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

By

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

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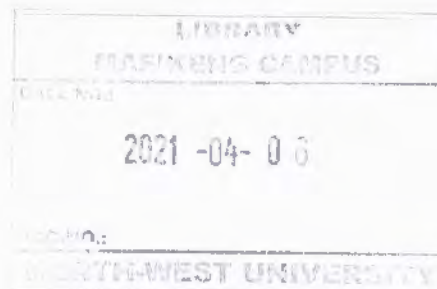
Co-supervisor: Doctor Lubanza Ngoma

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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.....day of2017



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
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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, an observation study was performed before sampling, in order to understand the general practices and knowledge of street-vended meats in terms in food hygiene and safety. Data obtained from the observational study was analysed using the Statistical Package for the Social Sciences (SPSS) version 20.0. The analysis revealed that bad hygiene practices occurred more frequently than good hygiene practices. Exposure of food to dust and flies in the three streets studied provided the following results: 93.63% around the MTN-taxi rank; 77.42% around Bree Street (corner Claim Street); and 44.67% at Hancock Street (corner Claim Street). Washing of hands was poorly observed in the study. The frequency of the presence of stagnant water ($P>0.05$), exposure of food to flies and dust ($P>0.05$) and of the usage of polythene bags for serving food ($P<0.05$) was significantly different across the three streets sampled. The results revealed poor hygiene practices of street-vended foods. 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.

A total of 115 samples from street-vended foods such as 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 as well as molecular methods based on 16S rRNA (DNA extraction, PCR amplification, and sequencing). All the samples analysed based on biochemical test in this study were found to have been contaminated with different bacterial pathogens such as *Staphylococcus* spp, *Bacillus cereus* and *Bacillus* spp, an indication of the need for strict implementation and follow-up of food hygiene practices in order to reduce the risk of contamination of street foods, which emphasises a need to reduce the risk of transmission of pathogens on the consumption of street food. The total bacterial count obtained for the samples collected around MTN taxi-rank ranged from 9.6×10^{-2} to 1.9×10^{-2} cfu/ml, coliform count ranged from 2.9×10^{-2} to 1.0×10^{-2} CFU/ml in chicken gizzard and from 9.9×10^{-2} to 3×10^{-2} cfu/ml, and coliform count ranged from 2.9×10^{-2} cfu/ ml to $1.0 \times$

10^{-2} cfu/ml in beef intestine samples. For samples collected around Bree Street (corner Claim Street), the total bacterial count ranged from 9.1×10^{-2} cfu/ ml to 1.4×10^{-2} cfu/ ml while the total coliform count ranged from 2.4×10^{-2} cfu/ ml to 1.1×10^{-2} cfu/ ml in wors meat samples. The total bacterial count in chicken meat ranged from 9.7×10^{-2} to 1.1×10^{-2} CFU/ml while the total coliform count ranged from 3.0×10^{-2} to 1.0×10^{-2} CFU/ml.

For samples collected at Hancock Street (corner Claim Street), the total bacterial count ranged from 9.7×10^{-2} to 2.9×10^{-2} CFU/ml, while the total coliform count ranged from 2.9×10^{-2} to 1.1×10^{-2} CFU/ml in beef head meat samples. The total bacterial count of all the samples stood as 10^{-2} , which is classified as acceptable for ready-to-eat meat and meat products; the total coliform count also stood at 10^{-2} . 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 food samples with different bacteria such as *Kurthia* sp (7.143%), *Staphylococcus aureus* (25.0%), *Bacillus cereus* (10.71%), *Macrococcus caseolyticus* (14.29%), *Bacillus* sp (7.143%), *Bacillus thurigiens* (3.571%), *Staphylococcus vitulinus* (3.571%), *Bacillus subtilis* (3.571%), *Planomicrobium glaciei* (3.571%), *Planococcus antarcticus* (3.571%), *Citrobacter* sp (3.571%), *Staphylococcus equorum* (3.571%), *Enterococcus faecium* (3.571%) and *Enterococcus faecalis* (3.571%). This could be a potential public health danger. Some of the isolated bacteria are well-known to be the causative agent of food-borne diseases. There is, therefore, a need to improve the sanitation and hygienic condition conditions of street foods and to evaluate public health implication. The presence of *Staphylococcus aureus* in food is 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.

The antibiotic tests performed on the selected isolates revealed varying degrees of resistance to different antibiotics, and most of the bacterial isolated were found to be resistant to Ampicillin, Tetracycline and Chloramphenicol. The multidrug resistance observed among the bacteria in this study might be due to contamination from meat, or from the environment. These types of foods contaminated with multidrug resistance microorganisms could be potential vehicles for the transmission of foodborne infections among consumers and could cause serious public health problems.

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 multiple drug resistance. The surveillance of antimicrobial resistance needs to be strengthened on food pathogens.

LIST OF ABBREVIATIONS AND ACRONYMS

API	Analytic Profile Index
B. Cereus	Bacillus Cereus
B. Subtilis	Bacillus Subtilis
CFU	Colony forming units
CDC	Centres for Diseases Control and Prevention
CBD	Central Business District
CLIS	Clinical Laboratory Institute Standards
DNA	Deoxyribonucleic Acid
<i>E. Faecalis</i>	Enterococcus faecalis
<i>E. Faecium</i>	Enterococcus faecium
ETA	Exofoliative toxin
<i>et al.</i>	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 UNITS

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

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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 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 the street for 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 food contaminated by 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 economic reasons such as poverty, lack of adequate health care facilities, and the dearth of data regarding foodborne diseases (Organization, 2007). The safety of a 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 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), and 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, with 1.8 million people dying from diarrheal diseases globally (Jahan, 2012). More than 200 known diseases are transmitted through food by a variety of agents such as bacteria, fungi, viruses and parasites (Oliver *et al.*, 2005a). According to public health and food safety experts, each year, millions of illnesses in the United States and throughout the World can be traced to foodborne pathogens. The Centres for Diseases Control and Prevention (CDC, 2003 2004) estimates 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 (Oliver *et al.*, 2005a). There is also growing confirmation that persons of smaller racial and ethnic groups agonise from rates of certain foodborne infections (Chang *et al.*, 2009; Quinlan, 2013; Shiferaw *et al.*, 2004).

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 considered 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, 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 food is a common part of city lifestyle due to high joblessness and restricted work opportunities (Bryan *et al.*, 1988; Von Holy & Makhoane, 2006). Eating of street food has grown over the years due to fast population growth, unemployment, poverty and availability of relatively low cost foods (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). Street foods offer a source of inexpensive nutrients to the majority of people mainly the low-income group in developing countries (Muzaffar *et al.*, 2009). Street foods are known to be popular due to their availability, low cost, variety and nutritional value (Rane, 2011). Street foods are supposed to be a main causal factor. In most instances, foods are prepared in unhygienic conditions by people not qualified in proper food handling techniques (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 at 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: *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 perfringens* and *Vibrio cholera* (CHO *et al.*, 2011; Elobeid *et al.*, 2014; Mankee *et al.*, 2005). In addition, exposure to the above organisms could contribute to the increased occurrence of multi-drug resistance in organisms posing a real risk to the public health of the general population (Guyen *et al.*, 2010; Harakeh *et al.*, 2005). Antimicrobial resistance is presently the greatest test 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). Food contamination with antimicrobial resistance bacteria can be a major threat to public health, as such determinants can be transferred to other bacteria of human clinical significance. 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. There is no specific and scientific data that can be used to develop the control of informal street foods to ensure their safety. It is thus, also possible that a lot of informal street vendors are frequently uninformed of good hygienic practices. Informal meat food preparation is frequently unhygienic and meat is easily contaminated.

In order to do so, 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 study addresses this need by investigating the bacteriological quality, food handling and hygienic practices of street-vended meat around Johannesburg CBD through observation. In addition, a molecular characterisation of foodborne pathogens in ready-to-eat meat sold in streets was carried out.

1.3. Research questions

Several vendors sell ready-to-eat meat in the streets of Johannesburg Central Business District (CBD) without proper hygienic measures taken. In addition, these meats are served on plates which are not properly cleaned. Most vendors also sell ready-to-eat in areas exposed to flies and dust. The questions raised in this study were: 1) what is the microbiological quality of the meats sold? and 2) does the behaviour of sellers increase the risk of the occurrence of foodborne diseases in Johannesburg?

1.4 Hypothesis

Many people consume ready-to-eat meat cooked and sold in streets around Johannesburg CBD on a daily basis. 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. In addition, population movement seen in Johannesburg might lead to the introduction of new strains than the one previously identified or muted; variants or new pathogens which can lead to a risk of foodborne diseases in the future. There is a need, therefore, to monitor and study the pathogenicity of such bacteria, antibiotic resistance and to identify new ones.

1.5 Problem statement

In many big cities, foodborne infections are a major concern, thus causing many deaths. Since Johannesburg is a metropolitan city with more than 3 million inhabitants, there is a need to investigate cases of foodborne diseases in the city. In addition, there is a strong movement of people on a daily basis in the CBD. These people eat meat sold on the street. The consumption of street-vended meats is done without taking into consideration hygienic conditions. There is a risk of contamination through common food-borne bacteria and diseases. To date, there is no data on the 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 Significant of study

This study will provide baseline information on ready-to-eat meats sold in streets around Johannesburg, informal markets and predominant isolates of bacterial serotypes for their toxicity and antimicrobial resistance profiles. It will also provide information on different bacterial isolates in 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 important number of *Escherichia coli* spp. (Cardinale *et al.*, 2005) point out those street foods are dangerous and could raise health risks. They also examined samples of foods from 148 street-restaurants in Dakar and found the presence of harmful bacteria in the food samples. The source 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 perfringens*, *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 reports, however, do not stratify the isolates into those obtained from individual patients and those mixed up in epidemics (GERMS-SA, 2008).

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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. 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).

Africa recorded the highest number of epidemics between the three regions (Africa, Europe and America). These areas are unique in many ways, such as seasonality, the size of eruptions and number of sicknesses and deaths associated with epidemics. The high occurrence of diarrheal amongst newborns and young children is an indication of the poor food hygiene situation in Africa (DeWaal & Robert, 2005). Though 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 consequent economic reduction (Duff *et al.*, 2003). In the United States, it has been reported that seven pathogens found in animal products such as *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 and lifestyle

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 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 as the food is cheap and easily accessible (Latham, 1965; Riet, 2002). Street foods play a vital role for consumers, particularly in middle and low income of 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 found 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 is the conservation of the local food culture (Proietti *et al.*, 2014; Proietti *et al.*, 2013).

2.3. Socio-economic value of street food and food security

The significant socioeconomic effect 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 both urbanisation and a high rate of daily commuting from rural to urban areas (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 < from farm to fork > 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 represents a good living for families involved in food vending, since it is considered part of the informal sector (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 heads of 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; Proietti *et al.*, 2014).

Street foods play a significant socio-economic role, in terms of work and income, for those involved in their preparation and/or selling, empowerment of the local economy and the protection of local cultures (Ohiokpehai, 2003). 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). The substantial socioeconomic effect of street food with respect to income and as a possible source of work, predominantly for women, street food vending is also thought to make food available at reasonable prices to low income groups. 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 (Burnens *et al.*, 1992). They are also typical Gram-negative pathogens which cause gastroenteritis (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 is at risk of foodborne illnesses (Aluko *et al.*, 2014). Many street-vended foods, poor food handling procedures, 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, the 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 (WHO, 2015). To ensure the protection of people against the hazard of unsafe food (Kumar Singh *et al.* 2016). 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). Street-vended foods are usually prepared in small corners, from where the food is sold, 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 (Manguiat & Fang, 2013).

The safety of street food in developing countries is still a major concern (Muinde & Kuria, 2005). It is widely acknowledged that street vendors in these countries often operate under conditions which are unacceptable for the preparation and selling of food (Aluko *et al.*, 2014; da Silva *et al.*, 2014; Muyanja *et al.*, 2011). There are some serious concerns regarding the safety of street food (Muinde & Kuria, 2005). These concerns are due to the fact 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), demographic characteristics such age and gender do not appear to play a role in 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). These authors also reported a positive correlation between the level of education and knowledge on food

safety (Soares *et al.*, 2012). Additionally, while some studies have found a significant correlation between the level of knowledge on food safety and attitude on food safety (Cuprasitirut *et al.*, 2011), others 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 (Cuprasitirut *et al.*, 2011), Shijiazhuangcity, 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). Furthermore, most food vendors ignore good food handling practices, exposing foods to dangerous conditions such as cross-contamination, unsafe storage and poor time temperature conditions (Hanashiro *et al.*, 2005). 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 (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).

In Nigeria, not much work has been done on the occurrence of pathogenic microorganisms in cooked food, taking into consideration the method of preparation and storage practices. However, the conditions of preparation and selling are sources of contamination, especially by germs involved in poisoning food (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

The majority of outbreaks of diseases related to street food were linked to the negligence of food handlers (WHO, 2002). About 15 cases of the outbreaks of foodborne diseases monitored 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

Vendors usually congregate in overcrowded areas, where there are high numbers of potential customers. Such areas usually provide limited access to basic sanitary facilities, such as running water, garbage disposal and clean toilets. 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 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, running water is not accessible, there are no toilet facilities, and vendor sell beside garbage

(which is a harbour for pests and rodents). Such contagion may result from communication 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

Most food vendors do not observe good food handling practices, exposing foods to dangerous conditions such as 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). 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 specify 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). The microbiological contamination of street-vended food is considered to be a significant contributor to foodborne diseases, and the consumption of certain street-vended foods is, somehow, at risk of contracting food-borne diseases (Organization, 2011a). 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).

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). These reports clearly indicate that people who patronise vendors of ready-to-eat street foods might be putting their health at risk (Manguiat & Fang, 2013).

The numerous advantages offered by street foods to food security should, nevertheless, 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).

Microbiologically contaminated food and drinking water are considered as important vehicles for the transmission of foodborne diseases throughout the world (Organization, 2011b). The microbiological contamination of street-vended food is considered to be a major contributor to foodborne infections, and consumers of certain street-vended foods are in one way or another, at risk of contracting foodborne diseases (Organization, 2011a).

Microbiological hazards are considered a greater challenge to food safety because harmful bacteria can either proliferate in the food or in the human body through consumption (Tent, 1999). The presence of specific microorganisms such as *E.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, in Nigeria, a number of foods, meats inclusive, have been reported to have a high incidence of bacteria (Clarence *et al.*, 2009).

Among the microorganisms found in street-vended food, *Salmonella*, *Staphylococcus aureus*, *Bacillus cereus* and *Clostridium perfringens* 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 perfringens* 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 perfringens* 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 and a 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 by microorganisms such as genus *Escherichia coli*, *Staphylococcus*, *Salmonella* and *Shigella* were identified from potato chips collected from Azezo and kebele (Bizuye *et al.*, 2014). In Ghana, a microbiological study conducted on the quality of food sold on and around Koforidua polytechnic campus, revealed *Escherichia coli*, *Salmonella* and *Staphylococcus*.

The level of hygiene consciousness of food vendors was found to be low (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,

Klebsiella 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* spp. was isolated from the stools 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 adopted on various continents based on feasibility to ensure the safety of food sold and consumed on streets (Sezgin & Şanlıer, 2016).

Street foods might upsurge 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).



It is known that poor personal and environmental sanitations contribute expressively to food contamination, thus resulting in foodborne illnesses (Bryan *et al.*, 1988). 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 due to the consumption of contaminated or toxic 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 100000 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).

Foodborne diseases (FB) constitute a widespread and growing public health problem, both in developing and developed countries (Bhattacharjya & Reang, 2014). The outbreak of a foodborne disease can occur due to ingesting food items contaminated with microorganisms and chemicals (Webb & Morancie, 2015). Each year, up to one-third of the population in

developed countries is affected by foodborne illnesses (Isara *et al.*, 2010). 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). 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). In some cases, the vending site lacks access to basic sanitary facilities such as clean running water, garbage disposal, and clean toilets, making it difficult for vendors to apply standard sanitary practices (Kubheka *et al.*, 2001).

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 illnesses result from the consumption of food containing pathogens such as bacteria, viruses, parasites or food contaminated by poisonous chemicals or bio toxins. Although the majority of cases of 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 programmes 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 and 155000 deaths 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* species are the second largest cause of foodborne illnesses after *Campylobacter* species (Mead *et al.*, 1999). *Salmonella* species 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. The indiscriminate use of antibiotics in animal feeds as growth promoters and therapeutic agents is a further threat to human and animal health (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 Mahmoud, 2011; Silva *et al.*, 2014).

