Microbial Quality, Strain Distribution and Enterotoxigenicity of Selected Food Borne Pathogens in Relation to the Hygienic Practices in Industrial area, Nairobi, Kenya

A research dissertation submitted in partial fulfillment of the requirements for the award of Master of Science degree in Food Safety and Quality of University of Nairobi.
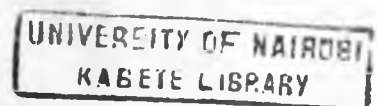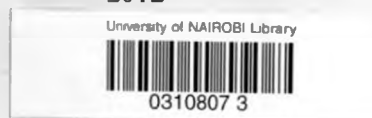
Gitahi Morris Githaiga

(Bsc. Food Science and Technology, University of Nairobi)

Department of Food Science, Nutrition and Technology, Faculty of Agriculture, College of Agriculture and Veterinary Sciences, University of Nairobi

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Declaration

This research dissertation is my original work and has not been presented for a degree or any other award in any other university.

Gitahi Morris Githaiga

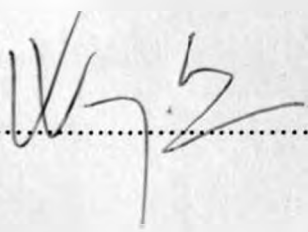Signed..Date......08/08/2012....

University supervisors' approval

This research dissertation has been submitted with our approval as university supervisors

Dr Patrick Kamau Njage

Signed................................Date......08/08/2012....

Dr John Wangoh

Signed................................Date......08/08/2012....

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List of acronyms and abbreviations

μg	Microgram
μg/ml	Microgram per millilitre
μl	microlitre
bp	base pair
cfu	Colony forming units
DNA	de-oxyneucleic acid
DSM2	German collection of microorganisms and cell cultures
EMB	Eosin Methylene Blue
EtBr	Ethidium Bromide
kbp	kilo base pair
KNBS	Kenya National Bureau of Statistics
Log cfu/g	logarithm colony forming units per gram
ml	Millilitre
mM	micro molar
PCA	Plate Count Agar
PCR	Polymerase Chain Reaction
PPE	Personal protective clothing
Rpm	revolution per minute
sd	standard deviation
TBE	Tris/Borate/EDTA
VRBA	Violet Red Bile Agar
PHPT	Department of Public Health Pharmacology and Toxicology, Nairobi University

Abstract

Street foods play a significant role in feeding the urban population with cheap, accessible and nutritious foods. Most street foods vendors are not trained on food hygiene and safety and operate in an unregulated business. Street food can lead to food poisoning and consequent food borne illnesses. Although studies on the safety of street foods have been carried out in most developing countries, not much has been done in Nairobi city in Kenya. This study was carried out to investigate microbial safety and hygiene practices in the vending of street foods in Nairobi city, Kenya. A total of 56 samples classified using seven modified FAO food groups from 29 vending stalls were evaluated. Standard microbiological methods were used for isolation, enumeration and identification of bacteria. No *salmonella* was detected per 25g in all food samples. *Escherichia coli* was qualitatively isolated in 3 food samples including mixed dish from Lunga lunga road and vegetables in Lunga lunga and Nanyuki roads. Vegetables from all locations had coliform levels (4.48 mean $\log_{10}$ cfu/g) that did not meet the quality standards (4.00 $\log_{10}$ cfu/g) of ready to eat food. The coliform counts ($\log_{10}$ cfu/g) were 3.84 in meats, 2.72 in mixed dishes;, 2.33 in legumes, 2.42 in starchy roots, 2.33 in cereals and below detection limits in beverages. *Enterococci* were detected ($\log_{10}$ cfu/g) in vegetables at 2.5, 2.40 in meats, , 2.04 in legumes, 2.44 in starchy roots 2.66 in cereals but below detection limit in mixed dishes and beverages. *Staphylococcus aureus* were detected ($\log_{10}$ cfu/g) in vegetables at 4.03, 3.45 in meats, 3.37 in legumes, 3.32 in mixed dishes and 3.27 in cereals. None were detected in starchy roots and beverages. Vegetable foods contained high microbial counts mostly greater than 4.0 $\log_{10}$ cfu/g) in all the food consumption food groups including Coliforms at 4.48 $\log_{10}$ cfu/g *Staphylococcus aureus* at 4.03 $\log_{10}$ cfu/g, total *Enterococci* at 2.50 $\log_{10}$ cfu/g. Vegetables however had acceptable total count of 4.71 $\log_{10}$ cfu/g against the

