PREVALENCE, SOURCE TRACKING AND ANTIMICROBIAL RESISTANCE OF SELECTED PATHOGENIC BACTERIA IN STREET VENDED FOODS SOLD IN KISUMU CITY, WESTERN KENYA.

BY

FLORENCE AWINO OUMA

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SCHOOL OF PUBLIC HEALTH AND COMMUNITY DEVELOPMENT
MASENO UNIVERSITY

DECLARATION

This thesis is my original work and has not been presented to any university for a degree or any other award.

Florence Awino Ouma

(PG/MSC/090/2008)

Signature:…… Date ……

SUPERVISORS:

This thesis has been submitted for examination with our approval as university supervisor(s).

Prof. Rose Kakai, PhD

School of Medicine

Maseno University,

Maseno, Kenya.

Signature……Date ……

Prof. David Onyango, PhD

Department of Zoology

Maseno University,

Maseno, Kenya.

Signature:…… Date ……

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ABSTRACT

The street food industry plays an important role in developing countries by meeting the food demand of the urban dwellers. There is an increase of food vending in Kisumu city due to poor economy and un-employment. Street foods are frequently associated with diarrheal diseases. The contribution of street-vended foods towards food borne infections is unknown in the city. Moreover, limited studies have been conducted in Kisumu city on pathogens associated with street-vended foods, potential sources and their resistance to commonly used antibiotics. Therefore, this study aimed at determining the prevalence, source tracking and antimicrobial resistance of selected pathogenic bacteria in street vended foods. This study adopted a cross sectional study design where quantitative approaches were employed. These approaches were used to investigate the prevalence, source tracking and antimicrobial resistance of selected microbes in street vended foods sold in the three open air markets within Kisumu city; Kondele, Kibuye and Oile. Systematic random sampling was used to select 62 street-vendors from whom food, water, soil and hand swabs samples were collected. Each market proportionately contributed to the sample size. A total of 248 samples were collected and analyzed using standard microbiological techniques for isolation and identification. The street-vended foods, water used for preparation of street-vended foods, swabs obtained from the vendors hands and soil from the vending environment were inoculated onto xylose lysine deoxycholate agar, hektoen agar and brilliant green agar for isolation of *Salmonella* and *Shigella,* Baird parker agar for *Staphylococcus aureus*, thiosulphate citrate bile salts agar for *Vibrio cholera* and violet red bile lactose agar for *E.coli* and Total *Coliforms*. This was followed by biochemical tests for identification using slide agglutination and homologous antisera. Eight antibiotics namely; ampicillin, amoxycillin, tetracycline, sulphamethoxazole, norfloxacin, nilidixic acid, erythromycin and chloramphenical were examined using standard disk diffusion method on Muller Hinton agar to determine antimicrobial resistance of the isolates. Microbial source tracking was carried out by swabbing of the vendors hands, testing of water used for food preparation and soil from the vending environment. Data was entered in Statistical Packages for Social Sciences (SPSS, version 19). Pearson correlation was performed to determine association between source and food contamination. Descriptive statistics were used to determine the prevalence and occurrences of pathogens in foods, hand swabs, soil, and water samples and to determine the antibiotic susceptibility levels. The data was then presented in tables and graphs. Results of this study demonstrated that the prevalence of enteric pathogens in street vended foods was; 74% Total *Coliforms,* 20% *Staphylococcus aureus* and 6% *E.coli*. *Salmonella. Vibrio* and *Shigella* were not isolated in any street food sampled. A further analysis by Pearsons' correlation indicated that *S. aureus* isolated from the vendors hands and foods was significantly correlated (r^2=0.076; *p= 0.03*). The study finding showed that antimicrobial resistance was observed in members of the *Enterobacteriaceae* and *S.aureus* isolates. Resistance to erythromycin was the most frequent (82.7%), sulfamethoxazole (43.2%), chloramphenicol (35.8%), amoxycillin and ampicillin (33.3%), tetracycline (22.2%) nalidixic acid (17.3%) and norfloxacin (16%). The results suggest that street-vended foods were contaminated with Total *Coliforms*, *E.coli* and *S. aureus* and these isolates exhibited some level of resistance to commonly used antibiotics. Therefore, regular testing and inspection of street-foods should be done by regulatory authorities. Health workers should carry out regular surveillance on resistance pattern of food borne pathogens.

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ABBREVIATIONS

A/A	Acid/Alkaline
AS	Australian Standard
ASPW	Alkaline Saline Peptone Water
ATCC	American Types of Cultures Collection
BGA	Brilliant Green Agar
BPW	Buffered Peptone Water
CDC	Centre for Disease Control and Prevention
CFU	Colony Forming Units
CLSI	Clinical and Laboratory Standard Institute
CPHP	Crop Post Harvest Programme
DCA	Deoxycholate Citrate Agar
ECDC	Early Childhood Development Centre
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organization
FSO	Food Safety Objectives
FSMS	Food Safety Management System
IMVIC	Indole, Methyl Red, Voges Proskaur and Citrate Utilization Tests
ISO	International Organization for Standardization
MRD	Maximum Recovery Diluents
NCTC	National Collections Types of Cultures
PAHO	Pan American Health Organization
PAS	Public Available Specification
RVS	Rappaport Vassiliadis Agar
TCBS	Thiosulphate Citrate Bile Sucrose Agar
TSI	Triple Sugar Iron
WHO	World Health Organization
VRBG	Violet Red Bile Glucose Agar
XLD	Xylose Lysine Deoxycholate Agar
MIC	Minimum Inhibition Concentration

TERMS AND DEFINITIONS

Aetiology: Cause or origin of a disease

Coliforms: Gram negative rod shaped *Enterobacteriaceae* commonly used bacterial indicator of sanitary quality of foods and water.

Contamination: unintended presence or introduction of an extraneous especially infectious material into food or environment.

Diarrhea: Passage of 3 or more loose or watery stools in 24 hours.

Enterobacteriaceae: Are a large family of gram-negative bacilli that normally inhibit the intestines of humans.

Isolate: a pure strain from a mixed bacterial or fungal culture.

Itinerant: A person moving from one place to another.

Pathogen: Infective agents, capable of causing disease.

Phenotypic: An observable physical or biochemical characteristic of an organism as determined by both genetic makeup and environment.

Prevalence: The total number of cases of a disease in a given population at a specific time.

Salmonellosis: Is a food borne illness caused by the genus *Salmonella* with symptoms like nausea, vomiting, abdominal cramps, diarrhea, fever and headache.

Shigellosis: Is a food borne illness caused by the genus *Shigella*.

Street vendor: A person who sells food to another on the streets without an established place of business.

Susceptibility: The likelihood of being affected or tendency to be affected by a specific organism

Street -vended foods: Refers to a wide variety of ready to eat foods and beverages (boiled maize, cooked beans, mandazi, boiled eggs, potato chips, samosas, ice-cream, boiled maize and beans, boiled groundnuts, fried Rastrineobola argentae (*R.argentae)*, home baked cakes, porridge, boiled sweet potatoes, roasted maize and *chapatis*) prepared along the streets and/or sold by vendors in the streets, markets and other public places for immediate consumption or can be taken away and eaten elsewhere.

Reference method: A method thoroughly investigated, clearly and exactly describes the necessary conditions and procedures, for the measurement of one or more property values. Accuracy and precision should be commensurate with its intended use to assess the accuracy of other methods for the same measurement.

Qualitative method: Method of analysis that detects either the presence or absence of the analyte in a certain quantity of a sample.

Quantitative method: Method of analysis whose response is the amount of the analyte measured e.g. enumeration in a mass or volume in a certain quantity of sample.

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CHAPTER ONE: INTRODUCTION

1.1 Background information

Street-vended foods are defined as those foods prepared on the street and ready to eat, or prepared at home and consumed on the street without further preparation (Martins and Anelich, 2000). The street food sector has been growing rapidly all over the world and Kenya is no exception. It is greatly acknowledged that street foods play a significant socio economic role in terms of employment potential, income for women, and in serving the food at reasonable prices to the lower and middle-income groups and even to the high income groups all over the world (Muzaffar *et al.*, 2009).

In many developing countries, a large proportion of ready to eat foods are prepared and sold at public places such as schools, markets, and along the streets. Unlike in developed countries where food preparation in terms of packaging, processing and handling is advance and safety is paramount, in developing countries traditional methods of food processing and packaging, improper storage and poor personal hygiene of food handlers are widespread especially among street vendors (Nicolas *et al.*, 2007). Types of vending sites includes, push carts, road side stalls and hawkers depending upon individual resources, type of food sold and availability of other facilities (FAO, 1990). Street food vendors benefit from a positive cash flow, often evade taxation and can determine their own working hours in this regard, they are likely not to observe critical health issues concerning food handling. Street food vending has been associated with food borne illnesses in many parts of the world.

Food borne illnesses of microbial origin are a major public health problems associated with street foods (WHO, 1984). In addition food borne illness is a major international health problem and an important cause of reduced economic growth (WHO, 1983). Street-vended foods are prone to contamination because they are sold in the open and are often not covered. The main health hazard associated with street foods is microbial contamination.

Microbiological studies from many developing countries, carried out on street vended food have revealed a high bacterial count, *Salmonella* species, *S. aureus* and members of the family *Enterobacteriaceae* as common pathogens found in such food items (Bryan et al., 1997; Mosupye and von Holy, 1999). *E.coli* and *Salmonella* are among the most common causes of gastroenteritis in humans (McComrmick *et al.*, 1993). *E.coli* is recognized as a good indicator of feacal contamination. *E.coli* and other groups of *Coliforms* may be present where there has been feacal contamination originating from warm-blooded animals (Chao *et al.*, 2003). *Salmonella* causes a wide range of human diseases such as enteric fever, gastroenteritis and bacteremia (Gaikwad and Parekh, 1984). It is estimated that food-borne Salmonellosis is responsible for 1.3 million illnesses annually worldwide, resulting in 16,000 hospitalizations and 600 deaths (CDC, 2006). In most developing countries there is scanty documented evidence on food borne infections.

A study done in South Africa reported that not much is known about street food and their contribution in causing food borne illnesses even though this forms a large sector of the

national economy in terms of providing employment and food sales (Steyn *et al.*, 2011). The same situation applies in Kenya, where information on microbial load and safety of street vended foods is unknown. In Kisumu town, the reported diseases which may be considered food-borne are generalized under gastroenteritis or dysentritis which rank third among the top ten diseases in inpatients cases and fifth in the outpatients list (Kisumu East District Development Plan, 2008-2012). Different studies in Kenya showed different level of contamination of street foods (Muinde and Kuria, 2005). For example, a study done in Kisumu found that most of the fish consumed in the town were contaminated with different bacterial species (Onyuka et al., 2011).

There are reported cases of diarrhea and typhoid outbreaks in Kisumu city; these have been closely linked to the limited supply of piped water and poor sanitary conditions, with higher concentration in the peri-urban areas where shallow well water and pit latrines provide alternatives to the conventional water and sanitation systems (Kisumu District Development Plan, 2002-2008). The health conditions are exacerbated by the limited access to the health facilities and relatively high cost of treatment, with majority of the poor resulting into unconventional 'home treatment' modes. It was established that even where the health facilities exist, they often lack drugs for treatment (Kisumu District Development Plan, 2002-2008). Thus many cases of diarrhea and typhoid infection in Kisumu city could be attributed to poor food handling and processing as much as they are associated with water and sanitation conditions. This is supported by the fact that poor sanitary condition has been found to contribute to food borne illnesses (Gitahi *et al.*, 2012).

According to studies done in Africa on street foods, their tremendous unlimited and unregulated growth has placed a severe strain on city resources, such as water, sewage systems and interference with the city plans through congestion and littering adversely affecting daily life (Canet and N'diaye, 1996; Chaulliac and Gerbouin-Renolle, 1996). The same can be said of Kisumu where most of the street food vendors occupy any space in disregard of availability of such facilities. Most of the street food vendors have had either no formal education or few years of schooling and therefore, lack knowledge on proper food handling and their role in the transmission of pathogens (Mensah *et al.*, 2002).

In most developing countries, stalls are often crude structures with no clean running tap water, toilets and adequate washing facilities available. Street-food vendors are often unlicensed, untrained in food safety, food hygiene and sanitation and work under crude unsanitary conditions (FAO, 1990). The vendors may practice poor personal hygiene and there is a possibility of food vendors being carriers and therefore could serve as a potential source of transmission of food borne illnesses. In addition foods cooked on the streets are subjected to cross contamination from various sources such as contaminated utensils, knives, raw foodstuffs, flies that are sporadically landing on the foods, by venders who occasionally serve using their bare hands (Bryan, 1988; Marks *et al.*, 1998; Gorris, 2005). From the initial contamination of raw foods with pathogenic bacteria, vending environment with poor toilet hygiene to subsequent contamination by vendors during preparation; therefore analysis of soil surrounding the vending environment, water used for preparation of foods and hand swabs from the vendors is critical for analyzing

the hazards associated with street foods. Consumers who depend on such foods are more interested in its convenience and usually pay little attention to its safety, quality and hygiene (Mensah *et al.*, 2002). Some consumers may be aware that food borne diseases could occur due to consumption of street food, however the majority disregard these health hazards (Bryan *et al.*, 1988). Therefore, the conditions under which street food preparation and vending occurs raise many concerns related to consumer's health. Studies done in Nairobi, Kenya have indicated that street vended foods are prepared in unsanitary conditions and can results into a source of food contamination and poisoning (Muinde and Kuria, 2005). Very little is known about the contribution of street food preparation, handling and packaging in food contamination in Kisumu city where street food vending is as common as Nairobi.

Several ailments caused by bacterial pathogens are cured by using different groups of antibiotics. These antibiotics are special class of chemotherapeutic agents obtained from living organisms. The emergence of antimicrobial resistance to members of the *Enterobacteriaceae* family is posing major problem in the management of bacterial infections (Ashok, 2008). Water and food contaminated with antibiotic-resistant bacteria is a major threat to public health, as the antibiotic resistance determinants can be transferred to bacteria of human clinical significance (Blake *et al.*, 2003). The prevalence of antimicrobial resistance has increased during the recent decades (Threlfall *et al.*, 2000). This could be partly due to selection pressure caused by indiscriminate use and misuse of antimicrobials including antibiotics given to veterinary animals (Bywater, 2004). Studies carried out in India have shown antimicrobial action of essential oils

against food borne pathogens isolated from food-vended fruit juice (Chandi *et al.*, 2014). Similarly other studies have also shown prevalence of spoilage microorganisms and their drug resistance in street vended foods in Bangladesh (Muzaffar *et al.*, 2009). According to Okeke and Sosa (2004) bacteria cause a significant proportion of infections in Africa and that antibiotic resistance is increasingly compromising the outcome of many infections that were, until recently, treatable in Africa.