2.9.2 Incidence of *E. coli* foodborne pathogens

Escherichia coli are members of the genus *Escherichia* within the family for 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 World Health Organisation's epidemiological updates, indicate that health

officials and the World Health Organisation (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 *Escherichia 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).

E. coli are an important indicator of faecal contamination in food and are commonly used to assess the hygienic quality of food products. Enterotoxigenic *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).

E. 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 *Escherichia 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).

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. Listeriosis, 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). The mortality rate of listeriosis is 25% worldwide (de Noordhout *et al.*, 2014) and with a hospitalisation rate of >95% (Scallan *et al.*, 2011); it ranks as the third most serious foodborne disease (Schlech & Acheson, 2000).

Susceptible populations (pregnant women and their unborn children, new-born babies, immunocompromised individuals and the elderly) with very high lethality rates, ranging from 20% to 30%, have been reported (Ayaz & Erol, 2010). The prevalence of food-borne diseases has increased in the last decades. *Listeria*, *Campylobacter*, and *Salmonella* species and *E. coli* O157: H7 have been isolated from the majority of outbreaks of food-borne diseases (Chemburu *et al.*, 2005). *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). *Listeria 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). 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



Most staphylococcal species are harmless and reside normally on the skin and mucous membranes of humans and other organisms (Guran & Kahya, 2015; Jørgensen *et al.*, 2005; Osman *et al.*, 2016). There are also well-known staphylococcal species responsible for a wide variety of diseases in animals and humans (Osman *et al.*, 2016; Vuong & Otto, 2002), their association with nosocomial infections in neonatal intensive care units and food poisoning in spite of the fact that they are also not classical food poisoning bacteria (Becker *et al.*, 2014; Chajęcka-Wierzchowska *et al.*, 2015; Cortes *et al.*, 2013; Tong *et al.*, 2015).

Staphylococcal toxins include toxic shock syndrome toxin 1 (TSST-1), exfoliative toxins (ETA to ETD), staphylococcal enterotoxins (SEs; SEA to SEE, SEG to SEI, SER) with demonstrated emetic activity, and staphylococcal enterotoxin-like (SEI) proteins. All the toxins listed above possess super antigenic activity and were designated as staphylococcal super antigens (SAGs) (Fowoyo & Ogunbanwo, 2016; Vasconcelos *et al.*, 2011). Staphylococcal food poisoning is an intoxication that results from the consumption of foods containing sufficient amounts of one (or more) of the above-mentioned toxins (Dinges *et al.*, 2000; Le Loir *et al.*, 2003). Enterotoxigenic CNS strains, including organisms responsible for outbreaks of food poisoning, have also been described by several authors (Batista *et al.*, 2013; Vasconcelos *et al.*, 2011). Additionally, it was reported that CNS may be a possible

reservoir of enterotoxin genes typically identified in *S. aureus* (Lawrynowicz-Paciorek *et al.*, 2007; Vasconcelos & de Souza, 2010).

S. aureus is a commensal and opportunistic pathogen that 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 *Staphylococcus aureus* enterotoxins every year, resulting in 1750 hospital admissions annually (Wieneke *et al.*, 1993).

2.9.6 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 World Health Organisation (WHO) has taken initiatives for estimation of the global burden of foodborne illnesses (Kuchenmüller *et al.*, 2009). The World Health Organisation and the US Centre for Diseases Control and Prevention (CDC) report, every year, a large number of people affected by foodborne illnesses (Busani *et al.*, 2005). Globally, an estimated 2 million people died 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. High risk groups for foodborne diseases include infants, young children, the elderly and immunocompromised persons (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 \$23billion. 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 Millennium Development Goals (MDG) (Jahan, 2012). There is an impact of foodborne illnesses on four out of the eight MDGs, these include MDG1 (eradication of extreme poverty); MDG3 (reduction in child mortality); MDG5 (improvement of maternal health); MDG6 (combating HIV/AIDS and other illnesses) (Jahan, 2012).

2.9 THE USE OF ANTIMICROBIAL AGENTS

Antimicrobial agents are widely used in human and veterinary medicine to control bacterial infections. Their usage for livestock can be for therapeutic, prophylactic, metaphylactic or growth promotion purposes. 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* species 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 cephalosporins 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*

Staphylococcus aureus is a gram positive coccus commonly present on the skin and mucous membranes of animals and humans (Tong *et al.*, 2015). 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 acquired infections (Boucher & Corey, 2008; Vandenesch *et al.*, 2003). *S. aureus* is known to cause a number of pathological conditions in humans and animals ranging from mild skin infections, bacteraemia, systemic diseases, and osteomyelitis to the more complicated toxic shock syndrome and staphylococcal food poisoning (Abdulgader *et al.*, 2014; Abdulgader *et al.*, 2015; Tong *et al.*, 2015). The emergence of MAR *S. aureus* 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*, whereas less attention is paid to other species (Gao *et al.*, 2012). Antibiotic resistant strains other than *S. aureus* were also found in food (Guran & Kahya, 2015) and genes encoding microbial resistance to Tetracycline, Erythromycin and β -lactams have been detected in CNS 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*, 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. 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 hands washing activities. Such information is valuable for improving hands washing practices, improve public health and reduce food contamination. It also allows for the identification of areas in need of improvement and subsequent targeted interventions in such areas. In this study, particular focus was on the attitude and practices of vendors on the main streets of Johannesburg (CBD) in order to assess possible public health risks for consumers.

Data collection for this was done through observations. Aspects taken into consideration were twenty critical aspects related to the general hygiene attitude and practices of street-vendors selling meat and meat products as well food hygiene and safety. These 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; Organization, 2002). Among aspects taken into consideration during this study were: the hygiene of vending sites; general hygiene practices of street-vendors; food hygiene; and hygiene of serving utensils.

3.2 OBJECTIVE OF THE STUDY

The purpose of this chapter is to provide an assessment of the general practices and attitudes of street-vendors selling ready-to-eat meat towards food safety before sampling for microbial analysis.

3.3 STUDY DESIGN AND METHODOLOGY

3.3.1 Study area

Observational data was collected from three different areas around Johannesburg Central Business District (CBD) as follows:

- MTN-taxi rank (considered as the main research site);
- Bree Street, corner Plein Street, considered as the second main site; and
- Hancock Street, corner Claim, considered the third main research site.

3.3.2 Observational study

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 and attitudes applied by vendors of street-vended foods towards the food safety of street-vended ready-to-eat meats sold in streets around Johannesburg Central Business District (CBD).

One hundred and fifteen observations were done in order to determine the general hygiene practices among street-vended meats around Johannesburg Central Business District. Recordings were done of all observations made on vending sites, food safety practices, and exposure of food to flies, dust, washing of hands and stagnant water at vending sites. A pre-structure check list form was used for the study (see appendix 1).

a) Hygiene of 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

Observations were performed to understand the general knowledge of vendors regarding the hygiene practices of street-vended meats at the 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) Food hygiene

In terms of food hygiene, the following were observed:

- 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) Hygiene of serving utensils

The observation focused on:

- 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 through the Statistical Package for the Social Sciences (SPSS) version 20.0. Bar charts, pie charts, and histograms were used to summarise the percentage frequencies of the following variables: hygiene practices at each street; and types of meat sold per street. Cochran's Q test was used in determining the difference between the three streets in terms of hygiene practices. Cochran's test was preferred in this study 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

The objective of this section was to observe and analyse the hygienic practices and attitudes of street-vendors selling ready-to-eat meat on the different streets of Johannesburg CBD. Results obtained and presented in this section 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 in Bree Street, no salubrious was observed. Exposure of foods to dust and flies was also observed in this study at different levels with 90.63% observations made at MTN-taxi rank (Figure 3.1), while at Bree Street, it was 77.42% (Figure 3.2), and 68.89% at Hancock Street (Figure 3.3). Handling money while serving food by vendors was also observed at 94.4 % at MTN taxi-rank (Figure 3.1), 92.31% at Bree Street (Figure 3.2) and 87.5% at Hancock Street (Figure 3.3). The presence of food debris on vendor's hands was also observed in this study at varying rates with 37.5% at MTN taxi-rank (Figure 3.1), 7.14% at Bree Street (Figure 3.2), and 41.67% at Hancock Street (Figure 3.1). The presence of stagnant water around vending sites was observed with 21.88% MTN taxi-rank (Figure 3.1) and 55.56% in Hancock Street (Figure 3.3). The presence of stagnant water was not observed in Bree Street. It was also observed that washing of hands after handling waste food was very low with 3.12% at MTN taxi-rank (Figure 3.1), 9.67% in Bree Street (Figure 3.2) and 2.22% at Hancock Street (Figure 3.3). The washing of hands with only water was observed with 3.44% at MTN taxi-rank (Figure 3.1), 12.9% in Bree Street (Figure 3.2) and 11.11% at Hancock Street (Figure 3.3).

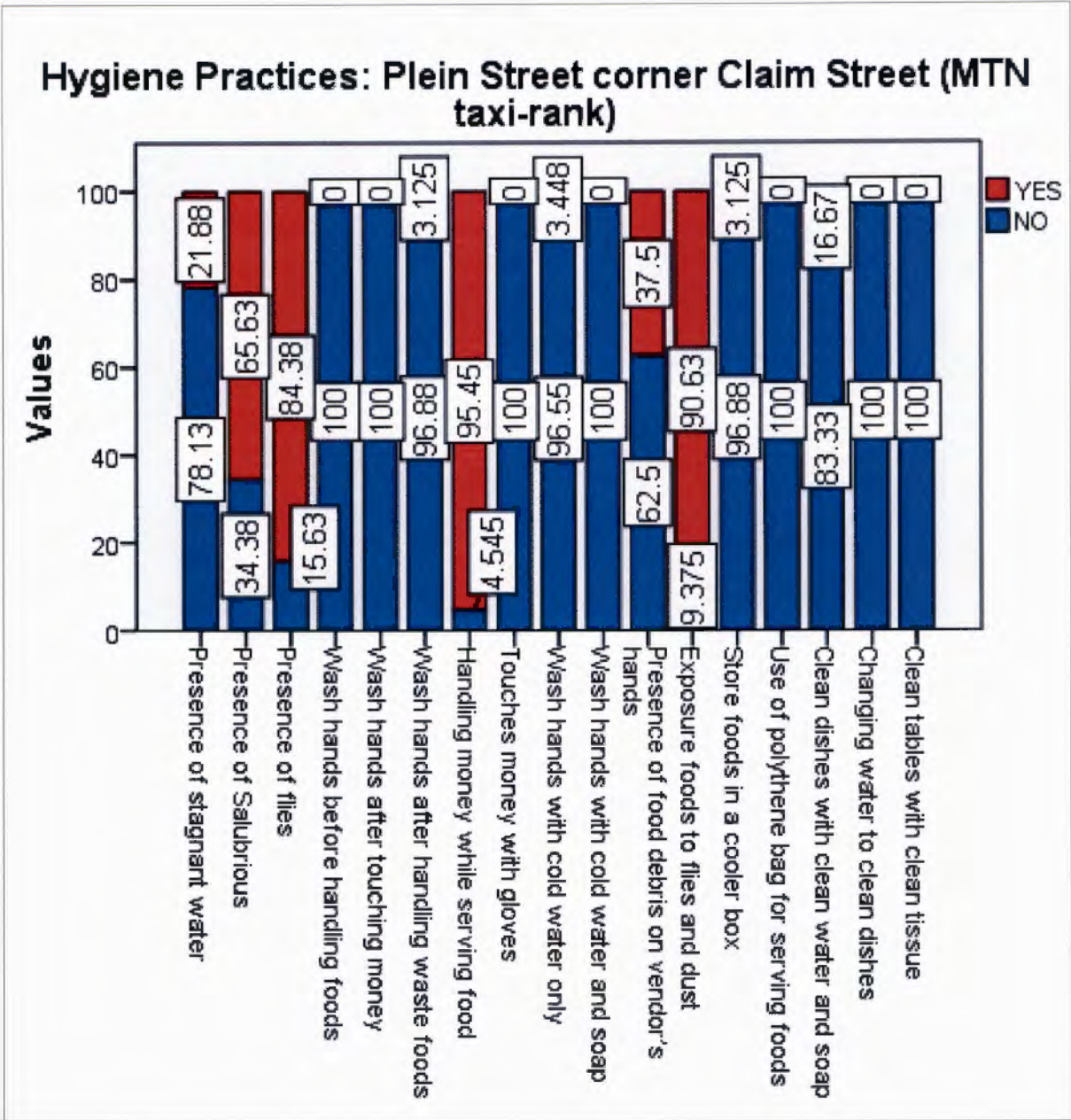


Figure 3.1: Summary of hygiene practices around MTN taxi-rank

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.

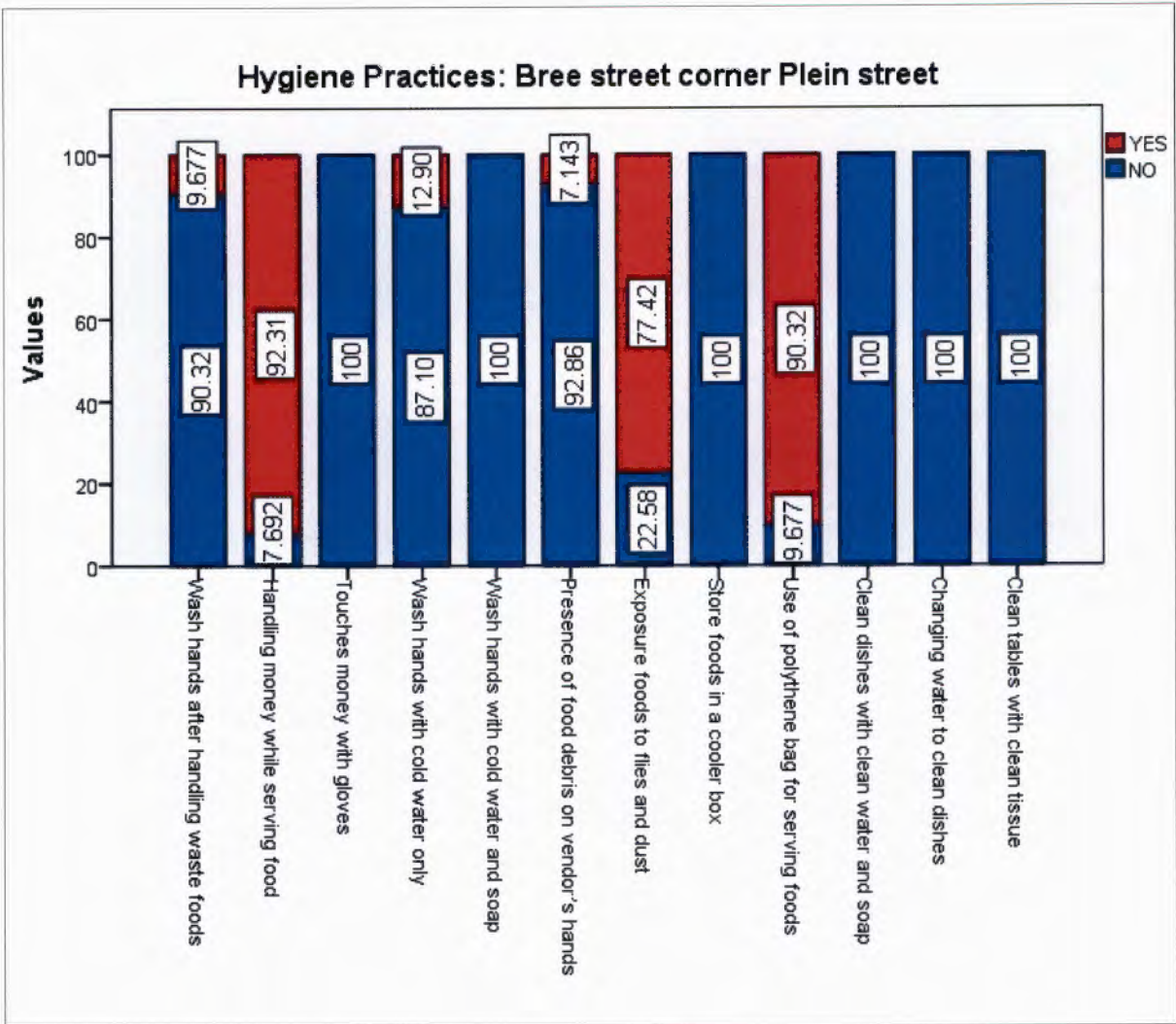


Figure 3.2: Summary of hygiene practices around Bree Street (corner Plein Street)

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 foods stood at 90.32%.