6.00 $\log_{10}$ cfu/g standard limits. Rep PCR revealed that isolates from each of *Enterobacter aerogenes, Enterococci* species and *Staphylococcus aureus* isolated from street foods of Industrial area in Nairobi and were related strains. The presence of staphylococcal enterotoxins (se); sea, seb, sec, sed, see, seg, sei & sej was also evaluated in *Staphylococcus aureus*. None of the isolates from street food possessed genes coding for production of the staphylococcal enterotoxins. It was noted that 94% of vending sites were exposed to potential contaminants. A total of 76% (22/29) of vendors did not have food handlers' medical certificate. Eighty eight per cent of the sites were clean. A total of 79% of the stalls were constructed using polythene bags. A total of 66% (19/29) vendors did not have protective clothing, 79% (23/29) vendors had no training on food hygiene, and 87% of vendors used polythene bags for packaging take away rations. However, 79% of the vendors reported no customer complaints concerning food safety. A total of 69% of vendors dump their wastes into Nairobi city council waste bins, while 79% (23/29) use the Nairobi city council sanitary facilities. It can be concluded that street foods evaluated from food groups consisting of cereals, legumes, starchy roots, beverages and some with meat were considered safe for human consumption as per the data. Vegetables had unacceptable contamination levels of coliforms and *Staphylococcus aureus*, while meat (fish) from Nanyuki road had unacceptable levels of coliforms. The relatedness of isolates from this study implies possibility of a common source of contamination such as contaminated processing water, or cross contamination of raw materials and cooked food by equipment or vendors. The occurrence of indicator microorganisms in most of the foods indicated a need for improvement in the processing environment hygiene of street food. There is need to provide water, sanitary facilities and waste collection services, training programs and educating the street food vendors.

1. Introduction

1.1 Background information

Street food is food obtained from a street side vendor, often from a makeshift or portable stall (FAO, 2007). Street food feeds millions of people daily with a wide variety of foods that are relatively cheap and easily accessible (Latham, 1997; Tambekar et al. 2011). Street food is intimately connected with take-out, junk food, snacks, and fast food (Lues et al. 2006). Street food is also regarded as tasty (Bhat and Waghray, 2000; Tambekar et al. 2011), distinguishable by its local flavor and can be purchased on the sidewalk, without entry into a building (Lues et al. 2006). There is a noticeable increase of food vendors in Kenya (Mwangi, 2002). This is apparent in Nairobi, where they sell both raw and cooked food items along the streets of Nairobi. This has been instigated by rapidly growing and changing food demands alongside the need to diversify and/or employ more income sources in the events of declining incomes (Mwangi, 2002). According to studies done in Africa on street foods, their remarkable 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 et al. 1996; Chaulliac and Gerbouin, 1996).

Food safety is the assurance that food will not cause harm to the consumer when it is prepared and eaten or consumed according to its intended use (FAO/WHO, 1997). Millions of people fall ill and many suffer from serious disorders, long-term complications or die as a result of eating unsafe food (FAO, 2007). Food borne and waterborne diarrhoea diseases are leading causes of illness and globally kill an estimated 2.1 million people annually, most of whom are children in developing countries (WHO, 2001). The high prevalence of diarrhoea diseases in many developing countries suggests major underlying food and water safety problems (WHO, 2011).

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A great proportion of these cases can be attributed to contamination of food and drinking water (WHO, 2011). Food borne illnesses are defined as diseases, usually either infectious or toxic in nature, caused by agents that enter the body through the ingestion of food (WHO, 2011). Street food vendors are often unlicensed, untrained in food hygiene and sanitation, and work under unsanitary conditions (FAO, 1997). Recent studies have indicated that ready to eat foods and food preparation surfaces may be reservoirs for microbial contamination (Mankee *et al.* 2005; Ghosh *et al.* 2007; Christison *et al.* 2008). FAO further stipulates that street foods raise concern with respect to their potential for serious food poisoning outbreaks due to improper use of additives, the presence of adulterants, environmental contaminants and improper food handling practices amongst street food vendors (FAO, 1997). Street foods in some African countries have been tested for various microorganisms of public health concern, including faecal coliforms, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* species and *Bacillus cereus*. *Escherichia coli* and *Staphylococcus aureus* were recovered in a significant proportion of the food, water, hands and surface swabs tested in Harare (FAO/WHO 2005).

1.2 Problem statement and justification

Although governments throughout the world are attempting to improve the safety of the food supply, the occurrence of food borne disease remains a significant health issue in both developed and developing countries (WHO, 2011). The global incidence of food borne disease is difficult to estimate, but it has been reported that in 2005 alone 1.8 million people died from diarrheal diseases. A great proportion of these cases can be attributed to contamination of food and drinking water (WHO, 2011). In countries where street food vending is prevalent, there is commonly a lack of information on the incidence of food borne diseases related to street-

vended foods (WHO, 2011). Due to lack of proper knowledge and guidance on street food vending, vendors prepare their foods in explicitly unhygienic and sanitary conditions (Muinde and Kuria, 2005; Sheth et al. 2005). Consumers who depend on such food are more interested in its convenience and usually pay little attention to its safety, quality, and hygiene (Mensa et al. 2002; Barrow *et al.* 2006).