Several mechanisms are known to induce antibiotic resistance in bacteria, but the most common type of resistance develops and transmits horizontally via conjugation of a plasmid. Likewise, evolution of multidrug-resistant (MDR) bacterial strains, may create serious threat which results in resistance to several antibiotics (Arslan and Ozdemir, 2008). Under these conditions, the treatment of such illnesses becomes difficult. In Kenya according to (Sang *et al.*, 1997), antimicrobial resistance surveillance has been conducted only at the institutional levels, with limited sharing of information and analysis of data. As a result, the actual scale of regional or national antimicrobial drug resistance is not well known. A study conducted in Asembo, in western Kenya revealed that more than half of the pathogens tested were not susceptible to empiric therapy and that a high percentage of cases of diarrhea is caused by antimicrobial resistance (Shapiro *et al.*, 1999).

In this study, some of the pathogens isolated from soil, hand swabs, food and water included *E.coli*, Total *Coliforms*, and *Staphylococcus aureus*. *Salmonella*, *Shigella* and *Vibrio* were also isolated from the soil samples. The food samples were collected from vendors with diverse serving and selling practices. Water used in the preparation of street

vended foods and samples of hand swabs from the same vendors were also collected and tested. Moreover, the susceptibility patterns of various bacterial isolates from several samples were assessed against the most commonly prescribed antibiotics due to their easy in availability and potency to various microbial infection namely; ampicillin, amoxycillin, tetracycline, sulphamethoxazole, norfloxacin, nalidixic acid, erythromycin and chloramphenicol to suggest effective curative measures for food borne ailments. This study was therefore designed to determine the prevalence, source tracking and antimicrobial resistance of selected pathogenic bacteria in street vended foods sold in Kisumu city, western Kenya.

1.2 Problem Statement

Increased reliance on street foods has been identified as one of the characteristics of urban food distribution system driven by changes in urban way of life and poverty in developing countries. This has been heightened by the daily movement from residential areas to working places that has created need among many working people to eat outside the home more often. However, street foods have raised concern with respect to their potential for serious food poisoning out breaks. In most developing countries there is scanty documented evidence on food borne infections. In Kisumu town, the reported diseases which may be considered food-borne are generalized under gastroenteritis or dysentritis which rank third among the top ten diseases in inpatients cases and fifth in the outpatients list. In Kisumu, the role played by street vended foods is unknown.

In the management of food-borne illnesses of microbial origin, a number of antibiotics are administered due to the susceptibility of the disease causing bacteria to these drugs.

More recently, reports of antibiotic resistance have been published which is in line with a general worldwide pattern of an increasing prevalence of antibiotic resistance, including multiple antibiotic resistances among many groups of bacteria. The local situation needs to be studied to provide information on the patterns of dispersion of antibiotic resistance among the pathogenic bacteria. While the immediate public health harm is from food-borne illness following ingestion of contaminated product, infection by antimicrobial resistant strains can lead to more severe diseases. This situation is further complicated by the potential of resistant bacteria to transfer their resistance determinants to resident constituents of the human micro flora including pathogens. Information on performance of food borne pathogens isolated from street vended foods to the commonly administered antibiotics is limited in Kisumu city.

1.3 Justification and Significance of the Study

Food borne illnesses resulting from the consumption of contaminated vended food may results in serious health complications in life. In this regard knowledge on sources of contamination and appropriate medication thereafter is necessary. Food borne diseases encompasses a wide spectrum of illnesses caused by multitude of microorganisms and those caused by chemical hazards and are a growing public health problem. Street foods are considered as a major health risk for causing food borne diseases, especially in developing countries, since the hygienic aspects of processing and vending operation are a major source of concern for food control. A study on analysis of public health risks associated with perishable food vending in Kisumu city recommended that a further research on microbial safety analysis of street vended foods be done to establish the

extent of contamination with respect to specific pathogenic microorganisms such as *Salmonella* and *Shigella* (Ooro, 2008).

The source of pathogens in street vended foods is an important assessment and will help to source tract the origin of contamination. The prevalence of antimicrobial resistance has increased during the recent decades (Threlfall *et al.*, 2000) partly due to selection pressure caused by indiscriminate use and misuse of antimicrobials including antibiotics given to veterinary animals (Bywater, 2004). The emergence of antimicrobial resistance to members of the *Enterobacteriaceae* family is posing major problem in the management of bacterial infections (Ashok, 2008). Water and food contaminated with antibiotic-resistant bacteria is a major threat to public health, as the antibiotic resistance determinants can be transferred to bacteria of human clinical significance (Blake *et al.*, 2003). Information gathered in this study will give the prevalence of enteric pathogenic microbial contamination of vended foods and their resistance to selected antibiotics. Policy makers may use this information to develop standards and policy on safety of street vended foods.

1. 4 Objectives

1.4.1 General objective

To determine the prevalence, source tracking and antimicrobial resistance of selected pathogenic bacteria in street vended foods sold in Kisumu city.

1.4.2 Specific objectives

i. To determine the prevalence of Total *Coliforms, E.coli, Staphylococcus aureus, Salmonella* species, *Shigella* species, *and Vibrio cholera* in street vended foods sold in Kisumu city.

ii. To identify the possible sources of microbial pathogens from the street vendors hands, water used in the preparation of street vended foods and soil from the surrounding vending environment in Kisumu city.

iii. To determine the resistance of the isolates to commonly used antibiotics in Kisumu city.

1.4.3 Research Questions

i. What is the prevalence of *Coliforms, E.coli, Staphylococcus aureus, Salmonella, Shigella* and *Vibrio* in street vended foods in Kisumu city?

ii. What are the possible sources of microbial pathogens in street vended foods in Kisumu city?

iii. What is the level of susceptibility of the isolates to the commonly used antibiotics in Kisumu city?

CHAPTER TWO: LITERATURE REVIEW

2.1 Prevalence of Pathogens in Street Vended Foods

Contamination of street vended food in many countries is common due to processing conditions, improper handling and prevalence of unhygienic conditions contributing substantially to food borne illnesses. Street-vended foods are defined as those foods prepared on the street and ready to eat, or prepared at home and consumed on the street without further preparation (Martins and Anelich, 2000). The types of street foods sold vary greatly between countries. However, most meals consist of the staple food served in various forms and in combination with side dishes such as stews, gravies and spices (Tomlins and Johnson, 2004). In addition, snacks such as dried meat, fish and cereal based ready to eat foods are also prepared and served. Street food vending is therefore a source of a wide range of foods that may be nutritionally important for various groups of the population.

There is a general perception that street-vended foods are unsafe, mainly because of the environment under which they are prepared and consumed, which exposes the food to numerous potential contaminants. Street food vendors usually take their products to their customers and therefore operate from such places as bus terminals, industrial sites, market places and other street corners where there are ready and numerous clients. In countries, where street food vending is prevalent, there is commonly a lack of information on the incidence or prevalence of food borne diseases related to the street vended foods. However, microbial studies on such foods in American, Asian and African countries have revealed increased bacterial pathogens in the food (Kumar *et al.*, 2006).

Some studies, however, have shown that food prepared on the street can also be safe, thereby providing alternative outlets for consumers (Von-Holy and Mokhoane, 2006).

There exist an ample discussion on street food vending in literature and a large portion of it is concentrated on the health and hygiene. In India, the street food trade is a growing sector with its expansion linked with urbanization and the need of urban populations for both employment and food. However, the microbiological status of popularly consumed street foods, general hygienic and vending practices are not known (Ghosh *et al.*, 2007). This therefore means that the knowledge on prevalence of microbial contamination of food is important in order to safeguard against food borne illnesses. According to studies done in Africa on street foods, their tremendous unlimited and unregulated growth has placed severe strain on city resources, such as water, sewage systems and interference with city plans through congestion and littering adversely affecting daily life (Canet and N'diaye, 1996) . This has also had an impact on the microbial quality of food vended in most of their streets and health of the people.

According to a bacteriological study in Ethiopia, it was found that street vended foods in Gondar city were contaminated with different pathogenic bacteria as a result of poor sanitary conditions among other factors posing a health hazard to the inhabitants (Derbew *et al.*, 2013). However, a study done in South Africa (Von-Holy and Mokhoane, 2006) found that street food vendors were capable of producing relatively safe food with low bacterial counts, although there was still need for proper hygiene conditions and access to basic sanitary facilities. On a similar note, a study done on street vended foods in Western African countries found several contamination mechanism and suggested improvement pathways (Barro *et al.*, 2006). They recommended the Food safety

objectives (FSO) concept developed by FAO and WHO to aid governments in elaborating guidance for street food production, vending and consumption to ensure acceptable microbial quality is maintained.

A profile of street food vending was conducted in Botswana (Ohiokpehai, 2003) where the focus was on the content and nutritional impact of street food for people where some segments of the population rely almost entirely on street foods for their everyday meals. In Ghana and elsewhere, food vendors are noted for selling foods and drinks at reduced prices, thus providing more affordable means for people to obtain nutritionally balanced meals outside the home (FAO/WHO, 1997; Marks *et al.*, 1998). Although street food has become an indispensable part of both urban and rural diets, some public health risks are associated with the consumption of street foods. While it is expected that street food meets the nutritional needs of consumers, it is also necessary to ensure its safety from contaminants and microorganisms to avoid food borne illnesses (Acar and Rostel, 2003).

There is a noticeable increase of food vendors in Kenya. This is clearly evident in Kisumu, where they sell both raw and cooked food items along the streets and markets. Kisumu city has high levels of skilled and unskilled employment with an employment rate of 30%, where 52% of the working population engages in informal activities with a monthly wage in the range of Kes 3000-4000 (Kisumu East District Development Plan, 2008-2012). Available statistics indicate that Kisumu, which is a net food importer, registers one of the highest incidences of food poverty with 53.4% of its population living below the food poverty line as compared to Nairobi (8.4%), Mombasa (38.6%) and Nakuru (30%) (Kisumu East District Development Plan, 2008-2012). The street food

vending business is therefore contributing a significant income inflow for households involved in selling these foods.

A study done in Nairobi Kenya (Muinde and Kuria, 2005) identified lack of basic hygiene and sanitary practices both in the case of serving as well as preserving food in this sector which may lead to food contamination with bacteria. Their study emphasized the need for the establishment of street food centers by the city council, training of street food vendors on hygiene, sanitation and the establishment of code of practice for the street food industry and the empowerment of public health officers. A similar study conducted in Mauritius echoed that of Muinde and Kuria by highlighting on the need for health education among food vendors (Subratty *et al.*, 2004).

Food safety rules and regulations in many African countries indicates that the informal food distribution sector often escapes formal inspection by regulatory authorities, mainly because most vendors operate without licenses and from un-designated places. Many of the vendors are itinerant. In some countries, such as Kenya, vendors operating from undesignated places are forcefully removed from the vending sites, mainly because their activities violate existing laws governing the sale of food. However, in many African countries, food control programmes still need to be strengthened (FAO/WHO, 1997). The food safety laws of Kenya chapter 242 and 254 respectively apply mostly to established food premises that have to be inspected before licensing. Part 2 section 3 of Cap. 254 prohibit the sale of unwholesome, poisonous or adulterated food. Cap 242, part 11 sections 131 also prohibit the sale of unwholesome food. These are general laws that do

not apply to street food only. Chapter 242, section 135 sub section A, states that the Minister for Public Health and Sanitation shall make rules requiring Medical examination of persons handling any particle of food that, it is unlawful for anyone to sell food without undergoing medical examination and issued with a medical certificate.

2.2 Aetiology of food-borne illnesses

Food borne illness is any syndrome resulting from the consumption of contaminated food. Microbial food-borne illness commonly occurs throughout the world. Food borne illnesses are caused by a variety of pathogenic bacteria, viruses, parasites, toxins and metals (Bryan, 1988). Bacteria are the major causative agents of food-borne illness in which 60% of cases are severe enough to require hospitalization (Mead *et al.*, 1999). It is estimated that about 2.1 million children in developing countries die due to diarrheal-related illnesses annually as a result of bacterial infections (Bern et al., 1992). It is also suspected that food or water is the vehicle for many of these illnesses (WHO, 2004). Because food is biological in nature and is capable of supplying consumers with nutrients; it is equally capable of supporting the growth of contaminating microorganisms especially bacteria.

Three types of bacterial food borne diseases are recognized: intoxication, infections, and toxicoinfections. Food borne bacterial intoxication is caused by ingestion of food containing preformed bacterial toxin, such as toxins produced by *Staphylococcus aureus* and *Clostridium botulinum*, resulting from bacterial growth in food. Food borne infection, on the other hand, is caused by ingestion of food containing viable bacteria such as *Salmonella* which then grow and establish themselves in the host, resulting in illness.

Food borne toxicoinfections result when bacteria present in food, such as *Clostridium pefringens,* are ingested and subsequently produce a toxin in the host. The majority of these are *Enterobacteriaceae,* including pathogens such as *Escherichia coli*, fecal *Coliforms*, and *Salmonella* spp., many of which are causal agents of human food poisoning (Murray, 2005).

Analysis of microbial load on the MacConkey agar is therefore a more reliable indication of whether a food is contaminated with potentially pathogenic bacteria (although some non-enteric bacteria such as *Staphylococcus aureus* also cause food poisoning). The enteric bacteria isolated on the selective agar can arise from a number of sources of contamination throughout the food production process (Hobbs and Roberts, 1993). Meat, poultry and eggs are frequently contaminated with bacteria such as *Coliforms* and *Salmonella* species on the farm. Inadequate storage or subsequent cooking then allows the bacteria to multiply and is more likely to induce disease when eaten. Enteric microbes can also be spread from person to person, via the feacal-oral route, due to inadequate hygiene following contact with feaces.

Handling raw food and inadequate cooling and reheating of cooked food are also common causes of high bacterial growth on food. Contaminated water flies and pets may also spread bacteria. The presence of pathogenic bacteria in food is a health risk, and although it increases the risk of becoming ill, there are other factors involved. One of these is the amount of bacteria on the food and the infective dose for each bacterium. Bacterial strains such as *E. coli* 0157: H7, which causes food poisoning that can progress

to serious complications such as kidney failure and death, has an infective dose of around 100 bacteria. Species of *Salmonella* require at least 100,000 bacteria to be ingested before food poisoning symptoms are seen (Muleta and Ashenafi, 2001), so very low numbers of viable organisms are unlikely to cause disease. Symptoms of food poisoning may vary in degrees and combinations and includes; abdominal pains, vomiting, headache and diarrhea. More serious cases can result in life threatening neurological, hepatic, and renal syndromes leading to permanent disability or death.