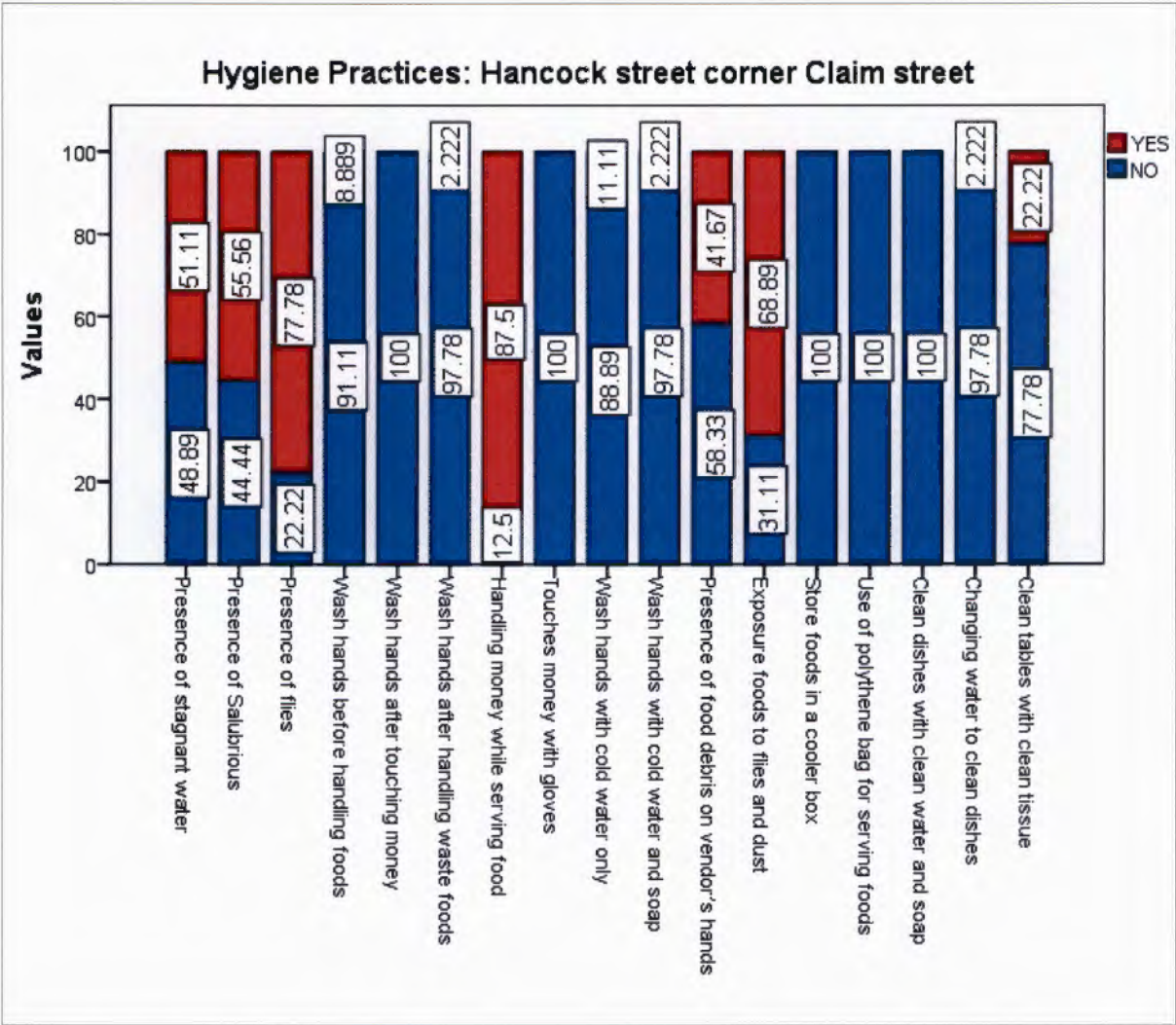


Figure 3.3: Summary of hygiene practices around Hancock Street (corner Claim Street)

Results presented in Figure 3.3 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.

Presence of stagnant water	Test statistics	
	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 and attitudes related to food safety and hygiene of street vendors around Johannesburg Central Business District (CBD) before sampling. It was observed in this study that presence of stagnant water represented 51.11% in at Hancock Street (Figure 3.3), 21.88% at MTN taxi-rank (Figure 3.1), while in Bree Street; the presence of stagnant water was not observed (Figure 3.2). The presence of salubrious was observed representing 65.63% at MTN taxi-rank (Figure 3.1), 55.56% at Hancock Street (Figure 3.3), while the presence of salubrious was not observed in Bree Street. The presence of flies represented 77.78% at Hancock Street (Figure 3.3) and 84.38% at MTN taxi-rank (Figure 3.1). This is an indication of the poor environmental and sanitation condition of vending places. It was also observed that food vending premises needed hygienic and sanitation improvement. These results differ to the findings of similar observations reported by Mensah *et al.* (2002) and Muinde *et al.* (2005) who conducted similar studies on street foods in Nairobi, Kenya, and Accra, Ghana. FAO (1995) 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. However, results obtained in this study are in agreement with the findings of other studies (Bryan *et al.*, 1997; Hanashiro *et al.*, 2005; Lues *et al.*, 2006; Mosupye & von Holy, 1999; Muinde & Kuria, 2005; Umoh & Odoaba, 1999). These researchers concluded that the accumulation of garbage which provides harborage for insects and pest around vending sites may expose food to contamination.

It was also observed that washing of hands before handling foods represented 8.88% at Hancock Street (Figure 3.3), while at Bree Street and MTN taxi-rank, washing of hands before handling foods was not observed (Figure 3.2 and 3.1). Handling money while serving foods stood at 95.45% at MTN taxi-rank (Figure 3.1), 87.5% at Hancock Street (Figure 3.3) and 92.31% at Bree Street (Figure 3.3). Washing of hands after handling waste food represented 2.22% at Bree Street (Figure 3.2) and 2.22% at MTN taxi rank Figure 3.1).

Washing of hands with cold water represented only 3.44% at MTN taxi-rank (Figure 3.1), 12.9% at Bree Street and 11.11% at Hancock Street (Figures 3.2 and 3.3). The presence of food debris on the hands of vendors stood at 37.5% at MTN taxi-rank (Figure 3.1), 7.14% at Bree Street (Figure 3.2), and 41.67% at Hancock Street (Figure 3.3). The results obtained in this study concur with the findings of Behling *et al.* (2010) who found that contact of the hands with foods represents a potentially important mechanism through which pathogens

may enter the food supply. These results also correlate with 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.

The results of this study further correlate with the findings of Ferro *et al.* (1993) and (Muinde & Kuria, 2005) who found 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, poor hygienic behaviour while serving or preparing food by street vendors can also be explained by the poor hygiene education of street vendors. 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.

It was observed that exposure of foods to flies and dust stood at 90.63% at MTN taxi-rank (Figure 3.1), 77.42% at Bree Street (Figure 3.2) and 68.89% at Hancock Street (Figure 3.3). These results are in line 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; Cuprasitrit *et al.*, 2011). These results also correlate with the findings of several 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 observed in the three Streets considered for the study that ready-to-eat meat is not stored in a cooler box but in a big casserole already prepared and ready to be sold. This situation can increase the risks of multiplication of microorganisms faster 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).

From the results obtained in this study, it was observed 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 % of them use a clean cloth to clean tables

after serving customers (Figure 3.3). These findings correlate with those of 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. In the absence of water and soap to clean utensils, street vendors in these areas apply unhygienic methods to clean utensils. A cleaning cloth is several times to clean utensils without water and soap. This process is done repeatedly until the cloth becomes completely dirty (full of oil). Another unhygienic method used by vendors is the fact that ready-to-eat meat is cut and served on the same table used by consumers to eat. In addition, waste food is handled with the same knife used to cut meat, and the same knife used from time to time to dish out the meat from the casserole, thus leading to cross-contamination. These results correlate with the findings of Bryan *et al.* (1988) who reported that major contamination of street food that occurs at vending sites are due to cross contamination, chopping, cutting, cooking and serving.

It was also discovered that 3.12% of vendors store foods in cooler boxes at MTN taxi rank (Figure 3.1), while 0% of vendors do at Bree and Hancock Streets (Figures 3.2 and 3.3). Food is preserved plastic buckets and casseroles. In some instances, the food was already cooked and ready to be sold. This result is in line with the findings of (Ekanem, 1998; Lues *et al.*, 2006; Martínez-Tomé *et al.*, 2000; Unnevehr, 2000; Walker *et al.*, 2003). In their study, they 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.

90.32% of vendors use polythene bags when serving customers at Bree Street (Figure 3.2). This result is in agreement with the finding of Mensah *et al.* (1997) who found that scoping food into polythene bags was one of the causes of diarrhoea from street food vending. Polythene bags are usually contaminated by food handlers and pathogens may invade the interior of polythene bags during packaging and poor handling practices of vendors (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. Other hygiene practices in Table 1 did not differ significantly across areas.



3.7 CONCLUSION

The observation study conducted before the sample collection revealed that in general, hygienic practices are poorly applied by majority of street vendors as well as poor environmental conditions around vending sites. Such poor behaviour may mostly be due to poor hygiene education and knowledge among street vendors. These findings prove that street vendors around Johannesburg CBD constitute a significant potential threat to human health. There is an imperative need for government and public health sectors 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 ANALYSIS

4.1 INTRODUCTION

Consumption of unclean food can cause gastrointestinal diseases. The number of bacterial population in food indicates the quality of the food. Excess bacteria may indicate the contamination of food poisoning bacteria, which can cause diarrhea, nausea, vomiting, fever, headache or even death.

4.2 Aim of the study

The main aim of the study was to use qualitative and quantitative approaches to inquiry in order to determine the molecular characterisation of food-borne pathogens in food sold in streets around Johannesburg CBD

4.3 Objectives of the study

The objectives specific of this study were to:

- Collect ready-to-eat meat sold in street of Johannesburg CBD and analyse for bacterial contamination;
- Evaluate the microbiology quality of ready-to-eat meats sold in streets around Johannesburg Central Business District (CBD);
- Identify bacterial isolates using preliminary biochemical tests (Catalase, Oxidase, voges proskauer, indole, and APIs) and to confirm isolates using PCR and sequencing;
- Classify and genotype all strains obtained; and
- Evaluate possible health risks encountered by consumers.

4.4 Significant of the study

This study will provide baseline information on ready-to-eat meats sold in streets around Johannesburg CBD and predominant serotypes for their antimicrobial resistance profiles as well as for different bacterial isolates in meats sold across different streets around Johannesburg CBD, Gauteng Province, South Africa.

4.5 Materials and methods

4.5.1 Study site

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

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

There are a number of street vendors and consumers in these streets.

4.5.2 Study design

The study was conducted from December 2015 to April 2016 in the Microbiology Laboratory of the Department of Animal Health, School of Agriculture, Faculty of Agriculture, Science and Technology, North-West University, Mafikeng Campus.

4.5.3 Collection of samples

115 meat samples were collected twice a week for 12 consecutive weeks (Table 4.1). 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. In the laboratory, the samples were kept in a deep freezer until 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 and gloves worn in hands under biological safety cabinet class II (Model, 4BI02, Serial, 4BI020158. South Africa). Twenty five grams of meat samples were weighed aseptically under biological safety cabinet class II 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 products were incubated aerobically for 24 hours in a checker incubator Labcon (Model, fsies p024, serial C2421, South Africa) (Apha, 1998).

a) Serial dilution

From homogenised inoculum product, 1 ml was pipetted and added in Eppendorf 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. In order to avoid any contamination, the process was performed inside Biological safety cabinet class II (Model, 4BI02, Serial 4BI02158).

4.5.5 Bacterial isolation and total bacterial counts

From the 10-fold dilutions of homogenates inoculum product, 1ml of 10^{-2} , 10^{-3} , 10^{-4} and 10^{-5} dilutions of homogenate products were pipetted from Eppendorf tube and plated on nutrient agar, using L-shape sterile glass rod and pour plate method. The process was performed inside Biological safety cabinet class II to avoid any contamination. Plates were taken and incubated aerobically at 37°C for 48 hours (Apha, 1998) in a room incubator (Ecr manufacturing, model, Su 131h, Serial 128538. South Africa). After an incubation period at

of 48 hours at 37°C, colonies were counted using colony counter (Model #81-520-000 Bioresupply: Perry hall, USA) and recorded as total bacterial counts and total coliform counts. The counts for each plate were expressed as colony forming units of the suspension (CFU/ml). Total coliform were isolated using MacConkey agar (Apha, 1998). For control and quality assurance, 1 ml of sterile nutrient broth was also pipetted and plated on sterile nutrient Agar and MacConkey, and plates were incubated aerobically at 37°C for 48 hours using room incubator. After the incubation period, the absence of growth on the control plate confirmed the sterility of the environment where samples were cultured.

4.5.6 Morphological examination of isolated bacteria

Isolated bacteria were subjected to sub-culture, on fresh nutrient Agar and plates aerobically incubated at 37°C for 24 hours. Identification of isolates obtained was performed based on colony morphologies, followed by streaking 3 times of each colony on fresh nutrient Agar; Gram's staining (Fawole & Oso, 2004). The characteristics of the morphology were recorded by observing and comparing the morphology as described in Bergey's Manual of systematic Bacteriology (Kerstens & Vancanneyt, 2005) and (Fawole & Oso, 2004).

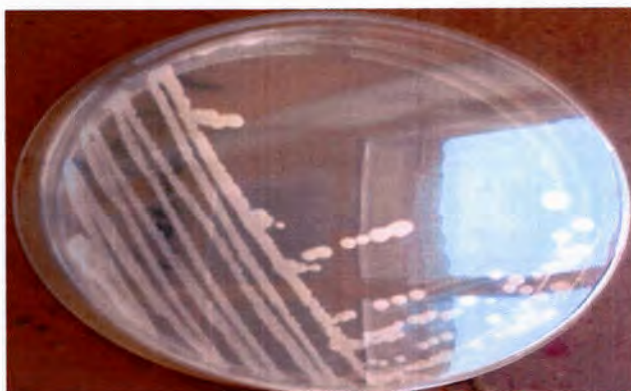


Figure 4.1: Plate showing isolated bacteria on nutrient agar after streaking

4.5.7 Biochemical test and cellular morphology

a) Gram's staining

Gram's staining was performed on each colony using standard methods and techniques. The Gram's stain was performed in order to get the two groups of bacteria (gram negative and gram positive), 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 seconds; and crystal violet was applied on the stain for two minutes followed by washing the slide with sterile water. Few drops of iodine solution were then applied on the stain for seconds 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 for 5 minutes, then the slide was washed again with running tap water. Finally, few drops of safrin solution were dropped on the slide as a counter stain for 2 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 performed according to (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 was released after 10 seconds, if the isolates were catalase negative, nothing was producing (Cheesbrough, 2006).

c) Oxidase test

An oxidase test was performed according to Ateba and Setone (2011) to identify bacteria that produce cytochrome oxidase. The isolates were subjected to sub-culturing on fresh nutrient and incubated at 37°C for 24 hours, using room incubator (ecr manufacturing, model su 131, serial 128538. South Africa). From a pure culture, after 24 hours of incubation, a single isolate bacterium was taken up with a sterile wire loop and smeared over oxidase strips paper

(Microbact identification kits, MBO266A). The result was read after 5 to 10 seconds. A positive result was indicated by 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 according to (Manga & Oyeleke, 2008). Bacterial cultures were inoculated in nutrient broth, using a sterile wire loop 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 product and shaken carefully. The presence of the red colour within 15-20 minutes was an indication of a positive result.

e) Indole test

Indole test was performed according to Hassan Ali *et al.* (2010). This test was performed using Kovaco's reagent. Nutrient broths 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 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

4.5.8 Bacterial identification using analytic profile index (API staph)

Twenty-five suspected bacterial isolates (according to biochemical tests results showing some characteristics of gram positive, such as *staphylococcus* spp. and *Micrococcus* spp.) were selected and subjected to API - staph for confirmation according to Downes & Ito (2001). The tests were performed according to the manufacturer's instruction (Bio-Merieux, France). 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.



