^-	V^+	V^+	V^-	*Bacillus* spp
CH 20 D2	Rod, circular, entire, raised, white, smooth	V^-	V^-	V^+	V^+	V^+	Citrobacter spp

Positive = (V^+), Negative = (V^-), Gram stain = Gr, Oxidase test= (Ox), Catalase test= (Cat),

VP = Voges proskauer test

IN = Indole test

Table 3: Preliminary results based on morphological and biochemical characteristics

Sample identification	Morphology	Gr	Oxi	Cat	VP	IN	Presumptive bacteria
BHM1D3	Cocci, circular, entire, convex, beige, shiny	V^+	V^+	V+	V^+	V^-	*Micrococcus caseolyticus*
BHM23D3	Cocci, circular, entire, convex, dirty white, rough	V^+	V^-	V+	V^+	V^-	*Staphylococcus aureus*
BHM23M	Cocci, circular, entire, convex, dirty white, rough	V^+	V^-	V+	V^+	V^-	*Staphylococcus aureus*
BHM2D2	Cocci, circular, entire, convex, dirty white, rough	V^+	V^-	V+	V^+	V^-	*Staphylococcus aureus*
BHM22D3	Rod, circular, entire, flat, cream, dull	V^+	V^-	V+	V^+	V^-	*Bacillus* spp
BHM1D4	Rod, circular, entire, flat, cream, dull	V^+	V^-	V+	V^+	V^-	*Bacillus* spp
BHM6D4	Cocci, circular, entire, convex, dirty white, rough	V^+	V^-	V+	V^+	V^-	*Staphylococcus aureus*
BHM17D3	Cocci, circular, entire, convex, beige, shiny	V^+	V^+	V+	V^+	V^-	*Micrococcus caseolyticus*
BHM17DM	Cocci, circular, entire, convex, dirty white, rough	V^+	V^-	V+	V^+	V^-	*Staphylococcus aureus*
BHM29D4	Rod, circular, entire, raised, white, smooth	V^-	V^-	V+	V^+	V^+	*Enterobacter* spp
BHM19D2	Rod, irregular, undolutate, flat, opaque	V^-	V^-	V+	V^+	V^-	*Bacillus cereus*
BHM22D2	Rod, circular, entire, raised, white, smooth	V^+	V^-	V+	V^-	V^-	*Enterobacter* spp
BHM24D3	Cocci, circular, entire, convex, dirty white, rough	V^+	V^-	V+	V^+	V^-	*Staphylococcus aureus*
BHM26D4	Cocci, circular, entire, convex, dirty white, rough	V^+	V^-	V+	V^+	V^-	*Staphylococcus aureus*
BHM28D2	Rod, circular, entire, raised, white, smooth	V^-	V^-	V+	V^+	V^+	*Enterobacter* spp
BHM29D1	Rod, circular, entire, raised, white, smooth	V^+	V^-	V+	V^-	V^-	*Enterobacter* spp
BHM20D3	Rod, circular, entire, raised, white, smooth	V^+	V^-	V+	V^-	V^-	*Enterobacter* spp

BHM21D4	Cocci, circular, entire, convex, dirty white, rough	V^+	V^-	V +	V^+	V^-	*Staphylococcus aureus*
BHM19D4	Rod, irregular, undolutate, flat, opaque	V^-	V^-	V +	V^+	V^-	*Bacillus cereus*

Positive = (V^+); Negative = (V^-); Gram stain = Gr; Oxidase test= (Ox); Catalase test= (Cat);