2.3 Sources of Food borne-pathogens

The major concern with street foods is their microbiological safety, mainly because vending is done in places that may have poor sanitation. Food that is safe to eat should be from a safe source and received from sources that have quality assurance and safety (Blake *et al.*, 2003). Concerns over the safety and quality of street-vended foods have been raised, because the vendors lack knowledge of basic food safety issues (Bryan *et al*., 1988). Personal hygiene practice of food vendors is the most important factor to be looked into in the production process (Theodore and Minor, 1976). Food service operators and cooks with cuts and wounds should not be allowed to prepare food because of the risks that they bring together with them. The potential spread of bacterial disease such as *Staphylococcus* is high if they are allowed to prepare meals. *Staphylococcus* bacteria cannot be eliminated but can be reduced through frequent hand washing with warm water and soap (Attekruse *et al.*, 1995). Street vending sites are usually stalls and carts that are inefficiently constructed, running water is not easily accessible, and hand and dish washing is done in the same bucket, sometimes without soap. Waste water is

usually discarded in streets and garbage is discarded in the nearby areas providing attraction of flies, the foods remains hence habour insects and rodents. In many cases toilets are not available, thus forcing the vendors to visit nearby areas for call of nature and return to their vending sites without washing their hands (Bryan, 1988).

Poor sanitation and hygiene practices as well as other such conditions and practices are likely to lead to cross-contamination of cooked foods (Ekanem, 1998). Food items received especially chilled items; the temperature should be below 5^0C, while frozen items should be below -10^0C, whereas for dry items the store rooms temperature should not exceed 27^0C (FDA, 2004). These conditions are not attainable in street vended foods. In other cases vendors buy raw materials from un authorized sources which may be contaminated with food borne pathogens or are unfit for human consumption due to other reasons (Dawson and Canet, 1991). *E. coli* is the most commonly isolated species of the family *Enterobacteriacae* in most vended food (Brown, 1995). Other less prevalent species include *Citrobacter*, *Enterobacter* and *Klebsiella* (Elumd et al., 1999; Edberg et al., 2000). Most *E. coli* are harmless and originate from the digestive systems of animals. *E. coli* are not always confined to the intestine, and their ability to survive for periods outside the body makes them an ideal indicator organism to test environmental samples (soil and water) for fecal contamination. However, some strains of *E. coli* such as *E. coli* O157:H7 can cause serious health concerns (Armstrong et al., 1996).

2.4 Antibiotic Susceptibility and Food Borne Bacteria

When a bacteria can tolerate higher concentrations of an antimicrobial than phenotypically related bacteria of the original strain (Acar and Rostel, 2003), it is defined as being resistant. Such isolates are phenotypically different from the wild type because of their acquisition of a resistance mechanism either by gene transfer or mutation (acquired resistance). These bacteria may originate from various sources, including animals, the environment and humans. These bacteria do not have primary reservoir in food animals, but can be spread from humans to food directly or indirectly through the environment, including water and by foodborne.

These resistant commensal bacteria may originate from various sources, including animals, the environment and humans. Commensal bacteria are those bacteria that live in or upon the host without causing disease. Mostly, this co-existence is of mutual benefit. However, many Commensal can cause disease if they enter body sites that are normally sterile or when the host's immune defense is impaired (Sharp, 1999). Commensal *E.coli* from the intestines of animals and humans contaminate food of animals origin, vegetables and water and may carry transferable resistance genes (Sunde and Nostrom, 2006). One of the best specific examples of spread of resistance genes from animal to human bacteria is through transposon-encoded streptothricin resistance (Tshape, 1994). Following the introduction of norseothricin for use as a growth promoter in pig production, resistance emerged in commensal *E.coli* of pigs and farmers, and later in the urinary isolates of *Salmonella, E.coli* and *Shigella* in humans causing disease in humans (Hammel *et al.*, 1986).

Antimicrobial-resistant food borne pathogens are acquired primarily through consumption of contaminated food or water (Mead *et al.,* 1999). The issue of antimicrobial resistance is of worldwide concern. The Early Childhood Development Centre (ECDC) in its review of 2005 data on communicable diseases in Europe, identified antimicrobial resistance as a major problem in European health care, and one that undoubtedly prolongs patients suffering, cost money and is responsible for the death of thousands of citizens each year (ECDC, 2007). Studies have shown that antimicrobial resistance in developing countries is most likely related to the frequent unrestricted use of over the- counter drugs without medical supervision (Grenet et al., 2004; Sang et al., 2011).

The emergence and spread of antibiotic resistance in bacteria is of medical importance and poses serious constraints on the options available for the treatment of many infections. This problem has been brought into prominence by the recent widespread outbreaks of enteric diseases caused by drug resistant organisms (Sang et al., 2011). Among enteric pathogens, major epidemics of infection with antibiotic resistant *Shigella* have occurred in Latin America, Asia and Africa (Nys et al., 2004; Hoge et al., 1998). High frequencies of antimicrobial resistance among members of the *Enterobacteriaceae* family isolated from clinical cases within the Lake Victoria region of Kenya have also been reported (Shapiro *et al.,* 1999; Onyango *et al.,* 2009). Generally studies within the Lake Victoria region have focused on the burden of diarrheal infections among human. In these studies the entero-bacterial species responsible for diarrheal infections have been found to be relatively resistant to commonly used antibiotics. Resistance to antibiotics in

food borne pathogens may create problems for disease or illness treatment, while antibiotic susceptibility leads to healing of illness which the organism caused.

Information on the magnitude of the public health burden due to resistant food borne pathogens shows that the situation is complex and differs by country (WHO, 2004). It is influenced by a number of variables such as antimicrobial use practices in farming, process control at slaughter houses, storage and distribution systems, the availability of clean water, and proper cooking and home hygiene, among others (WHO, 2004). While the immediate public health harm is from food borne illness following ingestion of contaminated product, infection by antimicrobial-resistant strains can lead to more intractable and severe disease (Martins and Anelich, 2000; Helms *et al.*, 2002). While there is much disagreement on the health burden imposed by resistance in food borne bacterial pathogens, it is generally agreed that the use of antimicrobials, whether for growth promotion, prevention, or treatment, can select for resistant bacteria pathogens, and that these pathogens can be transmitted on food originating from factories processing treated animals.

Several epidemiological studies have demonstrated antimicrobial use in animals and the subsequent isolation of resistant bacteria from the same animals. In addition, evidence shows that resistant isolates from food animals can reach humans via the food supply (Smith *et al.*, 1999; Fey *et al.*, 2000; Gupta *et al.*, 2003). However, additional quantitative information is needed on the magnitude and nature of the contribution of agricultural antibiotic use, and its impact on the severity of food borne bacterial disease (Smith *et al.*,

2002; Philips *et al.*, 2004) especially in Kenya. Even though most animal health pathogens remain generally susceptible to antimicrobials, emerging resistance phenotypes have been documented among several important zoonotic gram-negative bacteria pathogens.

Spread of resistance genes between bacteria colonizing animals and man has been shown. For example, some studies have shown that the same R plasmids can be transferred between bacterial strains from bovines and humans (Oppegaard and And-wateson, 2001). Some categories of food may often be contaminated with *E.coli*, including resistant isolates (Sunde and Nostrom, 2006), and these bacteria reside long enough in the intestines of humans to be able to transfer resistance genes to the residential flora. It is therefore highly probable that food is a vehicle for spread of resistance genes between different ecosystems.

2.5 Methods in Food Microbiology

Microbial analysis of foods is an integrated part of management of microbial safety in the food chain. Both control authorities and individual food business operators use microbial analysis for the purpose of monitoring of the actual situation and trend analysis in order to detect emerging trends (Umoh and Adoba, 1999). In this study standardized methods (e.g. ISO methods) were used as reference analytical methods for analysis. These standardized classical methods are still in use by many laboratories especially by regulatory agencies, because they are harmonized and validated methods in food diagnostics and thus overall well accepted (Rosmini *et al.*, 2004). These standardized

methods employs the use of selective nutritious broth or agar media to grow, isolate or enumerate the target organism while suppressing the indigenous background flora of the food they reside in (Umoh and Adoba, 1999).

However, a serious drawback is that, although they demand no expensive infrastructure and are rather cheaper in consumables, they are laborious to perform, demand large volumes of solid, liquid media, reagents and encompass time-consuming procedures both in operation and data collection. During the last decades interest has risen to the development of more rapid methods that reduces the time taken to obtain a microbiological test result (Feng, 1996). Other methods used to enumerate bacteria in food samples especially if the numbers are suspected to be low (>50/g) is the Most Probable Number (MPN) (Barro *et al.*, 2006) . With the MPN method dilutions of food samples are prepared as for plate count method. Serial dilutions are then transferred into appropriate liquid medium, after incubation the number of positive tubes for each dilution is counted. On the basis of the number of positive tubes for each dilution MPN is determined by use of standard MPN table and taking into account the dilution factor.

Total *Coliforms* are members of the *Enterobacteriaceae* and include genera that originate from feaces; called Fecal *Coliforms*, such as *Escherichia*, as well as genera that are not of fecal origin, called the non-Fecal *Coliforms* like *Enterobacter*, *Klebsiella* and *Citrobacter*. Coliforms are facultatively-anaerobic, rod-shaped, gram-negative, non-sporulating bacteria. These mostly harmless bacteria live in soil, water, and the digestive system of animals. The historical definition of this group has been based on the method

used for detection of lactose fermentation rather than on the tenets of systematic bacteriology (APHA, 2003). This study used Violet red bile lactose agar for the isolation of *E.coli* and Total *Coliforms*. Other studies have determined microbial load in *Enterobacteriaceae* using MacConkey agar and have reported reliable results (Hobbs and Roberts, 1993).

Fecal *Coliforms* are facultative anaerobes, rod-shaped, gram-negative, non-sporulating bacteria. They are capable of growth in the presence of bile salts or similar surface agents, they are oxidase negative, and produce acid and gas from lactose within 48 hours at 44 ± 0.5ºC. Though fecal *Coliforms* are usually not pathogenic, their presence may indicate the presence of other pathogenic bacteria for example, *Salmonella, Shigella,* and *Vibrio*. They are hence considered to be indicator organisms. Pathogens are typically present in such small amounts that it is impractical to monitor them directly.

Accurate determination of bacterial susceptibility to antibiotics is essential to the successful management of bacterial infections and to the comparative analysis of antimicrobial agents. This can be done by a number of techniques, which include the disc diffusion method, the broth dilution assay and the Epsilometer test (Etests). The effectiveness of antibiotics can be assessed by their ability to suppress bacterial growth, described by the MIC, or by their ability to kill bacteria, characterized by the minimal lethal concentration (MLC). MIC is usually derived by means of tests in solid media, whereas both MIC and MLC can be determined in broth dilution assays. A number of reports have been dedicated to comparing the effectiveness of these methods. Clinical

resistance cannot therefore, be predicted by in vitro tests alone. The Minimum Inhibitory Concentration (MIC) of a drug for a bacterium isolated from clinical samples is used for guidance purposes. A bacterium isolate is categorized as resistant when the obtained MIC of the drug is associated with a high likelihood of therapeutic failure for treatment with the drug. To facilitate the interpretation, threshold values or break points are defined by national or international committees on the basis, for example pharmacokinetics, clinical trials and microbiology. In this study antibiotic resistance of the isolates was determined by the disk diffusion method using the standard procedure of the Clinical and Laboratory Standard Institute (CLSI) 2009M2- A9. Similar study conducted in Nigeria on incidence of antibiotic resistance in some bacterial pathogens from street vended foods (Oladipo and Adejumobi, 2010) also employed the same method.

2.6 Operational Framework

Background information **Proximate Factors** **Outcome Factors**

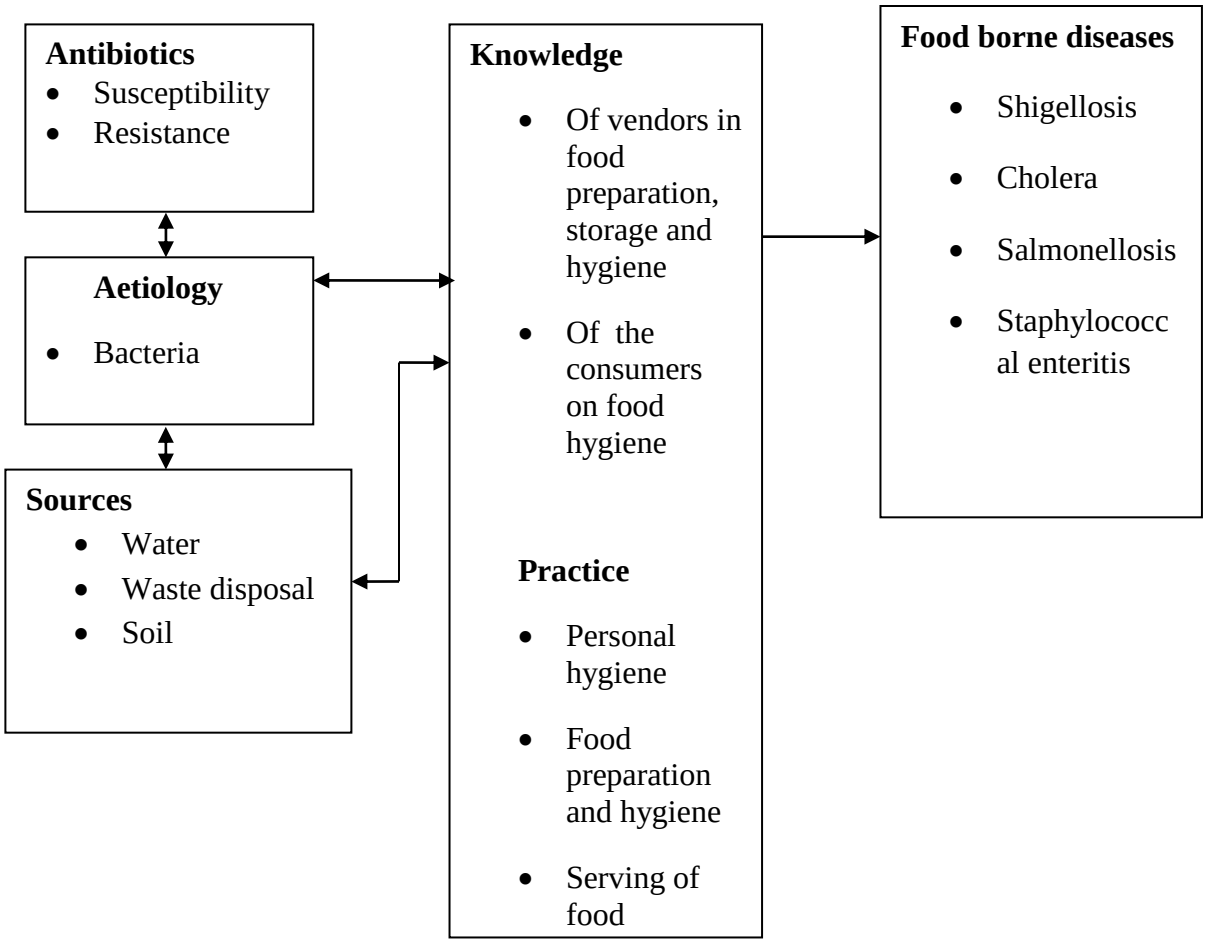


Figure: 2. 1: Operational Framework

Operational framework is divided into three categories namely; background information, proximate factors and outcome factors. Background information has sourced information on the global, regional and local perspective on street vended foods. The main causative agent of food borne illnesses under the three perspectives is bacteria. However some bacteria have developed resistance towards the common antibiotics. Sources of these causative agents are water, soil, and waste disposal.