Figure 4.3: Analytic profile index (IPA-Staph) for bacterial isolates

4.6 Molecular identification of bacterial isolates

Molecular identification has provided powerful tools to investigate microbial communities in the world at the level of species. This technique is very important as it provides informative insights about the 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 investigate molecular characterisation of 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 according to (Ngoma *et al.*, 2013). Pure isolated bacteria 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 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: 500g of agarose gel was weighed and mixed to 490 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 wells in the gel electrophoresis tank. Electrophoresis conditions were set 90 minutes at 80 voltage and 250 MA. Gel was then visualised under UV light at 420 nm 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 fragments 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 programme 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 programme DNA pair (Felsenstein *et al.*, 1989). Results were then showed as bootstrapped (1000 boot strap).

4.6.5 Phylogenetic 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 programme 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 neighbour 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 and coliform 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 unbalance 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 unbalance ANOVA was also used in determining the difference between the three streets in relation to coliform count as well as to determine 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 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 and Appendix 2.

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)

4.8.2: Determining the difference between the three areas in relation to bacterial 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 obtained in bacterial count in samples collected in 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	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

Table 4.6 shows that the sizes (N) are not equal; hence an unbalanced ANOVA was implemented. 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 ($p\text{-value} < 0.05$). This implies that the bacterial count is dependent on the street.

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 ^a	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 4.8: Summary of mean differences of bacterial count among different areas

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).

Table 4.8

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

² *Significant at 5% level

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

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.

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

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 4.10

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
	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

³Significant at 5% level

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

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

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

Table 4.12: Summary of mean coliform count according to different in type of meat analysed

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 ($p\text{-value} > 0.05$).

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

4.8.3 Morphology and biochemical characterisation of bacterial isolates

The morphological and biochemical characteristics of bacterial isolates from samples obtained from the three areas in this study were identified on the basis of cultural and cellular morphological (colony shape, margin, elevation, colour and texture) 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 4.13. Preliminary results based on morphology and biochemical characterisation for samples collected around MTN-taxi rank (Table 4.14) showed the results of morphology and biochemical tests for the samples collected around Bree Street (corner Plein Street). Table 4.15 shows the morphology and biochemical characteristics of samples collected at Hancock Street (corner Claim Street).

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

Table 4.13: 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 ⁻	<i>Bacillus cereus</i>
GIZ 0 D4	Cocci, ovoid, edges, raised, white colony, smooth	V ⁺	V ⁻	V ⁻	V ⁺	V ⁻	<i>Enterococcus faecalis</i>
GIZ 16 D4	Cocci, irregular, curled, raised, white, smooth	V ⁺	V ⁻	V ⁻	V ⁺	V ⁻	<i>Enterococcus faecalis</i>
GIZ 12 D4	Cocci, circular, entire, convex, beige, yellow	V ⁺	V ⁺	V ⁺	V ⁺	V ⁻	<i>Macrococcus caseolyticus</i>
GIZ29D4 L1	R Rod, circular, entire, raised, white, smooth	V ⁻	V ⁻	V ⁺	V ⁺	V ⁺	<i>Citrobacter spp</i>
GIZ 3 D1 L1	Rod, irregular, curled, raised, opaque, smooth	V ⁺	V ⁺	V ⁺	V ⁺	V ⁻	<i>Bacillus cereus</i>
GIZ 3 D3 L1	Cocci, circular, entire, convex, rough, golden	V ⁺	V ⁻	V ⁺	V ⁺	V ⁻	<i>Staphylococcus aureus</i>
GIZ 8M	Cocci, circular, entire, convex, shiny, yellow	V ⁺	V ⁻	V ⁺	V ⁺	V ⁻	<i>Staphylococcus aureus</i>
GIZ 15 D1	Cocci, irregular, curled, raised, white	V ⁺	V ⁻	V ⁻	V ⁺	V ⁻	<i>Enterococcus faecalis</i>
GIZ 27 D4	Cocci, circular, entire, convex, rough, opaque	V ⁺	V ⁻	V ⁺	V ⁺	V ⁻	<i>Staphylococcus aureus</i>
GIZ 17 D2	Cocci, circular, entire, convex, beige, shiny	V ⁺	V ⁺	V ⁺	V ⁺	V ⁻	<i>Macrococcus caseolyticus</i>
GIZ 17 D4	Cocci, circular, entire, curled, raised, white, smooth	V ⁺	V ⁻	V ⁻	V ⁺	V ⁻	<i>Enterococcus faecalis</i>
GIZ 2 D4 L1	Cocci, circular, entire, convex, rough, yellow	V ⁺	V ⁻	V ⁺	V ⁺	V ⁻	<i>Staphylococcus aureus</i>
GIZ 9 D3	Rods, circular, entire, raised, shiny, colorless	V ⁻	V ⁻	V ⁺	V ⁻	V ⁺	<i>Escherichia coli</i>
GIZ30DML1	Rods, irregular, flat, lobate, cream, smooth	V ⁺	V ⁺	V ⁺	V ⁺	V ⁻	<i>Bacillus subtilis</i>
GIZ 26 DM	Cocci, circular, entire, convex, rough, yellow	V ⁻	V ⁻	V ⁺	V ⁺	V ⁻	<i>Staphylococcus aureus</i>
B.I 19 D3	Rods, circular, flat, dry colony, cream, smooth	V ⁺	V ⁻	V ⁺	V ⁺	V ⁻	<i>Bacillus subtilis</i>
B.I 29 D2 L1	Cocci, circular, entire, convex, rough, opaque	V ⁺	V ⁻	V ⁺	V ⁺	V ⁻	<i>Staphylococcus aureus</i>
B.I 28 D1L1	Rods, circular, entire, raised, white, smooth	V ⁺	V ⁻	V ⁺	V ⁺	V ⁺	<i>Bacillus spp</i>
B.I 1 D4 L1	Rods, circular, entire, raised, white, smooth	V ⁺	V ⁺	V ⁺	V ⁺	V ⁻	<i>Bacillus spp</i>
B.I 3 D1	Cocci, circular, entire, convex, rough, opaque	V ⁺	V ⁻	V ⁺	V ⁺	V ⁻	<i>Staphylococcus aureus</i>
B.I 1 D3	Cocci, circular, entire, convex, rough, opaque	V ⁺	V ⁺	V ⁺	V ⁺	V ⁻	<i>Staphylococcus aureus</i>
B.I 19 D4	Cocci, regular, entire edge, flat, cream, smooth	V ⁺	V ⁻	V ⁻	V ⁻	V ⁻	<i>Enterococcus spp</i>
B.I 27 D2	Rods, circular, entire, raised, white, smooth	V ⁺	V ⁺	V ⁺	V ⁺	V ⁻	<i>Bacillus spp</i>
B.I 25 ML1	Rods, irregular undulolute , flat, opaque, smooth	V ⁺	V ⁺	V ⁺	V ⁺	V ⁻	<i>Bacillus cereus</i>
B.I 23 D3	Rods, circular, entire, raised, shiny, colorless	V ⁻	V ⁻	V ⁺	V ⁻	V ⁺	<i>Escherichia coli</i>
B.I 5 D3	Cocci, pinhead, entire, convex, yellow, smooth	V ⁺	V ⁺	V ⁺	V ⁺	V ⁺	<i>Macrococcus caseolyticus</i>
B.I 04 M	Cocci, circular, entire, convex, beige, shiny	V ⁺	V ⁺	V ⁺	V ⁺	V ⁺	<i>Macrococcus caseolyticus</i>

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

Table 4.14: 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 ⁻	<i>Bacillus</i> spp
Wo 3D1	Rod, circular, entire, raised, cream, dull	V ⁺	V ⁺	V ⁺	V ⁺	V ⁻	<i>Bacillus</i> spp
Wo 32 D 3	Rod, circular, entire, raised, cream, dull	V ⁺	V ⁺	V ⁺	V ⁺	V ⁻	<i>Bacillus</i> spp
Wo 2 D2	Cocci, circular, entire, convex, rough, yellow	V ⁺	V ⁻	V ⁺	V ⁺	V ⁻	<i>Staphylococcus</i> spp
Wo 10 D4	Cocci, circular, entire, convex, beige, shiny	V ⁺	V ⁺	V ⁺	V ⁺	V ⁻	<i>Macroccoccus caseolyticus</i>
Wo 29 D3	Rods, irregular, undoluate, flat, opaque	V ⁺	V ⁻	V ⁺	V ⁺	V ⁻	<i>Bacillus cereus</i>
Wo 5 D4	Cocci, circular, entire, convex, beige, shiny	V ⁺	V ⁺	V ⁺	V ⁺	V ⁻	<i>Macroccoccus caseolyticus</i>
Wo 12 D2	Rod, circular, entire, flat, cream, dull	V ⁺	V ⁻	V ⁺	V ⁺	V ⁻	<i>Bacillus</i> spp
Wo 8DM	Cocci, circular, entire, convex, rough, opaque	V ⁺	V ⁺	V ⁺	V ⁺	V ⁻	<i>Staphylococcus aureus</i>
Wo 12 D4	Cocci, circular, entire, convex, beige, shiny	V ⁺	V ⁺	V ⁺	V ⁺	V ⁻	<i>Macroccoccus caseolyticus</i>
Wo 0 D3	Cocci, circular, entire, convex, rough, opaque	V ⁺	V ⁻	V ⁺	V ⁺	V ⁻	<i>Staphylococcus aureus</i>
Wo 5 D5	Rod, irregular, undoluate, flat, opaque	V ⁺	V ⁻	V ⁺	V ⁺	V ⁻	<i>Bacillus cereus</i>
CH 11 D2	Cocci, circular, entire, raised, smooth	V ⁺	V ⁻	V ⁻	V ⁺	V ⁻	<i>Enterococcus faecalis</i>
CH 5 D3	Cocci, circular, entire, convex, beige, shiny	V ⁺	V ⁺	V ⁺	V ⁺	V ⁻	<i>Macroccoccus caseolyticus</i>
CH 4 D3 L1	Cocci, circular, entire, convex, dirty white, rough,	V ⁺	V ⁻	V ⁺	V ⁺	V ⁻	<i>Staphylococcus aureus</i>
CH 22 D1	Cocci, circular, entire, convex, dirty white, rough	V ⁺	V ⁻	V ⁺	V ⁺	V ⁻	<i>Staphylococcus aureus</i>
CH 25 ML1	Rod, circular, entire, flat, cream, dull	V ⁺	V ⁻	V ⁺	V ⁺	V ⁻	<i>Bacillus</i> spp
CH 3M	Cocci, circular, entire, convex, rough, yellow	V ⁺	V ⁻	V ⁺	V ⁺	V ⁻	<i>Staphylococcus</i> spp
CH 22 D2	Cocci, circular, entire, convex, beige, shiny	V ⁺	V ⁺	V ⁺	V ⁺	V ⁻	<i>Macroccoccus caseolyticus</i>
CH 0 D4	Cocci, regular, entire, flat, cream, smooth	V ⁺	V ⁻	V ⁻	V ⁻	V ⁻	<i>Enterococcus faecium</i>
CH 20 M	Rod, circular, entire, flat, cream, dull	V ⁺	V ⁻	V ⁺	V ⁺	V ⁻	<i>Bacillus</i> spp
CH 20 D2	Rod, circular, entire, raised, white, smooth	V ⁻	V ⁻	V ⁺	V ⁺	V ⁺	<i>Citrobacter</i> spp

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

VP = Voges proskauer test

IN = Indole test

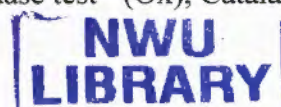


Table 4.15: Preliminary results based on morphological and biochemical characteristics

Sample identification	Morphology	Gr	Oxi	Cat	VP	IN	Presumptive bacteria
BHM1D3	Cocci, circular, entire, convex, beige, shiny	V ⁺	V ⁺	V ⁺	V ⁺	V ⁻	<i>Macrococcus caseolyticus</i>
BHM23D3	Cocci, circular, entire, convex, dirty white, rough	V ⁺	V ⁻	V ⁺	V ⁺	V ⁻	<i>Staphylococcus aureus</i>
BHM23M	Cocci, circular, entire, convex, dirty white, rough	V ⁺	V ⁻	V ⁺	V ⁺	V ⁻	<i>Staphylococcus aureus</i>
BHM2D2	Cocci, circular, entire, convex, dirty white, rough	V ⁺	V ⁻	V ⁺	V ⁺	V ⁻	<i>Staphylococcus aureus</i>
BHM22D3	Rod, circular, entire, flat, cream, dull	V ⁺	V ⁻	V ⁺	V ⁺	V ⁻	<i>Bacillus spp</i>
BHM1D4	Rod, circular, entire, flat, cream, dull	V ⁺	V ⁻	V ⁺	V ⁺	V ⁻	<i>Bacillus spp</i>
BHM6D4	Cocci, circular, entire, convex, dirty white, rough	V ⁺	V ⁻	V ⁺	V ⁺	V ⁻	<i>Staphylococcus aureus</i>
BHM17D3	Cocci, circular, entire, convex, beige, shiny	V ⁺	V ⁺	V ⁺	V ⁺	V ⁻	<i>Macrococcus caseolyticus</i>
BHM17DM	Cocci, circular, entire, convex, dirty white, rough	V ⁺	V ⁻	V ⁺	V ⁺	V ⁻	<i>Staphylococcus aureus</i>
BHM29D4	Rod, circular, entire, raised, white, smooth	V ⁻	V ⁻	V ⁺	V ⁺	V ⁺	<i>Enterobacter spp</i>
BHM19D2	Rod, irregular, undoluate, flat, opaque	V ⁻	V ⁻	V ⁺	V ⁺	V ⁻	<i>Bacillus cereus</i>
BHM22D2	Rod, circular, entire, raised, white, smooth	V ⁺	V ⁻	V ⁺	V ⁻	V ⁻	<i>Enterobacter spp</i>
BHM24D3	Cocci, circular, entire, convex, dirty white, rough	V ⁺	V ⁻	V ⁺	V ⁺	V ⁻	<i>Staphylococcus aureus</i>
BHM26D4	Cocci, circular, entire, convex, dirty white, rough	V ⁺	V ⁻	V ⁺	V ⁺	V ⁻	<i>Staphylococcus aureus</i>
BHM28D2	Rod, circular, entire, raised, white, smooth	V ⁻	V ⁻	V ⁺	V ⁺	V ⁺	<i>Enterobacter spp</i>
BHM29D1	Rod, circular, entire, raised, white, smooth	V ⁺	V ⁻	V ⁺	V ⁻	V ⁻	<i>Enterobacter spp</i>
BHM20D3	Rod, circular, entire, raised, white, smooth	V ⁺	V ⁻	V ⁺	V ⁻	V ⁻	<i>Enterobacter spp</i>
BHM21D4	Cocci, circular, entire, convex, dirty white, rough	V ⁺	V ⁻	V ⁺	V ⁺	V ⁻	<i>Staphylococcus aureus</i>
BHM19D4	Rod, irregular, undoluate, flat, opaque	V ⁻	V ⁻	V ⁺	V ⁺	V ⁻	<i>Bacillus cereus</i>

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

VP = Voges proskauer test; IN = Indole test

4.8.4 Overall bacterial isolates based on biochemical and morphology tests

The preliminary results obtained from morphology and biochemical tests showed that beef head meat samples were the most contaminated among all with 27.50% of contamination followed by chicken gizzard, beef intestine, wors and chicken meat with 23.20%, 17.40%, 17.40% and 14.50% respectively. In addition, the results obtained showed 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	Micrococcus 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: Overall 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 with *Staphylococcus aureus* from chicken meat and beef head meat samples; 11.11% were confirmed positive with *Staphylococcus haemolyticus*, from chicken gizzard and wors meat samples, while 40.74% were confirmed positive with *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	<i>Staphylococcus sp</i>
CH D3L1	<i>Staphylococcus aureus</i>
GIZ 17D4	<i>Staphylococcus haemolyticus</i>
Wo 23 M	<i>Staphylococcus saprophiticus</i>
Wo 30D1	<i>Staphylococcus sp</i>
BI 19D4	<i>Staphylococcus sp</i>
Wo 32D2	<i>Staphylococcus haemolyticus</i>
CH5 D3	<i>Staphylococcus xylosus</i>
GIZ 2DL1	<i>Staphylococcus haemolyticus</i>
CH 22D2	<i>Staphylococcus sp</i>
Wo 5D4	<i>Staphylococcus cohnii</i>
BI 27D2	<i>Staphylococcus xylosus</i>
Wo 20D2	<i>Staphylococcus lentus</i>
GIZ 17D2	<i>Staphylococcus sp</i>
GIZ 9D3	<i>Staphylococcus lentus</i>
CH D3 18	<i>Staphylococcus sp</i>
GIZ 2D4 L1	<i>Staphylococcus sp</i>
Wo10 D4	<i>Staphylococcus sp</i>
CH 22 D2	<i>Staphylococcus sp</i>
Wo 32 D3 L1	<i>Staphylococcus xylosus</i>
BI 1D3	<i>Staphylococcus lentus</i>
CH 22D1	<i>Staphylococcus xylosus</i>
BHM12D4	<i>Staphylococcus lentus</i>
Wo 27	<i>Staphylococcus spp</i>
Wo 5D4	<i>Staphylococcus sp</i>
CH5D3	<i>Staphylococcus spp</i>
BHM 23 D3	<i>Staphylococcus aureus</i>

4.9 Molecular identification of isolated bacteria

4.9.1 Genomic DNA of isolated bacteria

Fifty bacterial isolates were selected and subjected to PCR analysis. The results revealed a 1% (w/v) agarose gel representing 16S rDNA gene fragments (Figures 4.4, 4.5 and 4.6). The desired 1 kb base pairs fragments were obtained after running the gel on electrophoresis for 65 minutes. After running the gel, an automatic UV trans illuminator (UV tec, Sigma, Germany) was used to view the bands of the genomic DNA and photographed using a Bio profile gel documentation system. The purpose was to check the presence or absence of DNA bands and confirm successful extraction.