VP = Voges proskauer test; IN = Indole test

Appendix 4: Antibiotic resistance profiles of bacterial isolates

CH20M	*Kurthia sp*	2	16mm	20mm	2mm	22mm	27mm	17mm	19mm	24mm
B.I 3D1	*Staphylococcus aureus*	1	28mm	21mm	6mm	9mm	22mm	20mm	19mm	19mm
B.I04D2	*Bacillus cereus*	1	4mm	20mm	15mm	20mm	24mm	19mm	20mm	16mm
BHM23D3	*Macrococcus caseolyticus*	3	21mm	20mm	2mm	20mm	20mm	20mm	9mm	20mm
CH22 D1	*Macrococcus caseolyticus*	2	19mm	15mm	5mm	19mm	21mm	17mm	18mm	15mm
GIZ3D3L1	*Bacillus cereus*	1	5mm	19mm	4mm	19mm	17mm	16mm	16mm	11mm
W23 D3	*Bacillus sp*	2	7mm	21mm	11mm	13mm	19mm	21mm	11mm	3mm
B.I29D2L1	*Staphylococcus aureus*	1	31mm	21mm	21mm	21mm	18mm	19mm	16mm	17mm
GIZ3D1L1	*Lysinibacillus sp*	1	4mm	19mm	3mm	11mm	28mm	24mm	21mm	18mm
W8D1	*Bacillus thurigiens*	2	2mm	18mm	15mm	19mm	20mm	19mm	17mm	3mm
BHM17DM	*Macrococcus caseolyticus*	3	2mm	15mm	3mm	18mm	21mm	21mm	21mm	20mm
W12D4	*Staphylococcus vitulinus*	2	27mm	21mm	2mm	26mm	12mm	20mm	9mm	21mm
B.I19D3	*Bacillus subtilis*	1	2mm	17mm	3mm	20mm	18mm	19mm	15mm	3mm
CH 20M	*Kurthia sp*	2	27mm	21mm	4mm	23mm	26mm	18mm	20mm	25mm
W32D3L1	*Staphylococcus aureus*	2	36mm	30mm	23mm	21mm	29mm	25mm	17mm	21mm
BHM2D2	*Staphylococcus aureus*	3	31mm	27mm	3mm	5mm	11mm	20mm	5mm	20mm
CH4D3L1	*Staphylococcus aureus*	2	6mm	17mm	7mm	4mm	23mm	25mm	20mm	10mm
GIZ15D1	*Planomicrobium glaciei*	1	3mm	17mm	19mm	3mm	21mm	27mm	7mm	3mm
BHM1D4	*Bacillus sp*	3	4mm	18mm	14mm	5mm	21mm	20mm	11mm	21mm
W17D1	*Macrococcus caseolyticus*	2	4mm	19mm	15mm	11mm	22mm	20mm	12mm	9mm
BHM 23M	*Staphylococcus aureus*	3	29mm	18mm	3mm	14mm	25mm	21mm	17mm	21mm
CH0D4	*Bacillus cereus*	2	6mm	19mm	15mm	19mm	23mm	21mm	20mm	21mm
GIZ 30DML1	*Planococcus antarcticus*	1	6mm	13mm	4mm	13mm	21mm	21mm	15mm	11mm
BHM 29D4L1	*Citrobacter sp*	3	22mm	19mm	16mm	21mm	19mm	22mm	14mm	12mm
CH3M	*Staphylococcus equorium*	2	24mm	19mm	14mm	15mm	24mm	19mm	11mm	23mm
BHM23D3	*Staphylococcus*	3	9mm	20mm	4mm	21mm	23mm	18mm	9mm	11mm

	aureus									
GIZ 16D4	*Enterococcus faecium*	1	4mm	20mm	10mm	18mm	20mm	22mm	19mm	19mm
CH11D2	*Enterococcus faecalis*	2	4mm	18mm	17mm	16mm	17mm	10mm	18mm	5mm

5.2 Resistant, susceptible and intermediate resistance patterns of isolated bacteria

S-ID	Microorganisms	Stree t	AMP10	CN10	TE30	S3 300	S 300	CIP5	C30	E5
CH20M	*Kurthia sp*	2	R	S	R	S	S	I	S	S
B.I 3D1	*Staphylococcus aureus*	1	I	S	R	R	S	I	S	I
B.I04D2	*Bacillus cereus*	1	R	S	I	S	S	I	S	I
BHM23D3	*Macrococcus caseolyticus*	3	R	S	R	S	S	I	R	I
CH22 D1	*Macrococcus caseolyticus*	2	R	S	R	S	S	I	S	I
GIZ3D3L1	*Bacillus cereus*	1	R	S	R	S	S	I	I	R
W23 D3	*Bacillus* sp	2	R	S	R	I	S	S	R	R
B.I29D2L1	*Staphylococcus aureus*	1	S	S	S	S	S	I	I	I
GIZ3D1L1	*Lysinibacillus sp*	1	R	S	R	R	S	S	S	I
W8D1	*Bacillus thurigiens*	2	R	S	I	S	S	I	I	R
BHM17DM	*Macrococcus caseolyticus*	3	R	S	R	S	S	S	S	I
W12D4	*Staphylococcus vitulinus*	2	R	S	R	S	I	I	R	I
B.I19D3	*Bacillus subtilis*	1	R	S	R	S	S	I	I	R
CH 20M	*Kurthia sp*	2	R	S	R	S	S	I	S	S
W32D3L1	*Staphylococcus aureus*	2	S	S	S	S	S	S	I	I
BHM2D2	*Staphylococcus aureus*	3	S	S	R	R	R	I	R	I
CH4D3L1	*Staphylococcus aureus*	2	R	S	R	R	S	S	S	R
GIZ15D1	*Planomicrobium glaciei*	1	R	S	S	R	S	S	R	R
BHM1D4	*Bacillus* sp	3	R	S	R	R	S	I	R	I
W17D1	*Macrococcus caseolyticus*	2	R	S	I	R	S	I	R	R
BHM 23M	*Staphylococcus aureus*	3	S	S	R	I	S	S	I	I
CH0D4	*Bacillus cereus*	2	R	S	I	S	S	S	S	I
GIZ 30DML1	*Planococcus antarcticus*	1	R	I	R	I	S	S	I	R

BHM 29D4L1	*Citrobacter sp*	3	R	S	I	S	S	S	I	R
CH3M	*Staphylococcus equorium*	2	R	S	R	I	I	I	R	S
BHM23D3	*Staphylococcus aureus*	3	R	S	R	S	S	I	R	R
GIZ 16D4	*Enterococcus faecium*	1	R	S	R	S	S	S	S	I
CH11D2	*Enterococcus faecalis*	2	R	S	I	I	S	R	S	R

Key words: Area 1: MTN-taxi rank; Area 2: Bree Street (corner Plein Street); and Area 3: Hancock Street (corner Claim Street)