Sources are mainly attributed to poor practices on personal hygiene, food preparation, food services and failure to enforce food regulation policy. Some of the common problems faced by street food vendors globally, regionally and locally includes lack of knowledge of street food vendors on food preparation, food storage and also lack of knowledge of the consumers. Therefore, background information and proximate factors results in health outcomes of foodborne diseases.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study Design

This was a cross-sectional study which was done over a period of four months (March-July 2011). Across sectional study was chosen since it gives a snap shot of the situation within a given period of time. Quantitative and qualitative approaches were used for data collection, analysis and presentation. Commonly vended street foods were sampled and analyzed for the presence of pathogenic bacteria.

3.2 Study Site

The study took place in the open- air markets of Kibuye, Kondele and Oile in Kisumu city (Figure 3.1). These sites were chosen because of the higher concentration of food vendors serving most residents within Kisumu city. Kisumu City is the third largest city in Kenya, and is located on the Eastern shores of Lake Victoria, which is the second largest freshwater lake in the world. Kisumu city is a fast growing urban center currently with a population of approximately 500,000 (Kenya population census 2009). Annual temperature ranges between 17.3 to 32^{o}C. Kisumu city is located at latitude of 0.1 South, longitude of 34.76 East at an elevation/altitude of meters. The average elevation of Kisumu city is 1131 meters. Kisumu City has a number of hospitals and outpatient centers. As at January 2012, there were 62 health facilities in Kisumu city both, private, public, non –governmental and faith based organizations (Maoulidi, 2011).

Map Showing Markets within Kisumu City

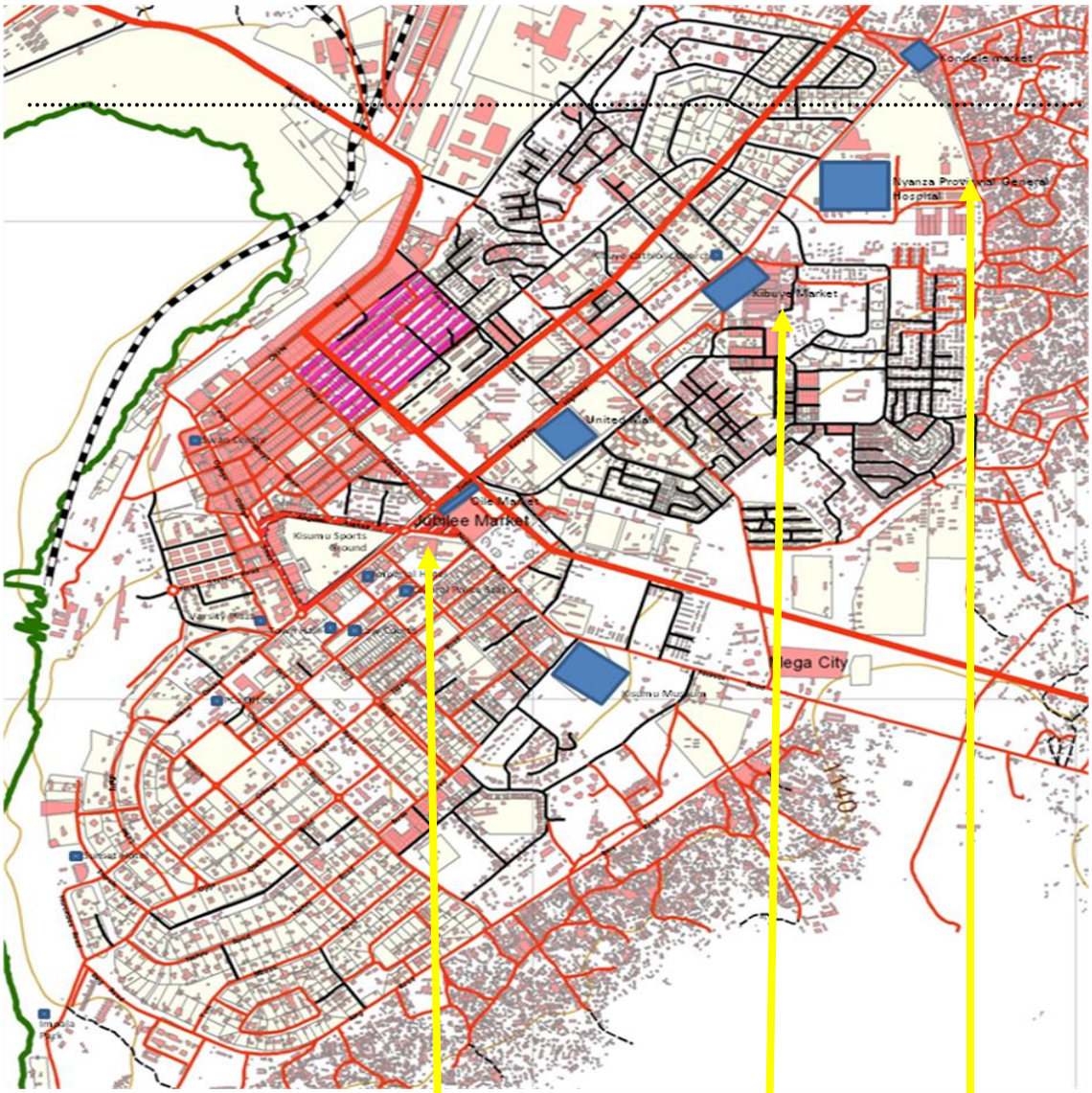


Figure: 3.1 Study Market

3.3 Study Population

The populations used in this study were 62 street vendors in the open- air markets of Kibuye, Kondele and Oile in Kisumu city. The markets were purposively selected due to their high population of street food vendors.

3.4 Sample Size Determination

Sample size was determined using Fisher's formula for sample size determination for a population less than 10,000 people (Mugenda and Mugenda, 1999).

$n=Z^2pq/d^2$

Where,

n = the desired sample size (if the target population is > 10,000).

Z = is the standard normal deviate at the required confidence level.

P = is the proportion in the target population estimated to have characteristics being studied.

q = 1-p =0.95

d = the level of statistical significance set ≤ 0.05

Z = Assuming 95% confidence interval Z =1.96

P = The current prevalence rate of sporadic bloody diarrhea in rural Western Kenya attributed with consumption of street vended foods was 5% as recommended by Brooks *et al*.,(2003). So, p = 0.05

$n = (1.96)^2 (0.050) (0.95)/0.05^2 =72.99 = 73$ when the study population is >10,000.

Kisumu municipal council records and a base line survey done indicated that there were approximately 422 street food venders in Kondele, Kibuye and Oile markets of Kisumu city. Since the target population is less than 10,000 (Mugenda and Mugenda, 1999) this informed the formula:

nf =n/1+n/N

Where,

nf = desired sample size when the population is less than 10,000

n = desired sample when the population is more than 10,000 =73

N =estimate of the population = 422

nf = 73/1+73/422 = 62.2 = 62 samples

A total of 62 street food vendors were recruited and from each vendor 4 samples were collected (soil from the vending environment, swab from the vendors hands, water used in preparation of vended foods and foods offered for sale each was collected making a total of 248 samples).

This was proportionately divided amongst the three study markets based on the population size as shown in (table 3.1)

Table: 3. 1 Sample size per study market (as per Kisumu municipal council records)

Vending site(s)	Population size	Sample size	Total samples Collected (food, soil, water and hand swab
Kondele	63	63/422×62 = 9	9×4=36
Oile	157	157/422×62 = 23	23×4=92
Kibuye	202	202/422×62 = 30	30×4=120
Total	422	62	248

3.5 Inclusion Criteria

In this study only ready to eat foods presented for sale at the time of samples collection were sampled for laboratory analysis. All vendors who consented at the time of sample collection were included in this study.

3.6 Exclusion Criteria

Foods not vended at the time of sample collection were not included in this study therefore samples were only collected the street food vendors present at that particular time. Venders who did not consent were also excluded from this study.

3.7 Study Limitation

Food vending is wide spread in almost every street within the city, but due to limited finances and time, only selected areas were studied. Also, different antibiotics are currently used to treat infections but due to limited finances only the selected antibiotics were subjected for susceptibility tests.

3.8 Sampling Procedure and Sample Collection

Systematic random sampling was used to collect samples from the three study sites Oile; Kibuye and Kondele markets. The population of the vendors and the required sample size per market was determined as indicated in Table 3.1. Informed consent of the food vendors was sought (appendix 4) and finally all the food vendors were numbered. Systematic random sampling was used based on the sample population against the required sample size. Based on the population of street vendors (422) and the calculated sample size (62) every seventh vendor was sampled to participate in the study. To determine the possible sources of food contamination, similar vendors' hands from where the foods were collected were swabbed and a total of 62 hand swabs were collected, 62 samples of soils from the similar vending environment were also collected and 62 samples of water used by the similar vendors for food preparation, washing and rinsing of hands were also collected and analyzed.

Samples of foods, soil and water each weighing 100g/ml-150g/ml were collected in a sterilized glass bottle and transported to the laboratory. Similarly swabs from the vendors hands were inoculated in a sterilized glass bottle containing 10ml of diluents. Samples

were collected at the beginning and the end of the market supply. All samples collected were stored in sterile containers kept at 4^{o}C and analyzed within 12 hours after collection. Aseptic techniques were applied during sample collection. Pathogens isolated from the soils, swabs, foods and water were subjected to commonly used antibiotics and categorized as resistant, susceptible or intermediate according to the interpretation of the zone standards recommended by Clinical Standard Institute (CLSI, 2009) (appendix 5) to determine the antimicrobial susceptibility.

3.8.1 Preparation of test samples (ISO 6887:2003)

Preparation of samples was done in accordance with ISO 6887 part 1: General rules for preparation of initial suspension and decimal dilutions for microbiological examination. For food samples 30g of test sample were weighed in a sterile Stoppard bottle of at least 500ml capacity. 270ml of maximum recovery diluent was then added for *Staphylococcus aureus, E.coli, Coliforms* and similar quantities of buffered peptone water for *Salmonella*, *Shigella* broth containing novobiocin for *Shigella*, and alkaline saline peptone water for *Vibrio cholera* and mixed thoroughly by shaking.

The mixtures were transferred into a sterile stomacher bags; and blended for 30 seconds. Using a fresh sterile pipette, 1ml of the emulsified sample (10^{-1}) was transferred into 9ml of sterile diluents to make (10^{-2}) dilution. This was repeated with further dilutions using fresh sterile pipette for each decimal dilution depending on the product. Soil samples were processed by transferring 30g into a pre-sterilized bottle. 270mls of sterile buffered peptone water was added and mixed for 2 minutes and then filtered through a pre-

sterilized 28μm-pore size nylon filter. The filtrate was then used to recover *Salmonella, Shigella, Vibrio cholerae, E.coli* and *Enterobacteriaceae* by direct plating on selective media. For water and swabs 10ml of samples were added to 90ml of buffered peptone water for *Salmonella*, and similar quantities for *Shigella* broth containing novobiocin for *Shigella*, and alkaline saline peptone water for *Vibrio cholera* and mixed thoroughly by shaking. The mixture was then analysed as per water methods (ISO6887-1, 1999).

3.8.2 Enumeration of *Staphylococcus aureus* in food and soil (ISO 6579:2002)

Using a sterile pipette, 0.1ml of the prepared sample (3.8.1) and the serial dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}) were transferred to the surface of prepared Baird parker agar plate in duplicate. The inocula was spread carefully starting from the lowest to the highest dilution as quickly as possible over the surface of the agar plates using a sterile glass spreader for each plate avoiding as far as possible contact with the side of the Petri dishes. The plates were covered and left on the bench for 15 minutes for absorption of inocula to take place. Also, one prepared and dried Baird parker plate was used as control for checking sterility. The inoculated plates were inverted and incubated at 37^0C. Typical colonies appeared as black shinning and convex surrounded by a clear zone that is partially opaque, 1mm to 1.5mm in diameter after incubation for 24hours and 1.5mm to 2.5mm in diameter after incubation for 48 hours. Atypical colonies appeared as grey colonies free of clear zones (ISO6579, 2002).

Confirmation was done using coagulase test. Five typical and five a typical colonies were transferred into a tube of 5ml Brain –Heart Infusion broth and incubated at $37^0C \pm 1^0C$ for

24 hours. Aseptically, 0.1ml of each culture was added to 0.3ml of rabbit plasma. The tube was examined after 4-6 hours for clotting of the plasma. Coagulase test was considered positive if the volume of the clot occupied more than half of the original volume of the liquid. The blank/control plasma showed no signs of clotting. The number of *Staphylococcus aureus* (N) present in the sample was calculated as shown:

$N=\Sigma a/v\ (n1+0.1n2)\ d$

Where:

Σa =Sum of confirmed *Staphylococcus aureus* colonies in all the dishes

V = Volume of inoculum applied

n1 =Number of dishes selected from the 1^{st} dilution

n2 = Number of dishes selected from the 2^{nd} dilution

d =dilution factor corresponding to the 1^{st} dilution

3.8.3 Detection of *Salmonella* species in food and soil (ISO 6579:2002)

In pre-enrichment stage the prepared food samples (3.8.1) were incubated at 37^0C for 16 - 20 hours, 10ml volumes of Rappaport- vassiliadis broth was inoculated with 0.1ml of the pre-enrichment culture. Similarly, 10ml of tetrathionate broth was inoculated with 0.1ml of pre- enrichment culture. Rappaport- vassiliadis broth was incubated at 41.5^0C for 18-24 hours, and the tetrathionate broth at 37^0C for 20-24hours. A loop was taken from each enrichment tube and streaked onto the surface of Brilliant Green agar and Xylose Lysine Decarboxylase agar. The plates were incubated at 37^0C for 18-24 hours. The plates were examined for the presence of typical bacterial colonies and confirmation was done using biochemical tests.

Typical *Salmonella* colonies were characterized as follows: on XLD agar typical colonies appeared black centres and a lightly transparent zone of reddish colour due to colour change of the indicator on BGA, pink colonies with bright red surrounding medium was observed. Confirmation was done by streaking selected colonies onto the surface of dried nutrient agar plates. Single isolated colonies were used for biochemical and serological confirmation. Biochemical confirmation was done on Triple Sugar Iron agar, Urea agar, Lysine Decarboxylation Medium, Tryptone water for and indole reaction.