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

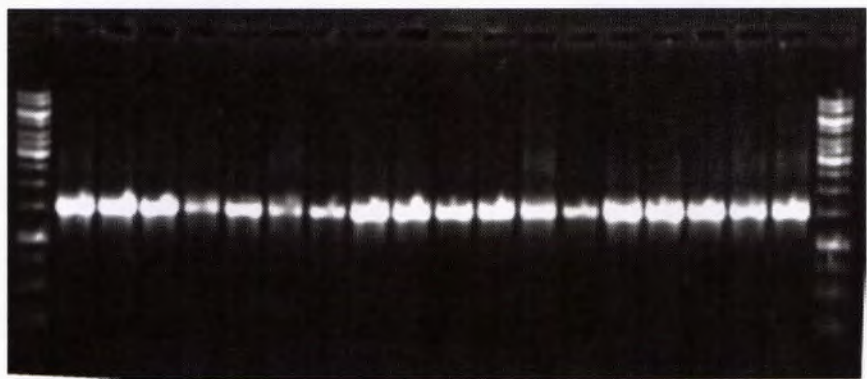


Figure 4.4 is an image of an Agarose gel (1%w/v) electrophoresis showing 16 rRNA gene fragments amplified from DNA extracted from isolated bacteria, Lane M = 1Kb DNA lader. Lanes 1-6 and 13 = Staphylococcus aureus, Lanes 7 = No similarity was found, Lanes 8 = Bacillus thuringiens, Lane 9 = Planomicrobium glaciei, Lane 10 = Bacillus sp, Lane 11-12 and = Macroccus caseolyticus, Lane 14 = Bacillus subtilis, Lane 15-16 = Bacillus cereus, 18 = Enterococcus faecalis

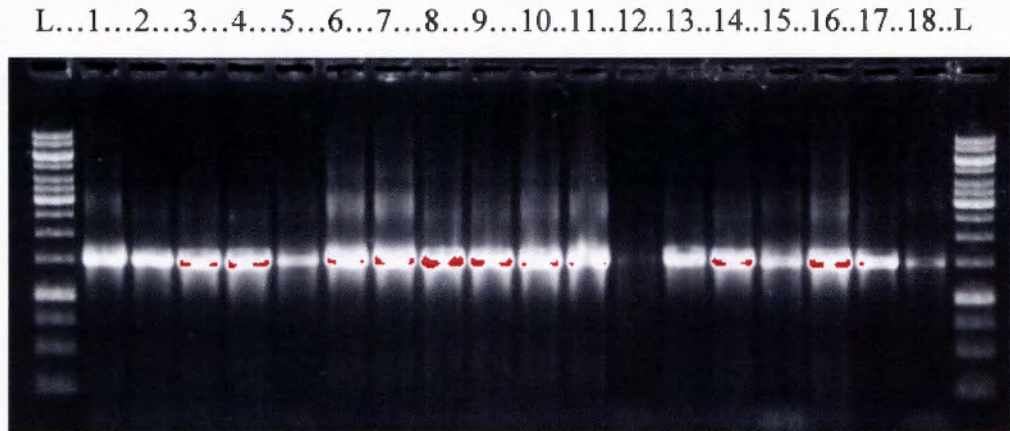


Figure 4.5: PCR products amplified from bacteria isolated from street-vended meat

Lane M, 1 Kb DNA ladder

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.

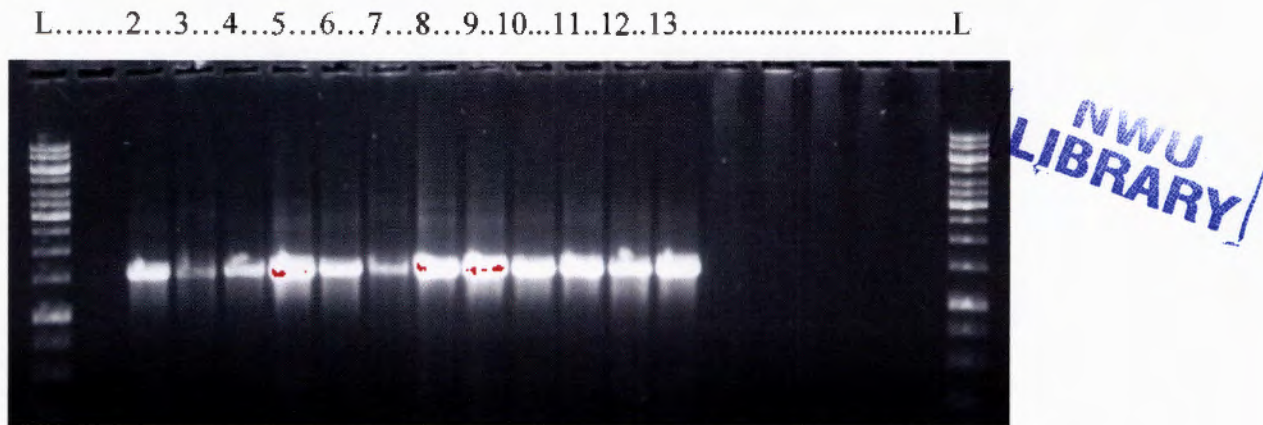


Figure 4.6: PCR products amplified from bacteria isolated from street-vended meat

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 identification and confirmation of results using the molecular method were based on 16S rRNA gene. The PCR amplification method of 16S rRNA gave some identification results as a conventional 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: Results of 40 bacterial isolates based on PCR product and sequence analysis bacteria and their accession number

Sample identity	Bacterial identity	Accession number	Identity%
BI29D2 I1	<i>Staphylococcus aureus</i>	KX447584.1	94%
GIZ 20 D1	<i>Staphylococcus aureus</i>	KX447584.1	95%
BHM2 D2	<i>Staphylococcus aureus</i>	KX447584.1	99%
CH4 D3	<i>Staphylococcus aureus</i>	KX447584.1	95%
BHM 23M	<i>Staphylococcus aureus</i>	KX447584.1	98%
Wo D3	<i>Staphylococcus aureus</i>	KX447584.1	96%
Wo 8D1	<i>Bacillus thuringienis</i>	EU161995.1	94%
GIZ 15D1	<i>Planomicrobium glaciei</i>	FB03B07	95%
Wo 23 D3	<i>Bacillus sp</i>	KX453917.1	96%
CH 22 D1	<i>Macroccoccus caseolyticus</i>	KP058399.1	97%
CH 22 D1	<i>Macroccoccus caseolyticus</i>	KJ783380.1	99%
CH 22 D1	<i>Staphylococcus aureus</i>	KX447584.1	94%
BI 19D3	<i>Bacillus subtilis</i>	KU936340.1	99%
GIZ 3D3L1	<i>Bacillus cereus</i>	KU977289.1	78%
BI 04D2	<i>Bacillus cereus</i>	KP235535.1	81%
CH5 D3	<i>Macroccoccus caseolyticus</i>	KC212015.1	95%
CH 11D2	<i>Enterococcus faecalis</i>	KJ571205.1	96%
GIZ 30DM L1	<i>Planococcus antarcticus</i>	KF318398.1	98%
Wo 32D3 I1	<i>Staphylococcus aureus</i>	KX447584.1	97%
BHM 1D3	<i>Macroccoccus caseolyticus</i>	KC212015.1	95%
BHM 23D3	<i>Enterococcus faecium</i>	KR858813.1	95%
BHM 23 D3	<i>Staphylococcus aureus</i>	BX571856.1	88%
BHM 17D3	<i>Macroccoccus caseolyticus</i>	KJ783380.1	97%
CH 3M	<i>Staphylococcus equorum</i>	DQ232735.1	96%
CH22 D2	<i>Macroccoccus caseolyticus</i>	KP181626.1	94%
CH5 D3	<i>Macroccoccus caseolyticus</i>	KP058399.1	97%

Wo5 D4	<i>Macrococcus caseolyticus</i>	KJ78380.1	95%
Wo10 D4	<i>Macrococcus caseolyticus</i>	KX062008.1	96%
CH25 ML1	<i>Planomicrobium glaciei</i>	JF411320.1	95%
BI3 D1	<i>Staphylococcus aureus</i>	KX447584.1	97%
BHM1 D4	<i>Bacillus sp</i>	JF701942.1	95%
Wo17 D1	<i>Macrococcus caseolyticus</i>	KP058399.1	95%
Wo3 D1	<i>Lysinibacillus sp</i>	LN483073.1	98%
CH20 M	<i>Kurthia sp</i>	CP013217.1	98%
BHM29 D4L1	<i>Citrobacter sp</i>	KT150059.1	83%
CH20 M	<i>Kurthia sp</i>	CP013217.1	97%
GIZ3 D1L1	<i>Lysinibacillus</i>	KT029127.1	95%
CH0 D4	<i>Bacillus cereus</i>	KP235535.1	82%
GIZ16 D4	<i>Enterococcus faecium</i>	FJ915708.1	94%
Wo12 D4	<i>Staphylococcus vitulinus</i>	JQ684238.1	97%

4.9.3 Phylogenetic tree of isolated bacteria

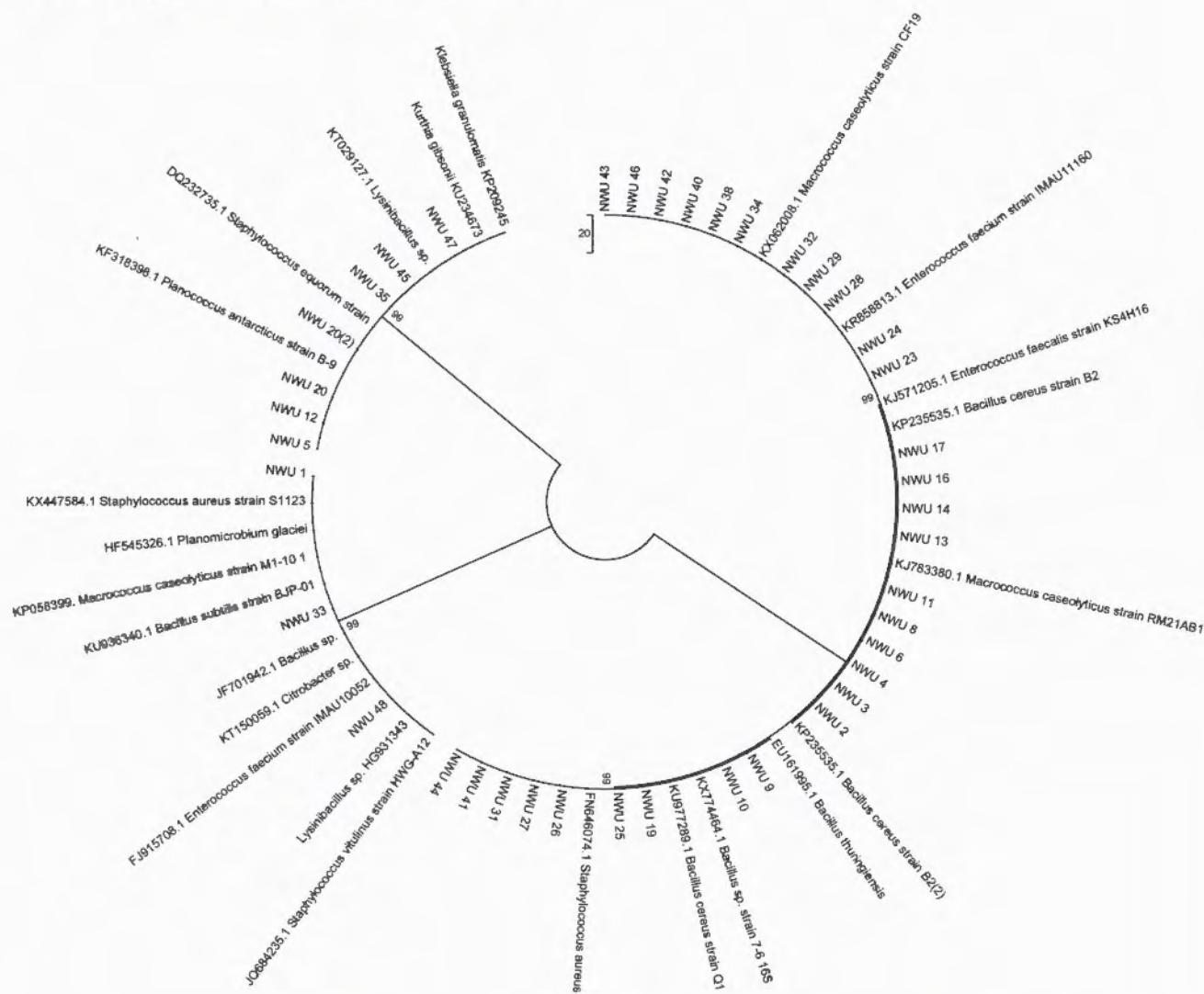


Figure 4.7 shows the Phylogenetic tree compared with 16S rDNA constructed using 40 isolates.

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

4.9.3 Prevalence of isolated bacteria

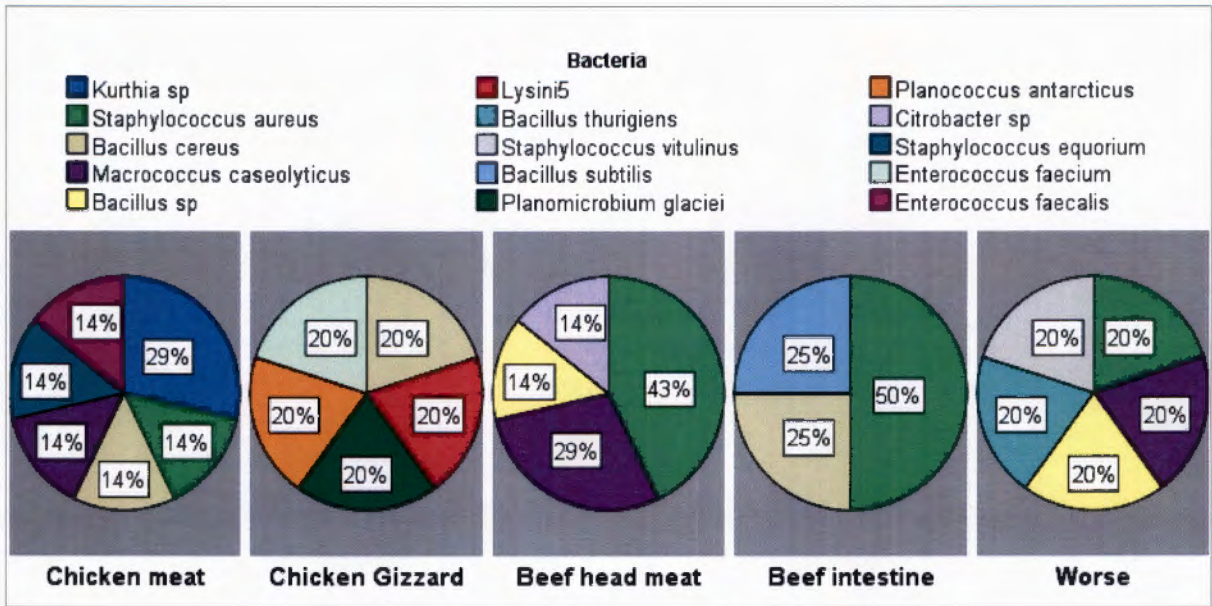


Figure 4.8: Percentage of bacterial isolates in each type of meat

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

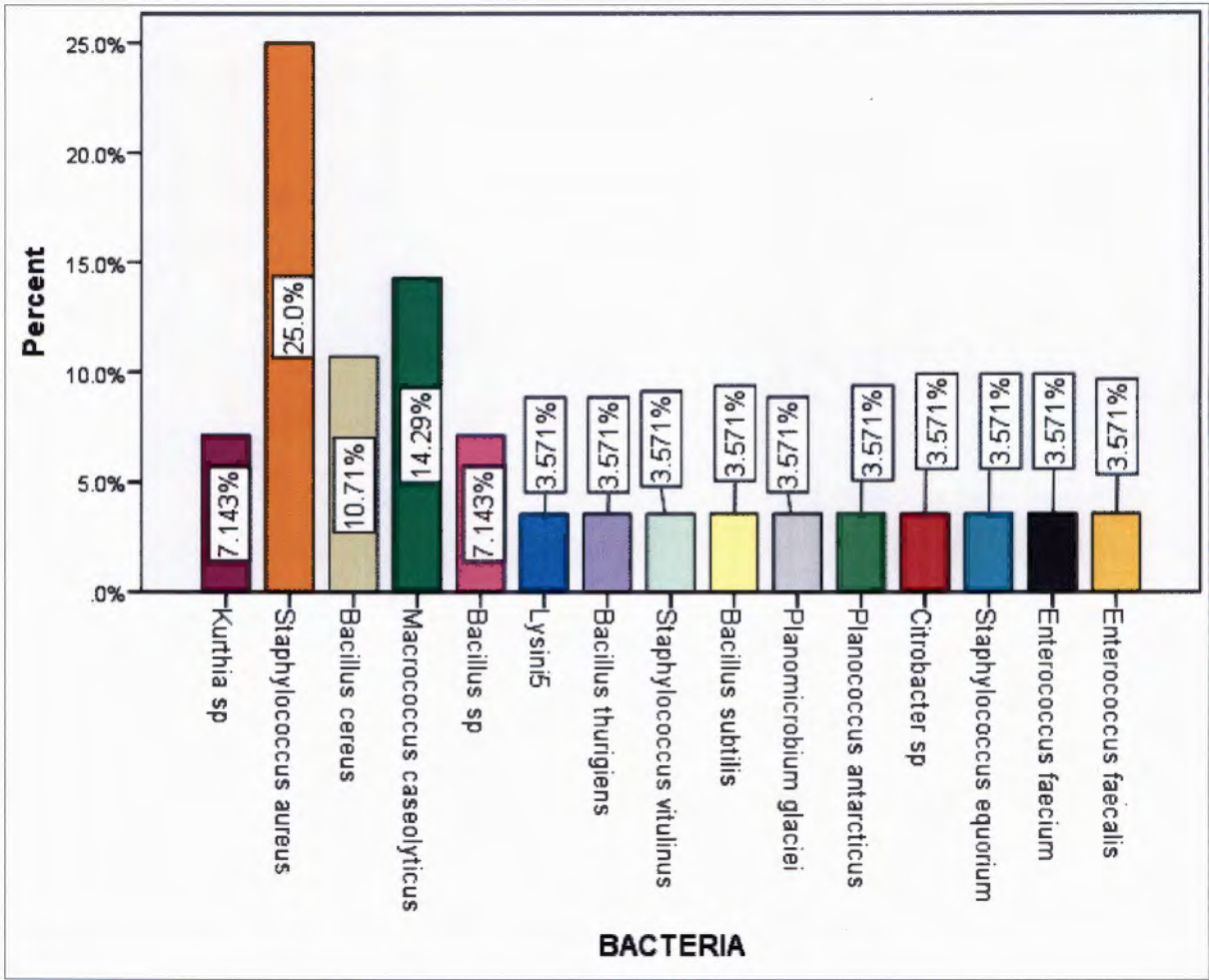


Figure 4.9: Prevalence of bacterial isolates around the three streets

The results obtained from PCR and sequencing summarised in Table 4.18 and Figures 4.8 and 4.9 show that a quarter of bacteria detected in meat samples were: *Staphylococcus aureus* (25%); *Macrocooccus 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 prevalent at 3.57% each.

4.10 Discussion

4.10.1 Analysis of bacterial and coliform counts

The values obtained for bacterial and coliform counts were presented by the street under investigation. The values obtained for bacterial count for chicken gizzard sold around MTN taxi rank ranged from 9.6×10^{-2} cfu/ml to 2.6×10^{-2} cfu/ ml total bacterial count and 2.9×10^{-2} cfu/ml to 1.0×10^{-2} cfu/ ml total coliform count for cooked beef intestine. Total bacterial count ranged from 9.9×10^{-2} cfu/ ml to 2.9×10^{-2} cfu/ ml, and for total coliform count, the values ranged from 2.9×10^{-2} cfu/ ml to 1.0×10^{-2} cfu/ ml (Tables 4.1 and 4.2). For the range of bacterial counts for wors samples collected at Bree Street (corner Claim Street), the values ranged from 9.1×10^{-2} cfu/ ml to 1.4×10^{-2} cfu / ml for total bacterial count and 2.4×10^{-2} CFU/ml to 1.1×10^{-2} CFU/ml for total coliform count. For chicken meat, the values ranged from 9.7×10^{-2} CFU/ml to 1.1×10^{-2} cfu/ ml for total bacterial count, and 3.0×10^{-2} CFU/g to 1.0×10^{-2} for total coliform count (Tables 4.3 and 4.4). For beef head meat, samples collected at Hancock Street (corner Claim Street), the values were respectively 9.7×10^{-2} cfu/ ml to 2.9×10^{-2} cfu/ ml for total bacterial count and 2.9×10^{-2} to 1.1×10^{-2} for total coliform count (Table 4.5).

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).

The results of this study revealed that there is no significant difference in the mean coliform count across the three areas $P > 0.05$ (Table 4.11) and no statistically significant difference in the mean coliform count across the type of meat $P > 0.05$ (Table 4.12).

Therefore, the total bacterial count of all the samples presented in Tables 4.1, 4.2, 4.3, 4.4 and 4.5 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).

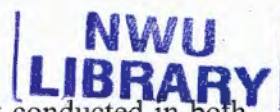
4.10.2 Analysis of isolated bacteria in meat samples and their prevalence

Molecular analysis based on 16s rDNA revealed a high frequency of occurrence of pathogenic bacteria such as *Staphylococcus aureus* (25.0 %), *Bacillus cereus* (10.71 %), *Bacillus spp.* (7.143 %), and other bacteria such as *Enterococcus faecalis* (3.571 %), *Enterococcus faecium* (3.571 %), *Lysinibacillus spp.* (3.571 %), and *Citrobacter sp* (3.571 %), *Macrococcus caseolyticus* (14.29 %), *Staphylococcus equorum* (3.571 %), *Staphylococcus vitulinus* (3.571 %), *Bacillus thuringiens* (3.571 %) and *Bacillus subtilis* (3.571 %) (Table 4.18 and Figure 4.9).

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 also correlate also 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).

In this study, *Staphylococcus aureus* was isolated in different meat samples at varying percentages: chicken meat (14%); beef head meat (43%); beef intestine (50%); and wors (20%) Figure 4.8. No *Staphylococcus aureus* was found in chicken gizzard (Figure 4.8). 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 high occurrence of *Staphylococcus aureus* in this study (25.0%) (Figure 4.9), 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. The observation study revealed poor hygienic practices by vendors (Figures 3.1, 3.2 and 3.3). 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 4.8 and Table 4.18). The overall count in all meat samples was 3.571% for both (Figure 4.9).

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 1 µg 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 butcherries 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).

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 4.8 and Table 4.18). The overall *Macrococcus caseolyticus* isolated in this study was 14.29% (Figure 4.9). No *Micrococcus caseolyticus* was found in chicken gizzard and beef intestine (Figure 4.8). The result is consistent with the findings of Baba *et al.* (2009), who isolated *Micrococcus caseolyticus* from cow milk, bovine organs and food (Baba *et al.*, 2009b).

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 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, some species of *Bacillus* were also isolated in the three different areas of the study, such as *Bacillus* sp (7.143%), *Bacillus cereus* (10.71%), *Bacillus subtilis* (3.571%) and *Bacillus thuringiensis* (3.571%) (Figure 4.9 and Table 4.18). These *Bacillus* species can cause food spoilage or food-borne illnesses (Cousin & Layfayette, 1989). Among *Bacillus* species, *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* was isolated from beef head meat and wors (Figure 4.7 and Table 4.18). The overall *Bacillus cereus* in this study was 10.71% (Figure 4.9). 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 result is also in line 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 concurs 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). *Lysinibacillus* spp. was also isolated in this study from chicken gizzard (20%) (Figure 4.8) and Table 4.18 and the overall in meat samples was 3.571% (Figure 4.9).

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). The presence of different species of *Bacillus* spp. 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).

Enterococcus faecalis (3.571%), *Enterococcus faecium* (3.571%) and *Citrobacter* spp (3.571%) were also isolated in this study (Figure 4.8 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).

However, 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 4.8 and 4.9 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).

Planococcus antarcticus (3.571%) was isolated from chicken gizzard and *Planomicrobium glaciei* (3.571%) isolated from chicken gizzard and chicken meat (Figures 4.8 and 4.9). Their presence in street food might be an indication that street food could be subject to a novel strain 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 *S. aureus*, *B. cereus* and *Bacillus* sp. The presence of *S. aureus* and *E. faecalis* 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 usage of antimicrobial drugs in food producing animals could result in a significant food safety (Petrovic *et al.*, 2010). It could also 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 study was to determine the antibiotic resistance profile of bacterial isolates and evaluate possible health risks encountered by consumers.

5.3 Materials and methods

5.3.1 Study design

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

5.4 METHODOLOGY

The paper disc diffusion method, according to the methods and techniques described by Kirby (Bauer *et al.*, 1966) was used in this study. Bacterial isolates were grown on nutrient agar at 37 °C for 18 to 24 hours. Bacterial suspensions were prepared and aliquots of 100 µl were spread over Mueller Hinton agar. Antimicrobial disks impregnated with Ampicillin (10 µg), Gentamicin (10 µg), Tetracycline (30 µg), Sulphonamides (300 mg), Streptomycin (10 µg), Ciprofloxacin (5 mg), Chloramphenicol (30 mg) and Erythromycin (5 mg) were obtained from Mast Diagnostics (United Kingdom). The disks (6 µm in diameter) were placed on the surface of the inoculated agar plates and the plates incubated at 37 °C for 18 to 24 hours.

After incubation, the antibiotic inhibition zone diameters (IZD) were measured (mm).

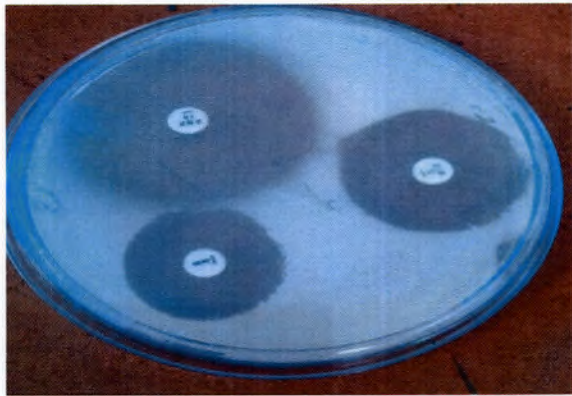


Figure 5.1: Antibiotic resistance profile of *Staphylococcus aureus* against Ampicillin, Erythromycin and Ciprofloxacin

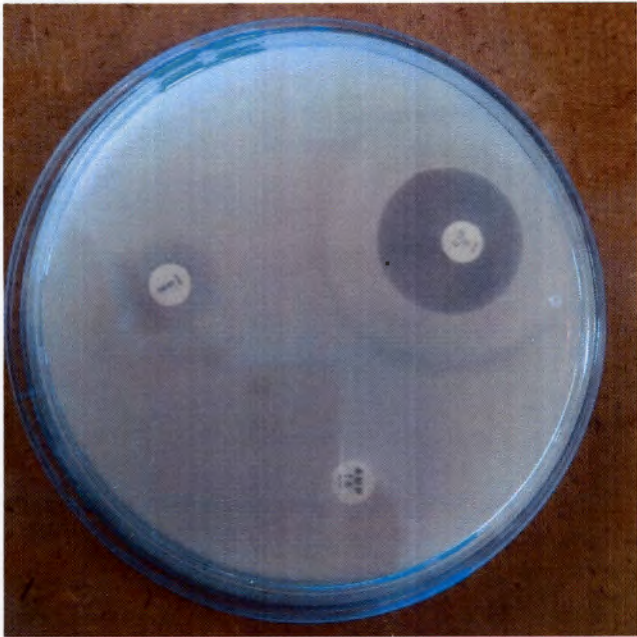


Figure 5.2: Antibiotic resistance profile of *Staphylococcus aureus* against Ampicillin, Erythromycin and Ciprofloxacin

5.5 STATISTICAL ANALYSIS

Cross tabulation was used to summarise bacteria counts according to the resistance 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 performed in this study was done with the help of 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	Intermediate
			s	I	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

Results obtained (see Appendix 3) were used to classify isolates as being resistant, intermediate or susceptible to a particular antibiotic using standard reference values followed by the recommendations of the Clinical Laboratory Institute standards (Wayne, 2009; Wikler, 2006; Wikler, 2007). Bacteria tested were represented as R (resistant), I (intermediate) and S (susceptible). Different bacteria tested in this study were *Macroccoccus caseolyticus*, *Enterococcus faecium*, *Enterococcus faecalis*, *Citrobacter* sp, *Bacillus* sp., *Bacillus cereus*, *Bacillus thuringiens*, *Bacillus subtilis*, *Lysinibacillus* sp, *Staphylococcus aureus*, *Staphylococcus vitulinus*, *Staphylococcus equorum*, *Kurthia* sp, *Planomicrobium glaciei* and *Planococcus antarcticus*. The isolated bacteria were tested with eight different antibiotics.