TSI

By means of an inoculating wire, single isolated colonies obtained from dried nutrient agar plate was streaked on the agar slope of TSI and stabbed on the butt. Incubation was done for 24 ± 2 hours at 37±1°C.

Typical *Salmonella* cultures showed alkaline (red) slopes with gas formation and acid (yellow) butts, with (in about 90% of the cases) formation of hydrogen sulphide (blackening of the agar).

Urea Agar

By means of an inoculating wire, single isolated colonies obtained from dried nutrient agar plate were streaked on Urea agar slope surface.

Incubation was done for 24±3 hours in the incubator controlled at 37±1°C and examined at three hours intervals.

Positive reaction resulted into, splitting of urea liberating ammonia, which changes the colour of phenol red to rose pink and later to deep cerise.

Lysine Decarboxylation Medium

Cultures obtained from dried nutrient agar plates were inoculated below the surface of the broth of Lysine Decarboxylation medium.

Incubation was done for 24±3 hours in the incubator controlled at 37±1°C.

A purple color after growth has occurred indicated a positive reaction. A yellow color indicated a negative reaction.

Medium for indole Reaction

Tubes containing 5ml of Tryptone water were inoculated using cultures obtained from dried nutrient agar plates.

Incubation was done for 24±3 hours in the incubator controlled at 37±1°C. After incubation; 1ml of the Kovacs' reagent was added. The formation of a red ring indicated a positive reaction. A yellow brown ring indicated a negative reaction.

Serological confirmation was done by observing agglutination reaction of O-antigen, Vi-antigen and H-antigens. If agglutination occurred the reaction was considered positive.

3.8.4 Detection of *Shigella* species in food and soil (ISO 21567:2004)

Using the prepared food sample (3.8.1) the following surface of selective agars were inoculated by means of a loop to obtain well isolated colonies: MacConkey agar with low selectivity; XLD agar with moderate selectivity and Hektoen enteric agar with greater

selectivity. The plates were incubated at 37^0C for between 20 and 24 hours. The appearance of different *Shigella* species is attached in (appendix 1).

Typical and suspect colonies were selected from each of the three selective agars using a sterile loop and streaked on nutrient agar, then confirmed using biochemical and serological tests. Biochemical involved inoculation on the following specified media: TSI agar (TSI slopes), semi-solid nutrient agar for motility tests. On TSI typical *Shigella* cultures showed a yellow butt (acid formation) and no gas bubbles, no change in the colour of the slant (no utilization of lactose or sucrose) and no hydrogen sulphide production. *Shigella* species were non-motile. Serological confirmation was carried out using agglutination tests. The manufacturer instructions were followed precisely for preparing antisera and conducting agglutination tests. The agglutination results were observed against a dark background and if necessary with the aid of a magnifying lens. In cases where all tests were negative and biochemical tests were characteristic of *Shigella* a suspension of pure cultures was heated in a water bath at 100^0C for 60 minutes and a repeat of the agglutination tests was done.

3.8.5 Detection of *Vibrio cholera* in food and soil (ISO/TS 21872-1:2007)

In selective enrichment 1ml of the prepared food sample (3.8.1) was transferred into a tube containing 10ml of Alkaline Saline Peptone Water (ASPW) and incubated at 37^0C for 18±1 hour. From the culture obtained in the ASPW, a sampling loop was inoculated on the surface of a Thiosulphate Citrate Bile Salt (TCBS) agar plate. The plates was inverted and incubated at 37^0C for 24±3 hours. *Vibrio cholera* form yellow colonies on

TCBS. The selected colonies were inoculated onto the surface of saline nutrient agar and incubated at 37^0C for 24±3 hours. The pure cultures were used for biochemical confirmations.

Presumptive identification was done using oxidase test and microscopic examination. In oxidase test, a portion of pure culture was taken from the saline nutrient agar and streaked onto the filter paper moistened with oxidase reagent, in positive tests the colour turned violet or deep purple within 10 seconds. In microscopic examination a film for gram staining was prepared and examination of morphology and gram reaction observed, similarly a drop of the culture was covered with a cover slip and examined for motility under the microscope. *Vibrio cholera* appeared as oxidase –positive, gram-negative and motility test was positive. Presumptive positive colonies were selected for biochemical confirmation using saline TSI agar, and indole. Typical reaction showed an acid slant (yellow) and an acid butt (yellow) without formation of gas or hydrogen sulphide.

3.8.6 Enumeration of *E.coli* and *Coliforms* in food and soil (ISO 7251:2005)

Using a sterile pipette, 1ml of the prepared sample (3.8.1) was transferred to two sterile petri dishes. About 15-20 ml of the Violet brilliant red lactose medium (VBRL) was poured into each petri dish. The time elapsing between the end of the preparation of the initial suspension (of the 10-1 dilution) and the moment when the medium is poured into the dishes did not exceed 15 minutes. Immediately after pouring, the plates were kept at horizontal position, taking care not to wet the lid, gently and thoroughly the medium and inocula were mixed by rocking. The mixture was allowed to solidify, with the petri dishes

standing on a cool horizontal surface. After complete solidification of the mixture, a covering layer of 10ml of the VRBL medium was added then let to cool for *E.coli*. Also a control plate, with 15-20 ml of the medium for checking sterility was prepared. The prepared dishes were inverted and incubate in an incubator set at 37±1°C for 18 - 24hour.

Characteristic *Coliform* colonies appeared as purplish red, 0.5mm in diameter or greater and sometimes surrounded by a reddish zone of precipitated bile. Typical *E.coli* colonies appeared as purplish red, 0.5mm in diameter or greater surrounded by a reddish zone of precipitated bile. Where spreaders were occurring, each was counted as a single colony, provided that the outer edge of each spreader could be defined, rejecting plates where 25 percent or more of the medium is occupied by spreading organisms.

For *E.coli* confirmation a colony was transferred to trypton water and incubated at 44^0C $\pm1^0$C for 24 hours. A drop of kovac's reagent was added to the mixer. The change of colour to cherry red ring confirmed the presence of *E.coli.*

The number of typical *E.coli* colony forming units (A) per milliliter or per gram was calculated by multiplying the mean of the duplicate counts of the selected dilution by the reciprocal of the dilution.

The number of confirmed *E.coli* (B) from the doubtful colonies was calculated using the following equation:

$B = (a/b \times D)\, d$

Where;

a = the number of doubtful colonies confirmed to be *E.coli*

b = is the total number of doubtful colonies submitted to the confirmation test

D = is the mean counts of doubtful colonies from duplicate plates of the selected dilution

d = is the reciprocal of the dilution

The total number of *E.coli*, colony forming units (cfu) (N) per milliliter or per gram was calculated by adding (A) to (B)

N = (A) + (B)

Where two different dilutions give plates with typical counts within the range 15 - 150 colonies, a weighted mean of count obtained was calculated as cfu per milliliter or per gram.

The number of typical *E.coli* present in the sample as the weighted mean was calculated from two successive dilutions using the following equation:

$N_1 = \Sigma C1/v(n1+0.1n2)d$

Where;

Σ 1 *C* is the sum of the typical colonies counted on all the dishes retained from two successive dilutions, one of which contains at least 15 colonies;

V is the volume of the inoculum applied to each dish, in milliliters

n1 is the number of dishes retained at the first dilution;

n2 is the number of dishes retained at the second dilution;

d is the dilution factor corresponding to the first dilution retained.

3.8.7 Detection of *Salmonella* in water and swabs (ISO 6340:1995)

Water and swab samples prepared in (3.8.1) were poured into a sterilized 500ml capacity funnel to the 100ml mark and filtered through a sterile filter membrane 0.45µM. The filter was then placed using a sterile forceps in 100ml of sterile buffered peptone water and incubated at 37^0C ±1^0C for 16 – 20 hours. From the enrichment culture 10ml of Rappaport Vassiliadis broth was inoculated with 0.1ml of pre-enrichment culture similarly, 10ml of Selenite Cystine broth was inoculated with 1.0ml of pre-enrichment culture. RVS medium was incubated at 41.5^0C±1^0C for 24 hours and SBB at 37^0C±1^0C for 24 hours. After incubation a loop from each enrichment tube was streaked onto the surface of Brilliant Green Lactose broth and Xylose Lactose Deoxycholate agar to obtain well isolated colonies. The plates were then incubated at 37^0C ±1^0C for 18 – 24 hours. Typical *Salmonella* colonies appeared as red colonies with or without black centers on XLD and pink colonies with bright red surrounding on BGA. Further confirmatory tests on the biochemical and serological were done as per (3.8.3) (ISO6340-1, 1995).

3.8.8 Detection of *Vibrio cholera* in Water and Swabs (ISO/TS 21872)

Water and swab samples prepared in (3.8.1) were poured into a sterilized 500ml capacity funnel to the 100ml mark and filtered through a sterile filter membrane 0.45µM. The filter was then placed using a sterile forceps in 100ml of sterile Alkaline Saline Peptone Water (ASPW) and incubated at 37^0C ±1^0C for 16 – 20 hours. From the culture obtained in the ASPW, a sampling loop was inoculated on the surface of a Thiosulphate Citrate Bile Salt (TCBS) agar plate. The plates was inverted and incubated at 37^0C for 24±3 hours. *Vibrio cholera* form yellow colonies on TCBS. The selected colonies were

inoculated onto the surface of saline nutrient agar and incubated at 37^0C for 24±3 hours. The pure cultures were used for biochemical confirmations. Further confirmatory tests were done as per (3.8.5) (ISO/TS21872, 2007).

3.8.9 Enumeration of *Staphylococcus aureus* in Water and Swabs (ISO 5944:2001)

Water and swab samples prepared in (3.8.1) were poured into a sterilized 500ml capacity funnel to the 270ml mark and filtered through a sterile filter membrane 0.45µM. The filter was then placed using a sterile forceps on the surface of prepared Baird agar plate and left on the bench for 15 minutes for absorption of inoculums to take place. One prepared and dried Baird parker plate was used as control for checking sterility. The inoculated plates were inverted and incubated at 37^0C ±1^0C for 16 – 20 hours. Typical colonies appeared as black shinning and convex surrounded by a clear zone that is partially opaque, 1mm to 1.5mm in diameter after incubation for 24hours and 1.5mm to 2.5mm in diameter after incubation for 48 hours. Atypical colonies appeared as grey colonies free of clear zones. Confirmation tests and calculations on the number of colonies forming units were done as per (3.8.2).

3.8.10 Detection of Shigella species in water and swabs (ISO 21567-1:2004) Water and swab samples prepared in (3.8.1) were poured into a sterilized 500ml capacity funnel to the 100ml mark and filtered through a sterile filter membrane 0.45µM. The filter was then placed using a sterile forceps in 100ml of sterile *Shigella* broth containing Novobiocin and incubated at 37^0C ±1^0C for 16 – 20 hours. The pre-enriched sample was

inoculated by means of a loop on the surface of selective agar plates to obtain well isolated colonies: MacConkey agar with low selectivity; XLD agar with moderate selectivity and Hektoen enteric agar with greater selectivity. The plates were incubated at 37^0C for between 20 and 24 hours. The appearance of different *Shigella* species is attached in (Appendix 1). Typical and suspect colonies were selected from each of the three selective agars using a sterile loop and streaked on nutrient agar, then confirmed using biochemical and serological tests as per 3.8.4.

3.8.11 Enumeration of *Coliforms* and *E.coli* in water and swabs (ISO 9308-1:2000)

Water and swab samples prepared in (3.8.1) were poured into a sterilized 500ml capacity funnel to the 100ml mark and filtered through a sterile filter membrane 0.45µM. The filter was then placed using a sterile forceps on the surface of prepared Violet Red Glucose agar and left on the bench for 15 minutes for absorption of inoculums to take place. One prepared and dried VRBG plate was used as control for checking sterility. The inoculated plates were inverted and incubated at 37^0C $\pm1^0$C for 16 – 20 hours. Typical colonies appeared as pink to red or purple (with or without precipitation haloes). *E.coli* colonies appeared as pink to red or purple with precipitation haloes. Sub culturing of selected colonies was done by selecting five characteristic colonies at random and streaking each onto already dried Nutrient agar plates. Since certain *Enterobacteriaceae* causes discoloration of the medium therefore, where no characteristic colonies was present five whitish colonies were chosen for confirmation and incubated at 37^0C$\pm1^0$C for 24hours ±2

hours. Further biochemical tests and calculations were done as indicated in 3.8.6 (ISO9308-1, 2001).

3.8.12 Quality control

Fresh reference cultures/working stocks from NCTC, batch numbers NCTC 04840 *S. poona*, NCTC 10885 *Shigella sonnei*, NCTC 9001 *E. coli*, NCTC 06571 *S. aureus* NCTC 10006 *Enterobacter aerogenes*, NCTC 10975 *Proteus mirabilis*, NTCT 9528 *Klebsiella aerogenes*, NCTC 9750 *Citrobacter fremundii* and NCTC 1134 *Vibrio cholerae* were subjected to the same procedure as the test samples. The reference culture showed appropriate/characteristic features and reactions as earlier described. A test was regarded as invalid if the results were not the same as the reference culture

3.8.13 Antimicrobial susceptibility testing

Antibiotic resistant of the isolates in this study was determined by the disk diffusion method using the standard procedure of the Clinical Standard Institute (CLSI, 2009). The isolates were classified as susceptible, intermediate, or resistant according to interpretation of the zone diameter standards recommended by (CLSI, 2009). The isolates from different foods, swabs, soil and water namely: Total *Coliforms*, *E.coli*, *Staphylococcus aureus*, *Salmonella*, *Vibrio cholerae*, *Shigella*, were subjected for susceptibility test using eight antibiotics namely; ampicillin, amoxycillin, tetracycline, sulphamethoxazole, norfloxacin, nalidixic acid, erythromycin and chloramphenicol was performed using the standard Kirby-Bauer disk diffusion method on Muller Hinton agar (Oxoid UK). Using a sterile swab the broth dilution containing the bacteria was spread on

the surface of Muller Hinton agar plates, the disks were then pressed onto the inoculated plate surfaces and incubated at 37^0C for 18 - 20 hrs. The diameters in millimeters of clear zones of growth inhibition around the antimicrobial agent disk, including the 6mm disk diameter was measured using precision calipers (Clinical and Laboratory Standard Institute (CLSI), 2002. A standard reference strain of *E.coli* (ATCC 25922) was used as a control. The breakpoints used to categorize the isolates as resistant, susceptible or immediate to each antimicrobial agent were those recommended by Clinical Laboratory Standards Institute (CLSI) (CLSI, 2009) (appendix 4).

3.9 Data Processing and Analysis

3.9.1 Data Storage and Sorting

Data was stored in Laboratory data forms (appendix 2) which included the following; all the details necessary for the identification of the sample; all references used; the counts of microorganisms per gram of the sample, and an appropriate statement if the count is approximate or probably underestimated; the presence of spreaders and any circumstances or unusual observations made during sampling and the course of the tests that may have had an effect on the result. The data obtained from the laboratory was coded to avoid incompleteness during entry. Following the completion of sample analysis, systematic organization of raw data was done to facilitate data analysis.

3.9.2 Data Analysis and Presentation

Statistical analyses were performed using Statistical Packages for Social Sciences (SPSS, version 19). Descriptive statistics and frequencies were used to determine the prevalence

and occurrences of pathogens in food samples, swabs samples, soil samples and to determine the susceptibility levels. The data was then presented in tables, and graphs. To determine if there was a relationship between the source and the food contamination Pearson correlation was used. The level of statistical significance was set at *p-value* $\leq$ 0.05.

3.10 Ethical Consideration

Clearance was obtained from Maseno University, School of Graduate Studies and Municipal Council of Kisumu Medical Health Department (appendix 5). Street food vendors were informed of the purpose of this study and they were assured of total confidentiality and anonymity. In this regard informed written consent (appendix 3) was obtained from the vendors before including them in the study. Data collection tools and samples were coded to conceal the street vendor's identity.

CHAPTER FOUR: RESULTS

4.1 Prevalence of selected pathogenic bacteria in street vended foods

This study investigated the prevalence, possible sources of contamination and antibiotic susceptibility of isolates from *Enterobacteriaceae* family (Total *Coliforms, E.coli*, *Salmonella* and *Shigella*) *Staphylococcus aureus* and *Vibrio cholera* in street vended foods sold in Kibuye, Kondele and Oile markets within Kisumu city. A total of 248 samples were collected and analyzed using ISO methods for microbiological analysis of food and water.

The results of this study shows that amongst the foods sampled from the three markets (Kondele, Oile and Kibuye), total *Coliforms* was more prevalent (74%), followed by *S.aureus* (20%) and *E.coli* (6%) (Figure 4.1).

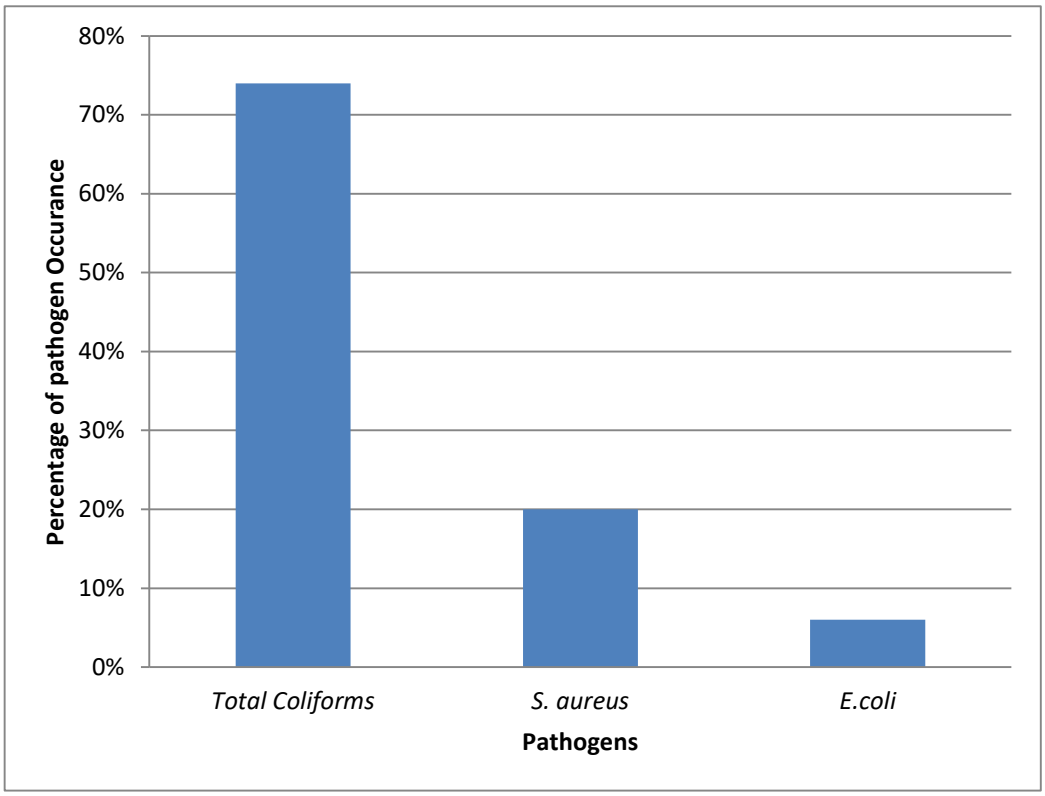


Figure 4.1: Percentages of pathogens occurrence in the sampled foods from the three markets

Figure 4.2 shows that Total *Coliforms* was the most prevalent in three markets Kibuye (79%), Oile (72%) and Kondele (77%). S. *aureus* was the second most prevalent pathogen isolated in Kibuye (16%), Kondele (20%) and Oile (20%). *E.coli* was the least prevalent from the three markets Kibuye (4%), Kondele (2%) and Oile (7%).

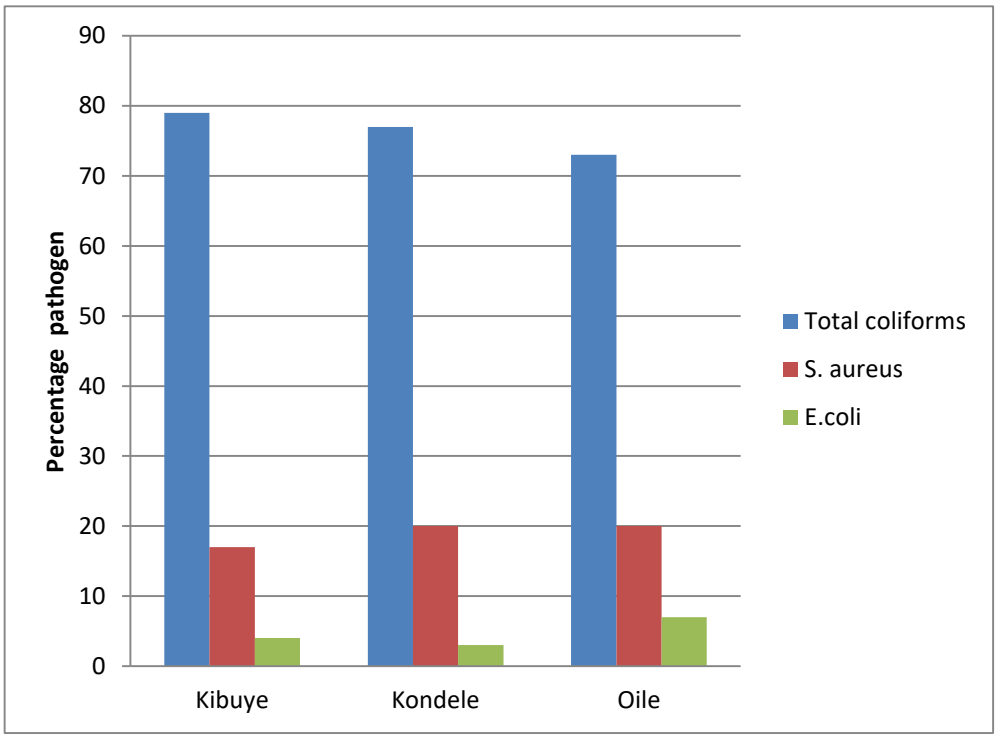


Figure 4.2: Percentages of pathogens occurrence in the sampled foods per markets

A further descriptive analysis of the data revealed that all the 62 foods samples from the three markets were contaminated with *Coliforms*. *S.aureus* contamination ranged between (50% - 100%) except in boiled eggs where *S. aureus* was not isolated. *E.coli* was noted absent in boiled maize and beans, home baked cakes and in porridge. However *E.coli* isolated from ice-cream, *R. agentae,* boiled groundnuts and boiled egg ranged between (50% - 100%). *E. coli* was noted absent in home baked cakes, chapatis, mandazi, porridge, potato chips, boiled maize and beans. (Table 4.1)

Table 4.1: Proportion of Bacteria Pathogens Isolated from Foods

Type of food collected	% of bacteria isolated		
	E.coli	***S.aureus***	***Coliforms***
Fried *R. argentea*	100%	100%	100%
Boiled maize and beans	0%	67%	100%
Ice cream	100%	100%	100%
Mandazi	50%	100%	100%
Cooked beans	50%	50%	100%
Home baked cake	0%	67%	100%
Porridge	0%	50%	100%
Boiled groundnuts	100%	100%	100%
Boiled eggs	100%	0%	100%
Boiled sweet potatoes	100%	100%	100%
chips	100%	100%	100%
Samosa	100%	100%	100%
Fried fish	100%	100%	100%
Home baked cake	0%	50%	50%
Boiled maize	67%	100%	100%
Chapati	0%	100%	100%
Boiled groundnuts	100%	100%	100%
Boiled sweet potatoes	100%	100%	100%
Roasted maize	0%	50%	100%
Mandazi	0%	100%	100%
Chips	67%	100%	100%
Porridge	0%	0%	100%

Among the sampled foods from the three markets, ice-cream had the highest number of counts with a mean of (4949 c.f.u), followed by samosas (3864 c.f.u), fried *R.argentea* (2928 c.f.u), potato chips (820 c.f.u), home baked cakes (337 c.f.u), boiled maize (309 c.f.u), porridge (246 c.f.u) and mandazi (162 c.f.u) (Figure 4.3). The mean colony counts per food at Oile, Kondele and Kibuye shows that samosas and ice cream were the most contaminated (Appendix 7).

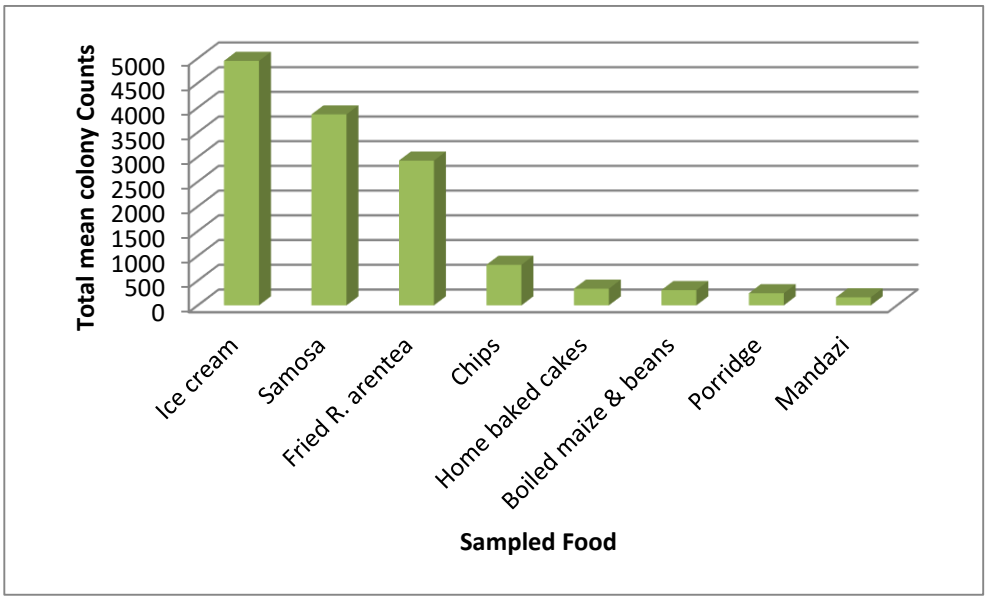


Figure 4.3: Mean colony counts in the sampled foods from the three markets

Figure 4.4 shows that Oile market recorded the highest mean colony counts for *Coliforms* (1546 c.f.u) followed by Kibuye market (1221c.f.u) and Kondele market (941c.f.u). Oile recorded the highest mean for *Staphylococcus aureus* (396 c.f.u), followed by Kondele (310 c.f.u) and Kibuye (277 c.f.u). Oile had the highest counts in E.coli (152 c.f.u), followed by Kibuye (74 c.f.u) and Kondele (32 c.f.u).

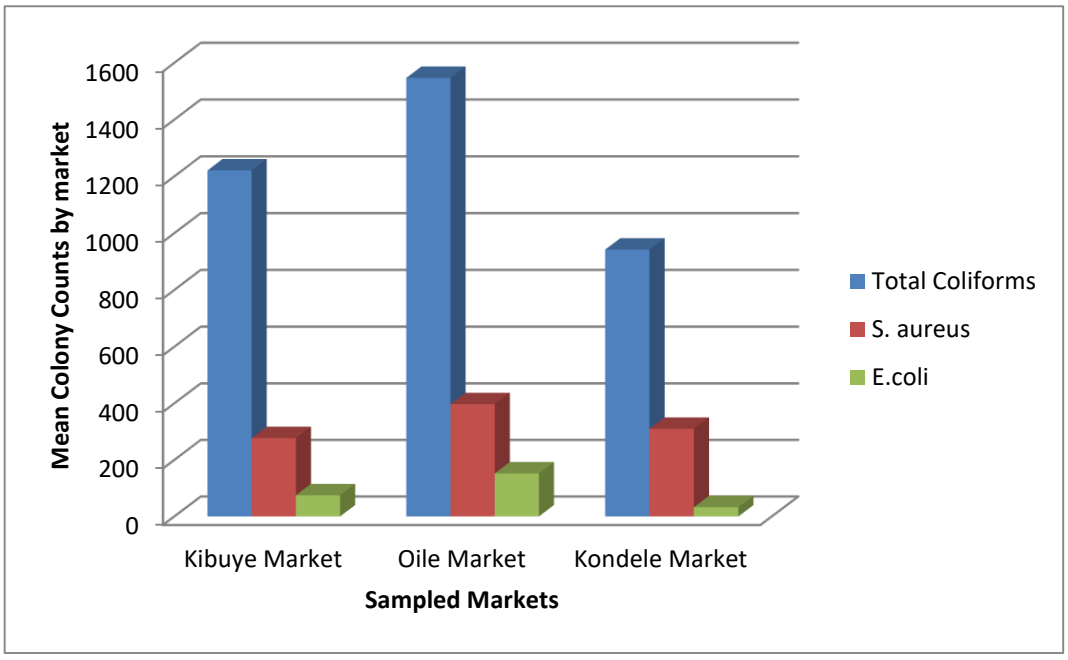


Figure 4.4 Mean colony counts by market for foods

4.2 Possible sources of contamination of street vended foods

Figure 4.5 shows the vending environment where fish is prepared and sold in Kondele market. Results of pathogens isolated from soil, swab and water is shown in (Table 4.2) and the relationship between the sources and the food contamination is shown in (Table 4.3).