5.2 Resistant, susceptible and intermediate resistance patterns of isolated bacteria

S-ID	Microorganisms	Street	AMP10	CN10	TE30	S3 300	S 300	CIP5	C30	E5
CH20M	<i>Kurthia sp</i>	2	R	S	R	S	S	I	S	S
B.I 3D1	<i>Staphylococcus aureus</i>	1	I	S	R	R	S	I	S	I
B.I04D2	<i>Bacillus cereus</i>	1	R	S	I	S	S	I	S	I
BHM23D3	<i>Macrococcus caseolyticus</i>	3	R	S	R	S	S	I	R	I
CH22 D1	<i>Macrococcus caseolyticus</i>	2	R	S	R	S	S	I	S	I
GIZ3D3L1	<i>Bacillus cereus</i>	1	R	S	R	S	S	I	I	R
W23 D3	<i>Bacillus sp</i>	2	R	S	R	I	S	S	R	R
B.I29D2L1	<i>Staphylococcus aureus</i>	1	S	S	S	S	S	I	I	I
GIZ3D1L1	<i>Lysinibacillus sp</i>	1	R	S	R	R	S	S	S	I
W8D1	<i>Bacillus thurigiens</i>	2	R	S	I	S	S	I	I	R
BHM17DM	<i>Macrococcus caseolyticus</i>	3	R	S	R	S	S	S	S	I
W12D4	<i>Staphylococcus vitulinus</i>	2	R	S	R	S	I	I	R	I
B.I19D3	<i>Bacillus subtilis</i>	1	R	S	R	S	S	I	I	R
CH 20M	<i>Kurthia sp</i>	2	R	S	R	S	S	I	S	S
W32D3L1	<i>Staphylococcus aureus</i>	2	S	S	S	S	S	S	I	I
BHM2D2	<i>Staphylococcus aureus</i>	3	S	S	R	R	R	I	R	I
CH4D3L1	<i>Staphylococcus aureus</i>	2	R	S	R	R	S	S	S	R
GIZ15D1	<i>Planomicrobium glaciei</i>	1	R	S	S	R	S	S	R	R
BHM1D4	<i>Bacillus sp</i>	3	R	S	R	R	S	I	R	I
W17D1	<i>Macrococcus caseolyticus</i>	2	R	S	I	R	S	I	R	R
BHM 23M	<i>Staphylococcus aureus</i>	3	S	S	R	I	S	S	I	I
CH0D4	<i>Bacillus cereus</i>	2	R	S	I	S	S	S	S	I
GIZ 30DML1	<i>Planococcus antarcticus</i>	1	R	I	R	I	S	S	I	R
BHM 29D4L1	<i>Citrobacter sp</i>	3	R	S	I	S	S	S	I	R
CH3M	<i>Staphylococcus equorum</i>	2	R	S	R	I	I	I	R	S
BHM23D3	<i>Staphylococcus aureus</i>	3	R	S	R	S	S	I	R	R
GIZ 16D4	<i>Enterococcus faecium</i>	1	R	S	R	S	S	S	S	I
CH11D2	<i>Enterococcus faecalis</i>	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)

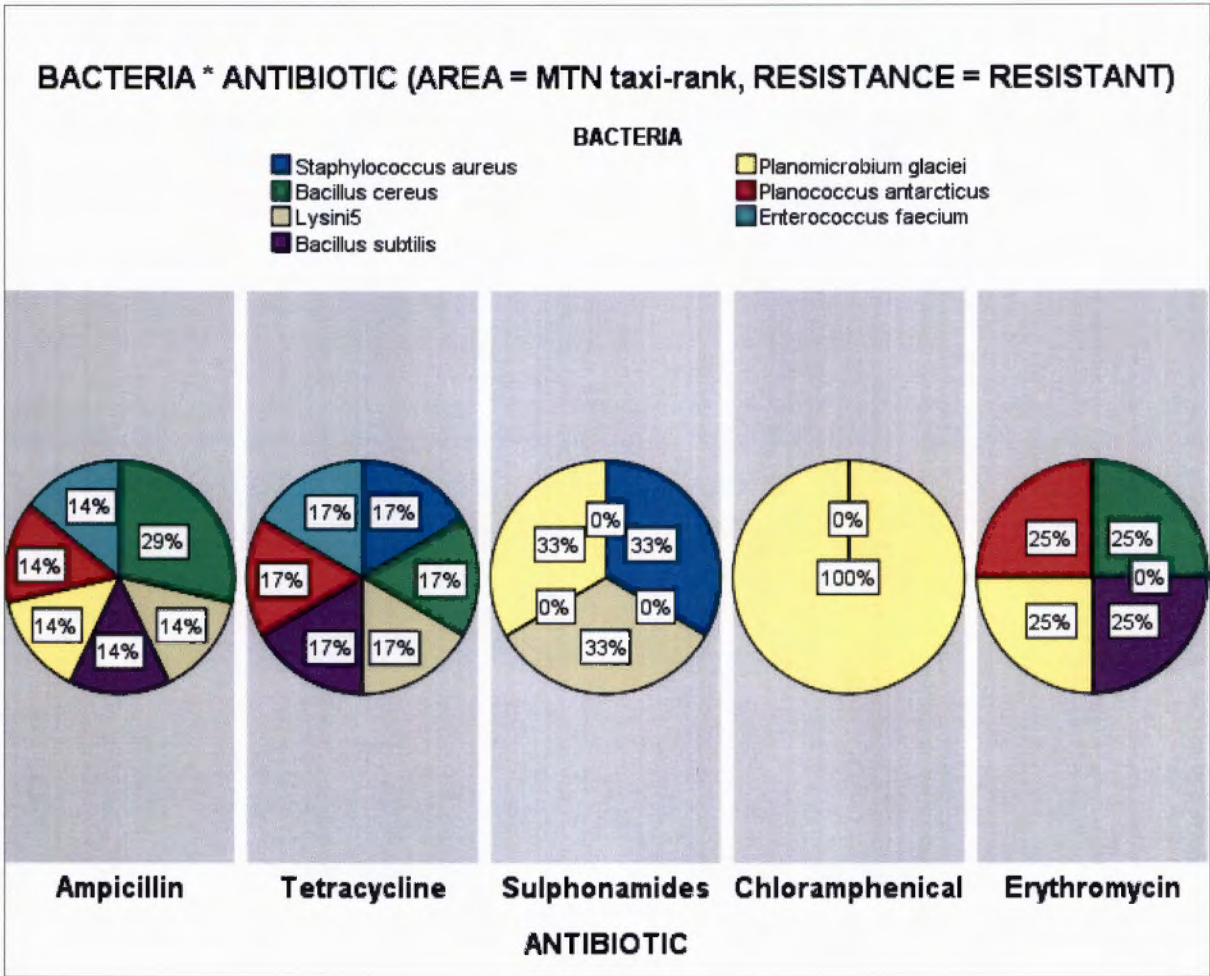


Figure 5.3: Summary of antibiotic resistance profile among the bacterial isolates according to the area of sampling.

The Figure shows that *B. cereus* represented 29%, while the other bacteria represented 14% and were 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.

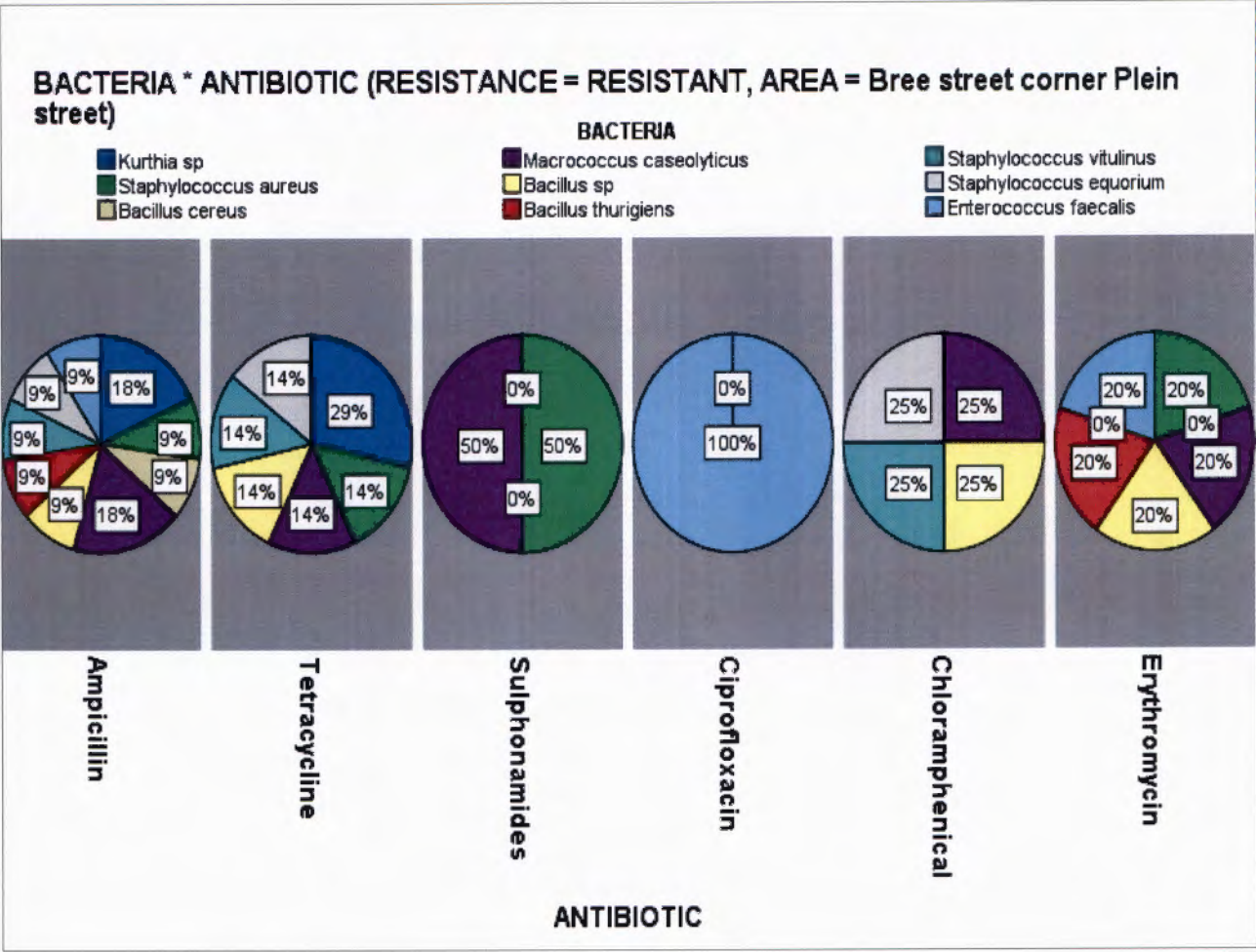


Figure 5.4: Summary of antibiotic resistance profile among the selected bacteria according to the Street of sampling

Figure 5.4 shows that all bacterial isolates were resistant to Ampicillin, *Kurthia* sp (18%), *M. caseolyticus* (18%), 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%)

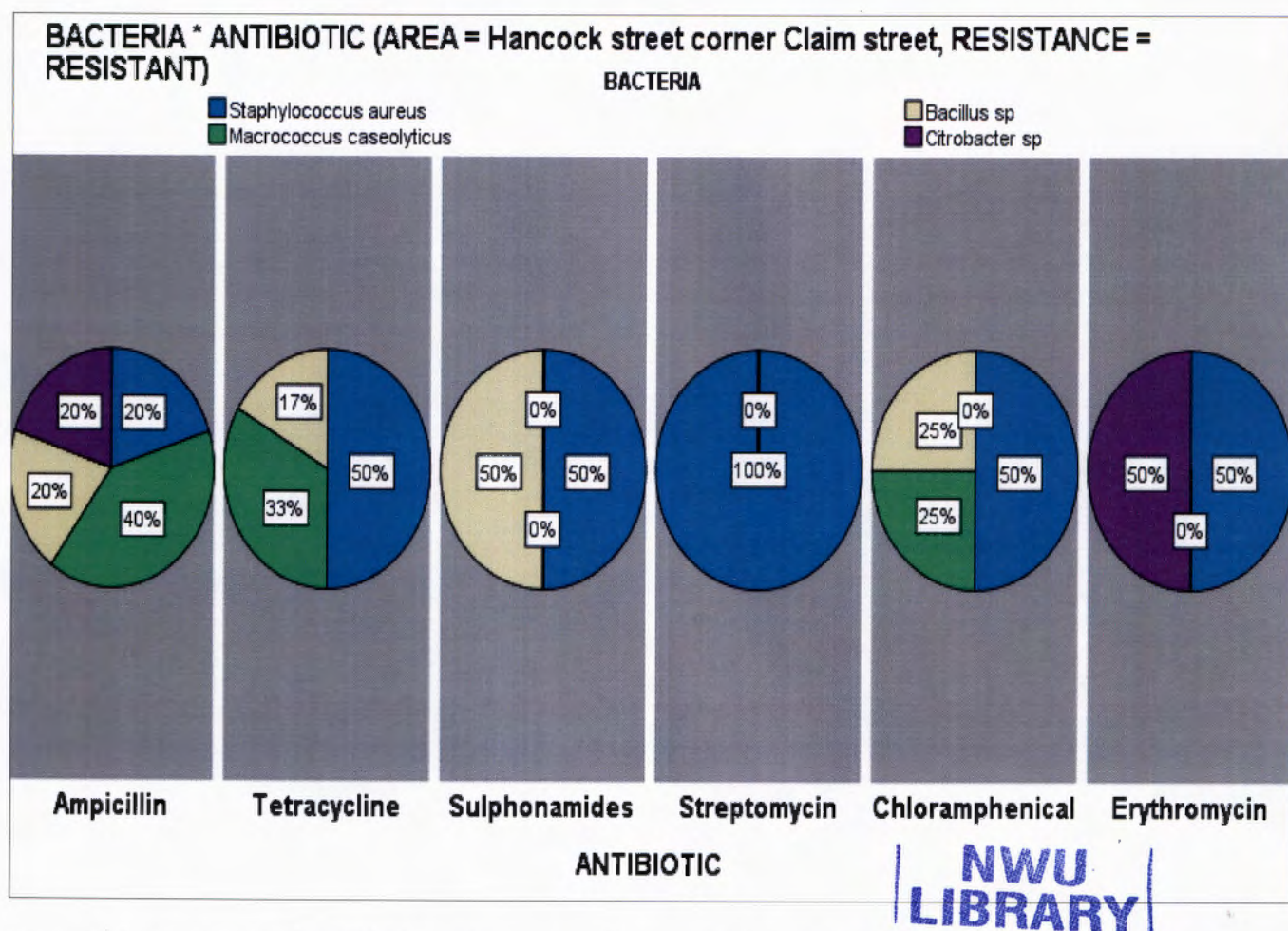


Figure 5.5: Summary of antibiotic resistance profile among the selected bacteria according to the street of sampling

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

Table 5.3: 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)

BACTERIA															
	<i>Kurthia</i> sp	<i>Staphylococcus aureus</i>	<i>Bacillus cereus</i>	<i>Macrocococcus caseolyticus</i>	<i>Bacillus subtilis</i> sp	<i>Lysinibacillus acillus</i>	<i>Bacillus thuringiensis</i>	<i>Staphylococcus vitulinus</i>	<i>Bacillus subtilis</i>	<i>Planomicrobium glaciei</i>	<i>Planococcus antarcticus</i>	<i>Citrobacter</i> sp	<i>Staphylococcus equorum</i>	<i>Enterococcus faecium</i>	<i>Enterococcus faecalis</i>
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
Ciprofloxacin	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 5.4: Multidrug resistance

Table 5.4 shows that *Staphylococcus aureus* was resistant to six out of eight antibiotics, followed by *Macroccoccus 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)

	BACTERIA							
	Kurthia sp	Staphylococcus aureus	Bacillus cereus	Macroccoccus 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 5.5: Occurrences of bacteria showing intermediate profile

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 4 summarises the count and types of antibiotics where the different bacteria displayed intermediate profile.

BACTERIA														
<i>Kurt hia sp</i>	<i>Staphylo coccus aureus</i>	<i>Baci llus cere us</i>	<i>Macro coccus caseolyt icus</i>	<i>Baci llus sp</i>	<i>Lysi ni5</i>	<i>Bacill us thurig iens</i>	<i>Staphylo coccus vitulinus</i>	<i>Baci llus subti lis</i>	<i>Planoc occus antarcti cus</i>	<i>Citrob acter sp</i>	<i>Staphylo coccus equorium</i>	<i>Enteroc occus faecium</i>	<i>Enteroc occus faecalis</i>	
Ampicillin	0	1	0	0	0	0	0	0	0	0	0	0	0	
Gentamici n	0	0	0	0	0	0	0	0	1	0	0	0	0	
Tetracycli ne	0	0	2	1	0	1	0	0	0	1	0	0	1	
Sulphona mides	0	1	0	0	1	0	0	0	1	0	1	0	1	
Streptomy cin	0	0	0	0	0	0	1	0	0	0	1	0	0	
Ciprofloxa cin	2	4	2	3	1	1	1	1	0	0	1	0	0	
Chloramp henicol	0	3	1	0	0	1	0	1	1	1	0	0	0	
Erythromy cin	0	5	2	3	1	0	1	0	0	0	0	1	0	

(1, 2, 3, 4 and 5 represent the number of bacteria found with intermediate profile to different antibiotics)

Table 5.6: Overall bacteria with intermediate profile

Table 5.6 shows 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).

BACTERIA								
	<i>Kurthia</i> sp	<i>Staphylococcus aureus</i>	<i>Bacillus cereus</i>	<i>Macroccus caseolyticus</i>	<i>Bacillus</i> sp	<i>Lysinibacillus</i>	<i>Bacillus thurigiens</i>	<i>Staphylococcus vitulinus</i>
List of Antibiotics that the bacteria were intermediate to	Ciprofloxa cin	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						
	<i>Bacillus subtilis</i>	<i>Planococcus antarcticus</i>	<i>Citrobacter</i> sp	<i>Staphylococcus equorum</i>	<i>Enterococcus faecium</i>	<i>Enterococcus faecalis</i>
List of Antibiotics that the bacteria are intermediate to	Ciprofloxacin Chloramphenicol	Gentamicin Sulphonamides chloramphenicol	Tetracycline chloramphenicol	Sulphonamides Streptomycin Ciprofloxacin	Erythromycin	Tetracycline Sulphonamides

Table 5.7: Summary of bacteria susceptible to antibiotics

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

BACTERIA															
	<i>Kurthia</i> <i>sp</i>	<i>Staphylo</i> <i>coccus</i> <i>aureus</i>	<i>Bacil</i> <i>lus</i> <i>cereus</i>	<i>Macro</i> <i>coccus</i> <i>caseolyticus</i>	<i>Bacil</i> <i>lus</i> <i>sp</i>	<i>Lysini</i> 5	<i>Bacil</i> <i>lus</i> <i>thuringiens</i>	<i>Staphylo</i> <i>coccus</i> <i>vitulinus</i>	<i>Bacil</i> <i>lus</i> <i>subtilis</i>	<i>Planomicrobium</i> <i>glaciei</i>	<i>Planococcus</i> <i>antarcticus</i>	<i>Citrobacter</i> <i>sp</i>	<i>Staphylo</i> <i>coccus</i> <i>equorum</i>	<i>Enterococcus</i> <i>faecium</i>	<i>Enterococcus</i> <i>faecalis</i>
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
Ciprofloxacin	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 5.8: Summary of bacteria susceptible to different antibiotics

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).

BACTERIA								
	<i>Kurthia</i> sp	<i>Staphylococcus aureus</i>	<i>Bacillus cereus</i>	<i>Macrococcus caseolyticus</i>	<i>Bacillus</i> sp	<i>Lysinibacillus</i>	<i>Bacillus thurigiens</i>	<i>Staphyloccus vitulinus</i>
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							
	<i>Bacillus subtilis</i>	<i>Planomicrobium glaciei</i>	<i>Planococcus antarcticus</i>	<i>Citrobacter</i> sp	<i>Staphylococcus equorum</i>	<i>Enterococcus faecium</i>	<i>Enterococcus faecalis</i>
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 5.9: Chi-Square test of association

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

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

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

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, antihiotic 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.

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

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 also 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 found that *S. aureus* was resistant to Ampicillin, Tetracycline and Chloramphenicol.

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 (Abdallah *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 *tetM* or *tetO* (Trzcinski *et al.*, 2000).

Staphylococcus vitulinus from the second research site, isolated in worst 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, 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 Fijałkowski *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, 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 in correlation with the one of this study related to Gentamicin and Streptomycin (Table 5.8), which was 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 (Figure 1). 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; Tahmasehi *et al.*, 2014; Turnbull *et al.*, 2004), who also reported the resistance of *B. cereus* to Ampicillin and Tetracycline.

Bacillus sp obtained from different research sites and meat samples were also tested for their resistance profiles. 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 agree with the finding of this study. However, Streptomycin and Gentamicin were found to be susceptible to *Bacillus* spp.

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 thuringiensis 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 (β -lactamase) secreted into the periplasmic space of the bacterium where it catalyzes hydrolysis of the β -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: Tetracycline; Ampicillin; Ciprofloxacin; Chloramphenicol; Streptomycin; Gentamicin; Sulphonamides; and Erythromycin. 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 are 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.

5.2 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.

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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 clothYes

No

Appendix 2: Bacterial count

Bacteria count by area

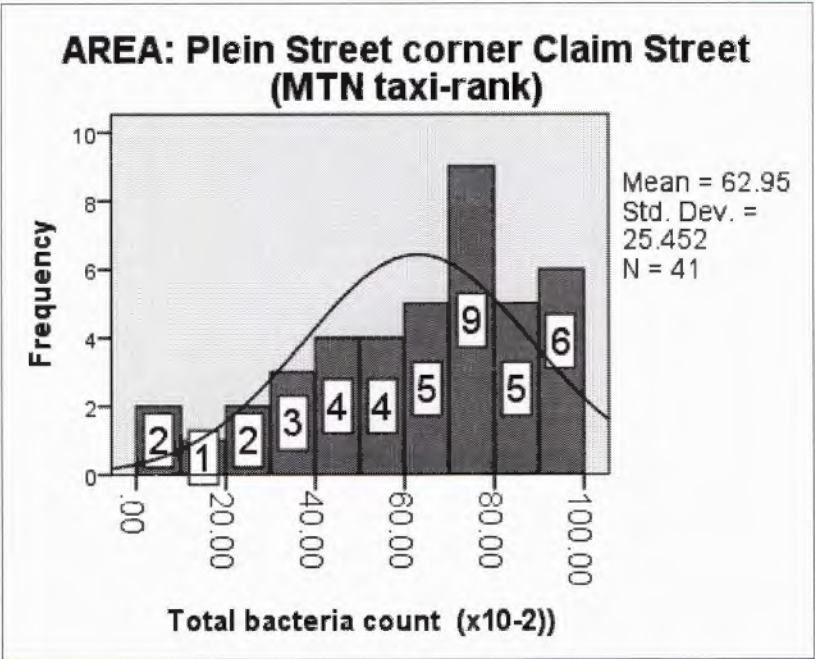


Figure 7 shows that the mean bacterial count at Plain Street (corner Claim Street) (MTN taxi-rank) is 62.95×10^{-2} with $N=41$ and the distribution of bacterial count is slightly skewed to the left, indicating that there are more upper counts of bacteria than lower counts. The modal class of bacteria count is 70×10^{-2} to $<80 \times 10^{-2}$.

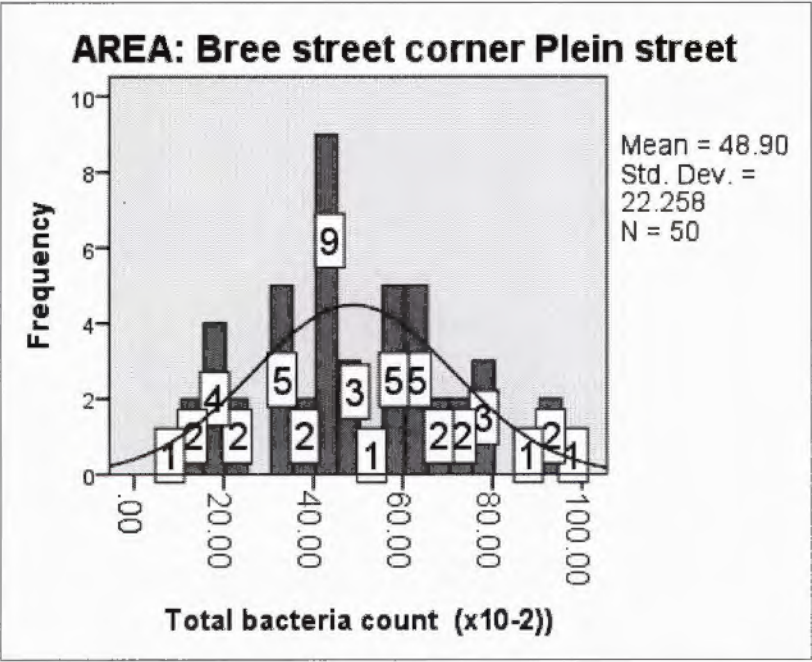


Figure 8 shows that the mean bacterial count at Bree Street (corner Plain Street) is 48.9×10^{-2} with $N=50$ and the distribution of bacterial count is approximately normal, indicating that there are almost equal upper counts of bacteria and lower counts. The modal class of bacterial count is 40×10^{-2} to $<45 \times 10^{-2}$.

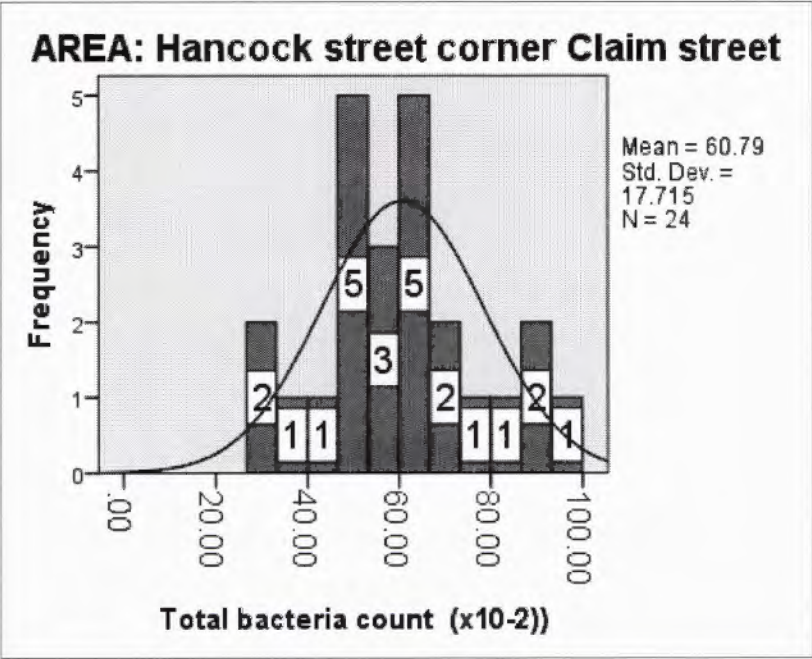


Figure 9 shows that the mean bacterial count at Hancock Street (corner Claim Street) is 60.79×10^{-2} with $N=24$ and the distribution of bacterial count is approximately normal, indicating that there are almost equal upper

counts of bacteria and lower counts. The modal classes of bacterial counts are 46.7×10^{-2} to $<53.3 \times 10^{-2}$ and 60×10^{-2} to $<66.7 \times 10^{-2}$.

Coliform count by area

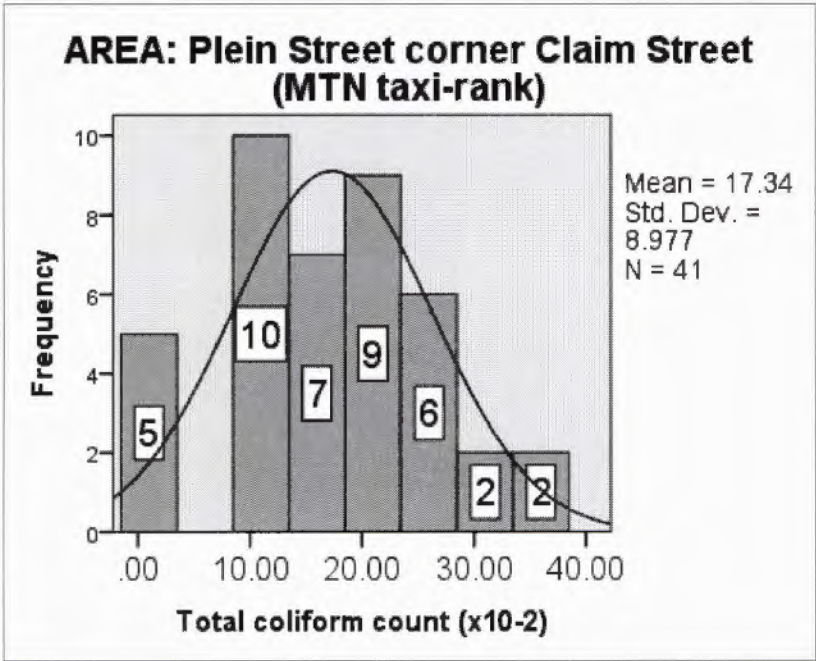


Figure 10 shows that the mean coliform count at Hancock Street (corner Claim Street) is 17.34×10^{-2} with $N=41$ and the distribution of coliform count is approximately normal, indicating that there are almost equal upper counts of bacteria and lower counts. The modal class of coliform count is 10×10^{-2}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

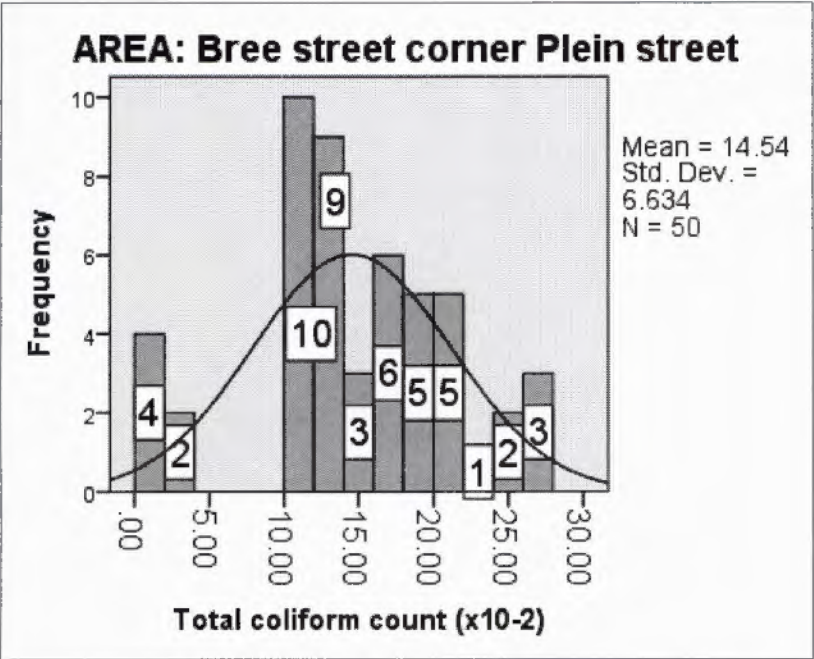


Figure 11 shows that the mean coliform count at Bree Street (corner Plain Street) is 14.54×10^{-2} with $N=50$ and the distribution of coliform count is approximately normal, indicating that there are almost equal upper counts of coliform and lower counts. The modal class of bacterial count is 10×10^{-2} to $<12.5 \times 10^{-2}$.

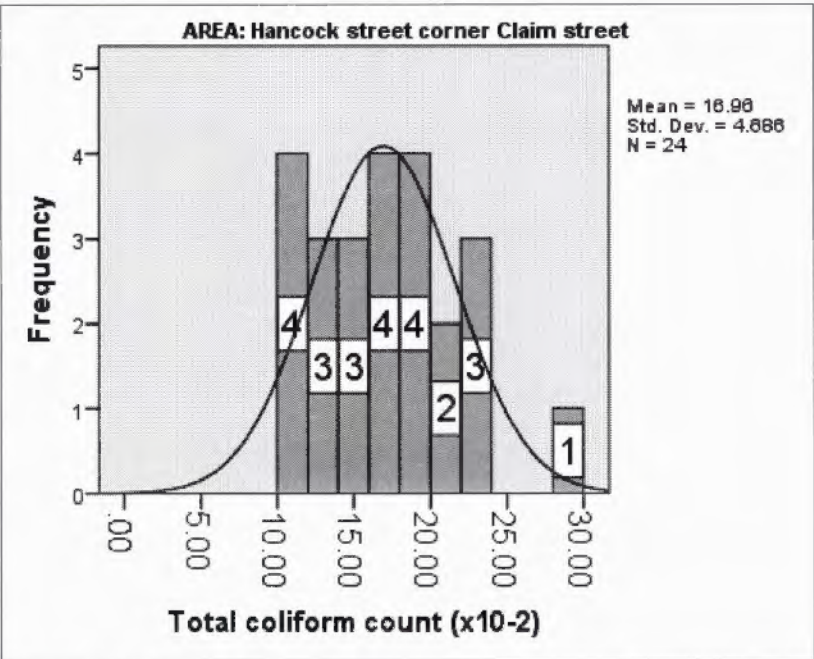


Figure 12 shows that the mean coliform count at Hancock Street (corner Claim Street) is 16.96×10^{-2} with $N=24$ and the distribution of coliform count is approximately normal, indicating that there are almost equal

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upper counts of coliform and lower counts. The modal classes of bacterial counts are 10×10^{-2} to $<12 \times 10^{-2}$, 16×10^{-2} to $<18 \times 10^{-2}$ and 18×10^{-2} to $<20 \times 10^{-2}$.

Appendix 3: Antibiotic resistance profiles of bacterial isolates

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

	<i>aureus</i>									
GIZ 16D4	<i>Enterococcus faecium</i>	1	4mm	20mm	10mm	18mm	20mm	22mm	19mm	19mm
CH11D2	<i>Enterococcus faecalis</i>	2	4mm	18mm	17mm	16mm	17mm	10mm	18mm	5mm