Figure 4.5: A picture showing vending market where fish is prepared in Kondele market, Kisumu. A picture taken on 12th, June, 2011

The results in (Table 4.2) shows that swabs collected from the vendors were contaminated with *E. coli*, *Total Coliforms* and *Staphylococcus aureus.* Kibuye market reported *E.coli* in swabs (30%), Oile (21%) and in Kondele *E. coli* was not isolated. Total *Coliforms* in Kibuye was (68%), Kondele (67%) and Oile (52%). *Staphylococcus aureus* contaminations in swabs were; Kibuye (57%), Kondele (57%) and Oile (39%). The prevalence of *E. coli* in water was- Kibuye (30%), Kondele (22%), and Oile (30%). *Coliforms* were- Kibuye (66%), Kondele (67%) and Oile (61%). *Staphylococcus aureus* contaminations in water were - Kibuye (53%), Kondele (44%) and Oile (39%). The results further showed that soil sampled around the vending environment were highly contaminated with pathogenic bacteria with results from Kibuye showing *E. coli* (63%), Kondele (78%) and Oile (78%). Total *Coliforms* results were Kibuye (83%), Kondele

(78%) and Oile (100%). *Staphylococcus aureus* contaminations in soils were Kibuye (93%), Kondele (100%) and Oile (100%). Other highly pathogenic *Enterobacteriaceae* detected in soils were *Shigella*, *Vibrio* and *Salmonella* which were not detected in foods, water and swabs. The prevalence of *Shigella* in Kibuye was (23%), Kondele (33%) and Oile (22%). *Salmonella* was Kibuye (53%), Kondele (56%) and Oile (48%). *Vibrio* in soil was found to be in Kibuye (3%), in Kondele (0%) and Oile (13%).

Table 4.2: Results of pathogens isolated from soil, swab and water by market

Market	**Sample type**	**N =**	***E. Coli***	***Coliforms***	***S. aureus***	***Shigella***	***Salmonella***	***Vibrio***
Kibuye	**Swabs**	30	9(30%)	20(67%)	17(57%)	0	0	0
	Soil	30	19(63%)	19(63%)	27(90%)	7(23%)	17(57%)	1(3%)
	Water	30	9(30%)	20(67%)	17(57%)	0	0	0
Kondele	**Swabs**	9	0	6(67%)	4(44%)	0	0	0
	Soil	9	7(78%)	7(78%)	9(100%)	2(22%)	4(44%)	0
	Water	9	0	6(67%)	4(44%)	0	0	0
Oile	**Swabs**	23	5(21%)	12(52%)	9(39%)	0	0	0
	Soil	23	18(78%)	20(87%)	23(100%)	4(17%)	11(48%)	3(13%)
	Water	23	5(21%)	12(52%)	8(35%)	0	0	0

Table 4.3 shows that there was a negative correlation between *E.coli* isolates in hand swabs and *E.coli* isolates in food and the correlation was insignificant (r^2= -0.009, P= 0.947), however *S.aureus* isolated in food and *S.aureus* isolated in swabs were significantly correlated (r^2= 0.276, P= **0.030**). There was no correlation between *Coliforms* in food and *Coliforms* in hand swabs (r^2=0.079, P= 0.540). *E.coli* isolated in food and *E.coli* in water showed a negative correlation(r^2= -0.073, P= 0.575), *S.aureus* in

food and *S.aureus* in water, *E.coli* in food and *E.coli* in water, *S.aureus* in food and *S. aureus* in water all showed a negative correlation. There was a weak correlation in *Coliforms* in food and *Coliforms* in water and in *Coliforms* in food and *Coliforms* in soil.

Table 4.3: Relationship between the possible sources and the food contamination

Source/sample	**Correlation co-efficient (r^2)**	**P value**
E.coli in hand swab verse *E.coli* in food	0.000081	0.947
S.aureus in food verses *S.aureus* in hand swab	0.076	**0.030**
Total *Coliforms* in food verses Total *Coliforms* in hand swab	0.0006	0.540
E.coli in food verses *E.coli* in water	0.00053	0.575
S.aureus in food verses *S.aureus* in water	0.019	0.286
Total *Coliforms* in food verses Total *Coliforms* in water	0.00056	0.561
E.coli in food verses *E.coli* in soil	0.017	0.311
S.aureus in food verses *S.aureus* in soil	0.017	0.317
Total *Coliforms* in food verses Total *Coliforms* in soil	0.00019	0.733

4.3 Antimicrobial Resistance of the Isolates

A further analysis shows that all pathogens isolated from food, soil,water and hand swabs were resistant to erythromycin except *S.aureus*. All the isolates showed some level of resistance to the selected antibiotics with *S.aureus* showing (100%) resistance to nalidixic acid and total *Coliforms* (22.2%) to norfloxacin. *E.coli* showed (100%) resistance to chloramphenicol, total *Coliforms* (44.4%) and *Shigella* (33.3%). Total Coliforms and *Shigella* showed (63.9%) and (33.3%) resistance to amoxycillin . *E.coli, Salmonella* and *S.aureus* were susceptible to amoxycillin. Resistance to tetracycline was total *Coliforms* (44.4%) and *E.coli* (10%). *Salmonella, Shigella* and *S.aureus* showed no resistance to tetracycline. *E.coli, Salmonella* and *Shigella* were susceptible to ampicillin whereas total *Coliforms* and *S.aureus* showed resistance level of (52.2%) and (55.6%) respectively to ampicillin(Figure 4.6).

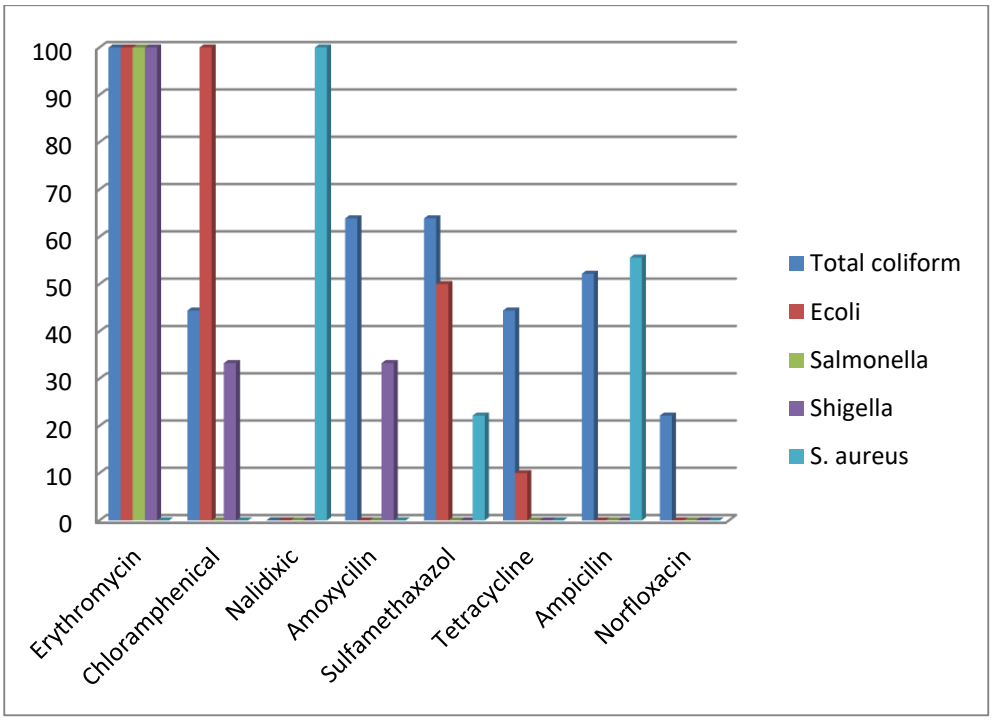
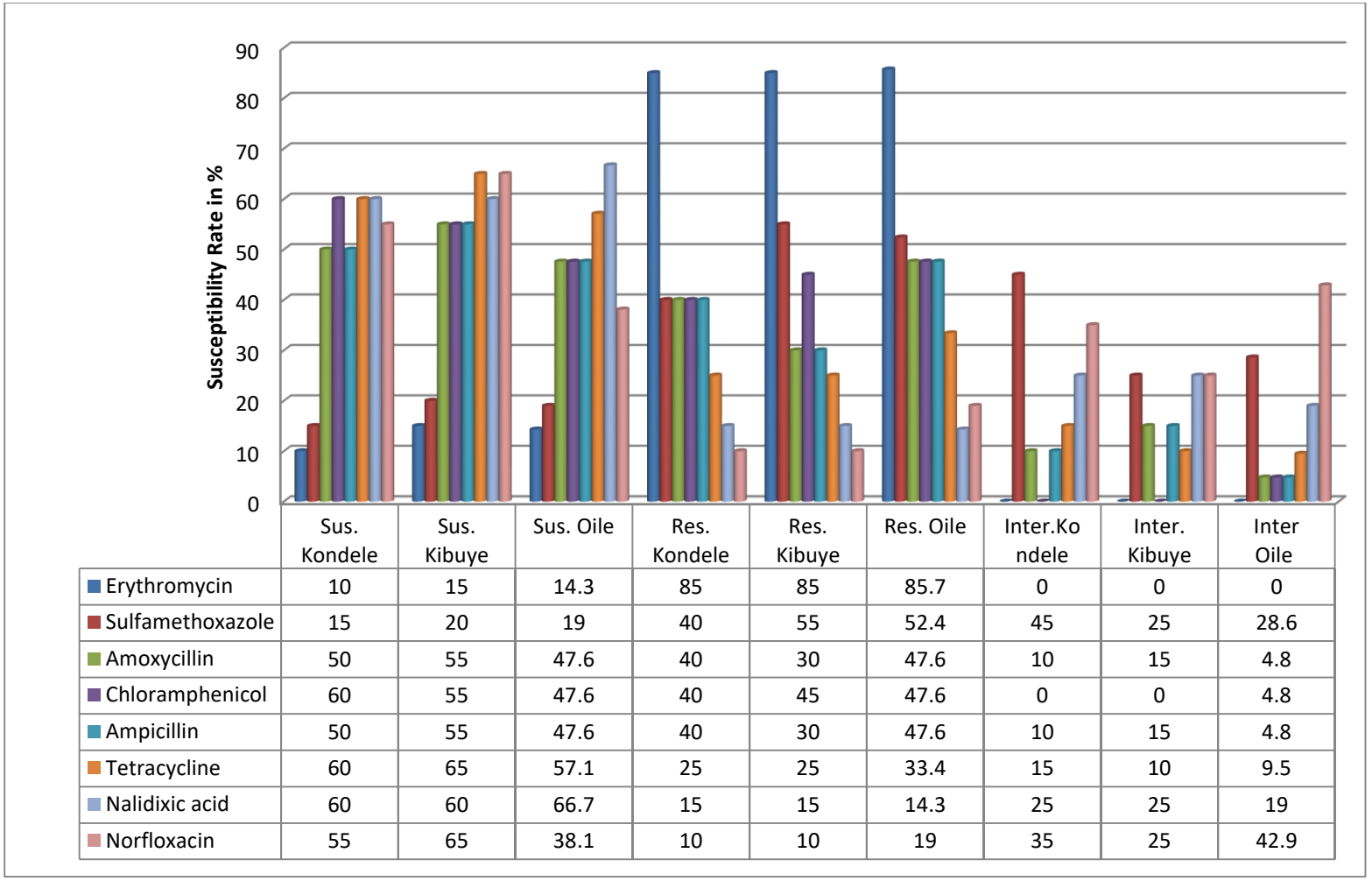


Figure 4.6: Resistance pattern of Total Coliforms, E. coli, Salmonella spp, Shigella spp and S. aureus to antibiotics

Figure 4.7 shows that most of the isolates from the three markets demonstrated similar resistance pattern to erythromycin Kondele (85%), Kibuye (85%) and Olie (85.7%). The isolates from the three markets showed least resistant to nalidixic acid Kondele (15%), Kibuye (15%), and Oile (14.3%) and norfloxacin Kondele (10%), Kibuye (10%), and Oile (19%).



	Sus. Kondele	Sus. Kibuye	Sus. Oile	Res. Kondele	Res. Kibuye	Res. Oile	Inter.Kondele	Inter. Kibuye	Inter Oile
Erythromycin	10	15	14.3	85	85	85.7	0	0	0
Sulfamethoxazole	15	20	19	40	55	52.4	45	25	28.6
Amoxycillin	50	55	47.6	40	30	47.6	10	15	4.8
Chloramphenicol	60	55	47.6	40	45	47.6	0	0	4.8
Ampicillin	50	55	47.6	40	30	47.6	10	15	4.8
Tetracycline	60	65	57.1	25	25	33.4	15	10	9.5
Nalidixic acid	60	60	66.7	15	15	14.3	25	25	19
Norfloxacin	55	65	38.1	10	10	19	35	25	42.9

Figure 2 Figure 4.7: Susceptibility of the isolates verses the markets

Key: S= Susceptible; I= Intermediate; R= Resistant

CHAPTER FIVE: DISCUSSION

5.1 Prevalence of Total *Coliforms, E.coli, Staphylococcus aureus, Salmonella* species*, Shigella* species and *Vibrio cholera* in street vended foods

Previous studies in Kenya have documented the microbial safety of street foods in industrial area, Nairobi (Muinde and Kuria, 2005; Gitahi *et al.*, 2012). This study provides the results of prevalence, source tracking and antimicrobial resistance of selected pathogenic bacteria isolated from street vended foods sold in Kisumu city, Kenya. Results of this study reported that street vended foods in Kisumu were contaminated with *Coliforms, S.ureaus* and *E. coli*. The study finding is comparable to a study which reported high prevalence of Total *Coliforms,* Feacal *Coliforms* and *Staphylococcus aureus* in street vended foods sold in India (Kumar *et al.*, 2006). However, other studies done in South Africa (Von-Holy &Mokhoane, 2006) found that street food vendors were capable of producing relatively safe food with low bacterial counts.

The findings of this study demonstrated that Total *Coliforms* (*Enterobacteriaceae* family) were the dominant bacteria in the sampled street foods. The presence of high counts in Total *Coliforms* can be linked to the fact that this bacteria is capable of growing at an environment with a high biological activity such as decomposing litter at (35^0C) and also in poor hygienic conditions of the premises as in the case of vending environment. The contamination could be introduced during handling and vending environment where by street vended foods are displayed and sold in the open air and handled by vendors with bare hands. This agrees with (Tembekar *et al.*, 2011).who found severe contamination of

displayed food through handling. The study findings are also comparable to that of Murray who reported that majority of food borne pathogens are *Enterobacteriaceae,* including pathogens such as and *Coliforms* (Murray, 2005). However a study conducted in Industrial area Nairobi Kenya, reported that Total *Coliforms* isolated from street vended foods was much higher 94% (Gitahi *et al.*, 2012) in all the food sampled. In this study Total *Coliforms* isolated from the three study markets was 74% in all the food sampled.

The presence of *Staphylococcus aureus* in the sampled foods might be explained by the fact that this organism forms part of the normal micro flora present on and in several parts of human body including the nose, skin or mouth of some carriers. This can be introduced into the street foods during handling, processing and vending (McComrmick *et al.*, 1993; Sandel and McKilly, 2004) The study finding is comparable to a study done in India which reported high prevalence of *Staphylococcus aureus* in street vended foods (Kumar *et al.*, 2006). In this study *Salmonella, Shigella* and *Vibrio cholera* were not detected in all the foods sampled. This may be because their numbers were too low to be detected by the conventional methods or the organisms were absent in the food samples. The absence of *Salmonella species* could also be due to the ingredients in which the prepared foods sampled in this study did not include animal products. The result of this study is in agreement with the study done in Nigeria on the safety and quality evaluation of street foods sold in Zaria, which also reported no *Salmonella, Shigella* and *Vibrio cholera* in food samples collected from mobile food sellers(Umoh and Adoba, 1999; Soriani *et al.*,

2001). Similarly a study done in Industrial area, Nairobi Kenya on microbial quality of selected food borne pathogens in street vended foods also did not report the presence of Salmonella (Gitahi *et al.*, 2012).

The results of this study reported that only 6% of the sampled street vended foods were contaminated with *E.coli*. This finding disagrees with a study conducted in street food of Cape coast Ghana (Annan-Prah et al., 2011) where all sampled foods tested positive for *E. coli*. Other previous studies carried out in Ethiopia, Amravati city also reported higher percentages in the prevalence of *E.coli* 41% of all the food sampled (Tembekar *et al.*, 2011). The fact that feacal *Coliforms* (*E.coli*) were detected at 44^0C in the foods is an indication of potential presence of pathogenic bacteria. This could be introduced during food handling by the vendors themselves.

5.2 Possible sources of microbial pathogens in street vended foods

The results presented in this study showed that high counts in Total *Coliforms, E.coli and Staphylococcus aureus* were obtained from ice-cream, samosas, fried *R.argentia*, across the markets, while low counts were reported in porridge, *mandazi,* home baked cakes, maize and beans. The counts obtained in these foods were above the acceptable limits compared to the Kenyan standards specification limits for microbiological requirements (Appendix 6). The high counts were probably attributed to poor personal hygiene, poor handling and cross contamination from the vendors' hands and soils from the surrounding environment. The presence of high counts in ice-cream could probably be attributed to

insufficient heat treatment, unhygienic materials used for packaging, water being contaminated and lack of good processing practice not being followed during the production.

It could also be due to ice-cream, samosas and fish are high protein foods which forms a rich source of culture media favoring bacterial growth. The presence of high counts in *Staphylococcus aureus* may have resulted from insufficient pasteurization of milk or improper handling. This finding is in agreement with a study done in Mumbai, India (Warke *et al.*, 2000). The findings of this study indicated that ice-cream sold in small portions from bulk containers, exposed to the open air, had a high microbial load, indicating low hygienic quality of this product in many countries. It has also been previously stated that production of ice-cream locally on a small scale rather than industrially is also a major factor associated with contamination of ice-cream (Bostan and Akin, 2002).

The study results showed that all the markets sampled reported higher counts in Total *Coliforms*, followed by *Staphylococcus aureus* then lastly *E.coli*. Oile market reported the highest number of counts followed by Kibuye then Kondele. The reason for the high numbers of counts in Oile, Kibuye and Kondele was not determined by this study but it could be attributed to poor sanitation and lack of basic hygiene in the markets. Kisumu municipality authority therefore needs to enforce higher standards of hygiene in the markets. In this study samples were drawn from the three main possible sources of

contamination, swabs from the vendor's hands, water for food preparation, hand washing, drinking and soil from the vending environment were collected along with the food samples.

Results from this study reported that high counts were obtained from both soils, water and hand swabs samples. Higher counts in soils could be attributed to bacteria being the smallest and most numerous microorganisms in soil and are most critical in decomposing of organic residues and recycling soil nutrients. Highly pathogenic bacteria like *Salmonella* and *Shigella* were only recovered from the soils and not the other sources. From the results reported there was a significant correlation between *S. aureus* isolated from hand swabs and *S. aureus* isolated from food samples suggesting that hands may be a major source of contamination. Hand washing policy should be encouraged among the food vendors. The absence of *Salmonella* and *Shigella* isolated from soils in foods was an indication that there was no cross contamination of these pathogens from soil to the foods.

In a study conducted in Ghana by Annan-Prah et al (2011), food items were sold in the open-air which was dusty, near drainage gutters and some near garbage bins. Muinde and Kuria, (2005) reported about 85 % of the vendors prepared their foods in unhygienic conditions given that garbage and dirty waste were close to the vending stalls. Similar findings were reported in this study as higher counts were reported in soil from the

vending environment which could probably lead to contamination of vended foods since foods offered for sale are spread on the ground over a sack.

The correlation between the isolates in the foods and the isolates from the hand swabs can be attributed to unhygienic handling of the foods by the vendors resulting in cross contamination of the foods. This study is in agreement with other previous studies conducted which reported that enteric bacteria isolated on the selective agar can arise from a number of sources of contamination throughout the food production process (Hobbs and Roberts, 1993) and in other findings it was also reported that in many vending sites toilets were not available, thus forcing the vendors to eliminate their body waste in nearby areas and return to the vending sites without washing their hands (Bryan, 1988). such conditions are likely to lead to cross contamination of cooked foods (Ekanem, 1998).

5.3 Susceptibility of the isolates to commonly used antibiotics

The study findings showed that resistance to erythromycin occurred most frequently, followed by sulfamethoxazole, chloramphenicol, ampicillin, amoxicillin, tetracycline, nilidixic acid and lastly norfloxacin. High resistance to erythromycin in *Enterobacteriaceae* family is attributed to members of this family like most gram negative organisms being intrinsically resistant to low levels of erythromycin (Sabath *et al.*, 1986). *S.aureus* isolated from the foods, soil, hand swabs and water were highly susceptible to erythromycin and the results of this study indicated erythromycin will be

more effective in the treatment of *S.aureus* infections. This study identified no resistance to nilidixic acid and norfloxacin among the *Enterobacteriaceae* isolated and minimal resistance to chloramphenicol, amoxicillin, ampicillin and tetracycline.

All pathogens identified from the three markets showed similar resistance pattern an indication that the source of origin could be similar. Resistance of bacteria may possibly result from inappropriate or uncontrolled use of antibiotics in farming practices, so it is necessary to pay more attention to food hygiene practices to reduce or eliminate the risk from antibiotic resistance and pathogenic bacteria originating from food. The findings of this study are consistent with those reported previously in a study done in Nigeria which also reported resistance of food borne pathogens isolated from street vended foods (Oladipo and Adejumobi, 2010).

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

1. Street vended foods sampled from the three markets in Kisumu city (Oile, Kondele and Kibuye) were contaminated with *Coliforms, E. coli* and *Staphylococcus aureus* but not *Salmonella, Shigella* and *Vibrio.* Total *Coliforms* was the most prevalent contaminant in the three sampled markets.
2. *S. aureus* isolated from the vendor's hands was significantly correlated with *S. aureus* in street vended foods.
3. Antimicrobial resistance to the commonly used antibiotics occurred in all the pathogens isolated from street foods, soils, water and hand swabs from Oile, Kondele and Kibuye markets. Erythromycin exhibited the highest level of resistance to all the isolates.

Recommendations

1. There is need for regulatory authorities to carry out regular sampling and testing of the street vended foods to ensure microbial safety.
2. Kisumu municipal council should create regular programmes for health education to street food vendors to boost the vendors' knowledge on safe food handling practices and proper hygienic practices during food preparation, handling, storing, serving and more emphasis on proper hand washing with soap to reduce the incidences of food borne illnesses.
3. Health providers in Kisumu city should carry out regular surveillance on the resistance pattern of food borne pathogens to ensure the effective antimicrobial is administered.

Suggestions for future research

1. Further studies should be considered on the prevalence of other pathogenic bacteria on street vended foods especially *E.coli* 0157:H7, *Pseudomonas species* and *Listeria* species.
2. Further research should be done on microbial safety of selected food borne pathogens in relation to hygiene practices of street food vendors in Kisumu city.

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APPENDICES

Appendix 1. Description of *Shigella* colony morphology and colour on selective agars.

Bacterial species	Selective agars		
	MacConkey agar	XLD agar	Hektoen agar
Shigella sonnei	Colourless to pale pink, translucent, lactose negative	Translucent with red centre, same colour as the agar	Green and moist raised colonies
Shigella, other species	Colourless, translucent, lactose negative	Translucent with red centre, same colour as the agar.	Green and moist
Escherichia coli	Red with turbid precipitate in agar	Yellow, opaque, surrounded by yellow precipitate in agar	Red with zone of precipitate in agar.
Enterobacter cloacae	Red with turbid precipitate in agar	Yellow, opaque, surrounded by yellow precipitate in agar	Red with zone of precipitate in agar.
Klebsiella pneumoniae	Red with turbid precipitate in agar	Yellow, opaque and mucoid, surrounded by yellow precipitate.	Red with zone of precipitate in agar.
Salmonella	Colourless and translucent, agar yellow around colony.	Red with black centre.	Blue green, with or without black centre.
Proteus mirabilis	Colourless and translucent, agar yellow around colony.	Yellow, black centre, yellow agar with precipitate.	Blue green, with or without black centre.
Enterococcus faecalis	Red, small round colonies.	None or poor growth colonies yellowish.	None or poor growth colonies yellowish.

Appendix 2: Samples and data collection form

Name of product…….

Vendor identity……

Name and location of the market……..

……

Time of Collection……. Batch identity…….

Sample size …….

Sampling method……

Reasons for collecting samples……

……

Any remark……

Name of person collecting samples Designation Signature Date

Appendix 3: Letter of consent

Prevalence, source tracking and antimicrobial susceptibility of selected pathogenic bacteria in street vended foods sold in Kisumu city, April 2011

Letter of consent

Hello. How are you? My name is _____.
I am a student at Maseno University doing research on safety of street vended foods in this city. The research is a partial requirement for the completion of my course and will also be shared with the public health department municipal council of Kisumu to improve on the safety of street vended foods. I hereby seek your permission and cooperation to collect my samples. Whatever information gathered and the results obtained will be kept strictly confidential. I will only need 15 to 30 minutes of your time to complete my work.
Participation is totally voluntary and you can choose not to tell me anything. You can ask me to leave at any moment, and refuse to answer any or all questions asked. However, I hope that you will participate since your input may assist the policy makers in the development of standards as well as regulations regarding street vended foods.
May I have your agreement now?

Signature of interviewer: Date: / /
Day / month / year

Respondent agrees **Respondent does not agree**

Appendix 4: Performance standards for antimicrobial susceptibility testing (CLSI) 2009M2-A9

Antimicrobial agent	Potency µg	Resistant	Intermediate	Susceptible
Amoxycillin	20/10			
Enterobacteriaceae		≤13	14-17	≥18
Staphylococcus spp.		≤19	6	≥20
Ampicillin	10			
Enterobacteriaceae		≤13	14-16	≥17
Staphylococcus spp.		≤28	6	≥29
Vibrio cholera		≤13	14-16	≥17
Chloramphenicol	30			
Enterobacteriaceae		≤12	13-17	≥18
Staphylococcus spp.		≤12	13-17	≥18
Vibrio cholera		≤12	13-17	≥18
Erythromycin	15			
Staphylococcus spp.		≤13	14-22	≥23
Nalidixic acid	30			
Enterobacteriaceae		≤13	14-18	≥19
Tetracycline	30			
Enterobacteriaceae		≤11	14-12	≥15
Staphylococcus spp.		≤14	15-18	≥19
Vibrio cholera		≤14	15-18	≥19
Sulfamethoxazole	1.25-23.75			
Enterobacteriaceae		≤10	15-11	≥16
Staphylococcus spp.		≤10	15-11	≥16
Vibrio cholera		≤10	15-11	≥16
Norfloxacin	10			
Enterobacteriaceae		≤12	13-16	≥17
Staphylococcus spp.		≤12	13-16	≥17

Appendix 5: Permission to conduct research in Kisumu Municipal markets

MUNICIPAL COUNCIL OF KISUMU

Tel. Nos: Kisumu
Office: (057) 202 3812
Tel/Fax: (057) 202 3812
Email: townclerk_kisumu@yahoo.com

Town Hall, Court Road
P.O. Box 105-40100
Kisumu, Kenya

Our Ref: MOH/ATT/02

Your Ref: .....

Date: 8th September, 2011

TO WHOM IT MAY CONCERN

RE: PERMISSION TO CONDUCT RESEARCH IN MUNICIPAL MARKETS

Permission is hereby granted to **Florence Awino Ouma** to conduct the above mentioned activity within Municipality Markets.

Dr. Omwoyo Willis
MEDICAL OFFICER OF HEALTH

Appendix 6: Kenya standard specification on microbial limits

Product	**Kenya Standard (KS)No.**	**Microbial limits (ml/g)**			**Clause**
		Coliforms	E.coli	S.aureus	
Water	459 part-1 2007	Shall be absent	Shall be absent	Shall be absent	5.3
Ice-cream	36:1999	Shall be absent	Shall be absent	Shall be absent	6.6
Cakes	1042:2007	Shall be absent	Shall be absent	Shall be absent	6.2
Potato chips (French fries)	114	Shall be absent	Shall be absent	Shall be absent	4.7
Groundnuts	EAS 57	Shall be absent	Shall be absent	Shall be absent	6.4
General street – vended foods(Africa)	CAAC/GL 22R-1997	Shall be absent	Shall be absent	Shall be absent	5.8

Appendix 7: Mean counts (c.f.u/g) of pathogens per food at Oile, Kibuye and Kondele markets

Foods	**Oile Market**			**Kondele Market**			**Kibuye Market**			**Totals**
	E. coli	*S. aureus*	*Total coliforms*	*E. coli*	*S. aureus*	*Total coliforms*	*E. coli*	*S. aureus*	*Total coliforms*	
Ice Cream	180	1800	3200	0	0	0	202	1447	3267	**10096**
Samosa	385	295	3550	250	220	3200	300	345	2950	**11495**
Fried *R. argentea*	505	530	2150	0	0	0	317	130	2310	**5942**
Potato chips	30	60	1700	13	30	1607	0	23	104	**3537**
Home baked cakes	0	310	180	0	0	0	0	83	140	**713**
Boiled maize and beans	0	270	69	0	40	200	0	0	0	**579**
Porridge	0	10	390	0	0	170	0	60	195	**825**
Mandazi	22	130	98	0	130	60	0	0	130	**570**
Total	**1122**	**3405**	**11336**	**263**	**420**	**5237**	**819**	**2028**	**9096**	**3726**