Muinde and Kuria (2005) observed that street food vendors in Nairobi practice minimal hygienic and sanitary practices.

There lacks knowledge on the epidemiological importance and public awareness of street food which hampers precise scientific approach of the food safety problem (Rane, 2011). Mwadime (2001) reported varying levels of coliforms, *Staphylococcus aureus, Bacillus cereus and Clostridium perfringens* in food. However there were limited studies on specific hazards posed by microorganisms of public health concern in street food. There is need to study the strain distribution and pathogenicity of presumptive food pathogens and the relationship between their occurrence and the hygiene practices in Nairobi. This could reveal potential of food poisoning outbreaks relating to street food consumption and relate this to handling practices through evaluation of virulence/pathogenicity of the micro organisms isolated from street food matrices.

1.3 Overall objective

The overall objective of this study was to characterize the pathogenicity and diversity of selected food borne pathogens in street food, and relate them with hygienic practices in Industrial area, Nairobi, Kenya.

1.3.1 Specific objectives

i.   To investigate the microbial quality of street food with respect to, total viable counts, total coliforms, *Enterococci, Staphylococcus aureus,* total *Enterobacteriaceae. Escherichia coli* and S*almonella* in Industrial area, Nairobi.

ii.   To investigate the hygiene practices in the handling of street foods in Industrial area, Nairobi.

iii. To assess strain distribution of *Staphylococcus aureus, Enterobacteriaceae, Enterococci* and virulence of *Staphylococcus aureus* detected in street food in Industrial area Nairobi.

1.4 Hypothesis

Microbial safety is influenced by the hygiene practices during handling of street food in Industrial area, Nairobi city.

Selected food borne pathogens isolated from street food in Industrial area, Nairobi city possess genes coding for virulence/pathogenicity.

1.5Assumptions

The assumption was that the street food vendors would cooperate during the hygiene survey and sample collection. It was assumed that the factories in the Industrial area would be operating during the study period. These factories drive the demand and consumption of the street food in the area.

2.0 Literature review

2.1 Street food

Food and green groceries are available on the street for a fraction of the cost in a restaurant or a supermarket (FAO, 2007). This food is termed as 'street food' and the consumption is common among those in the low socio-economic bracket (Mensah et al. 2002). Street food is obtainable from a street side vendor, often from a makeshift or portable stall (FAO, 2007). Some street foods are regional, while others have spread beyond their region of origin (FAO, 2007). The food and green groceries sold in farmers' markets may also fall into this category, including the food exhibited and sold in fairs such as agricultural show and state fair (FAO, 2007). Most street foods are both finger and fast food. Finger food is food eaten directly using the hands, in contrast to food eaten with a knife and fork, chopsticks, or other utensils (Kay, 1999). Fast food is food that can be quickly prepared and served (Jakle, 1999).

2.2 Importance of street food in urban areas

In developing countries, a large proportion of ready to eat foods are sold on the street (Mensah et al. 2002). According to the Food and Agriculture Organization, 2.5 billion people worldwide eat street food every day (FAO, 2007). Increased reliance of street food has been identified as one of the characteristics of urban food distribution systems driven by changes in the urban way of life and poverty in developing countries (FAO, 1998). Street foods have already become a common feature of urban life (Hilda, 2002). The increasing poverty and time constraints to survive in developing countries indicate that the street food phenomenon will only increase (Hilda, 2002). With the increasing pace of globalization and tourism, the safety of street food has become one of the major concerns of public health, and a focus for governments and

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scientists to raise public awareness of (FAO, 2007). Street food feeds millions of people daily with cheap and easily accessible food (Latham, 1997). Increased reliance on street food has been identified as one of the characteristics of urban food distribution systems, driven by changes in the urban way of life and poverty in developing countries (FAO, 1998).

2.2.1 Nutritional benefits

The street food industry plays an important role in developing countries in meeting the food demands of the urban dwellers (Latham, 1997). Street foods play significant nutritional role for consumers, particularly for middle and low-income sectors of the population, who depend on street foods for their main food intake (Mensah et al. 2002; Dardano, 2003). FAO reports that street foods provide nutritionally balanced diets, sufficient in quantity and presenting options for variety and choice for consumers, particularly from middle and low-income sectors of the population, who depend heavily on them (FAO, 1997).

The contribution to the daily food intake of poor urban dwellers is scarcely quantified in energy and nutrients (Hilda, 2002). The foods have been shown to contribute a substantial proportion of the daily requirement of energy and protein (25% - 50%) for adolescents attending schools (Oguntona and Kanye, 1995) and urban market women (Oguntona and Tella, 1999) in Nigeria. They are reported to play a considerable role in the daily diet of low-income male urban workers in Hyderabad (Sujatha et al. 1997), urban construction workers in Nairobi (Korir et al. 1998) and Calcutta street traders (Chakravarty and Canet, 1996). Their nutritional value however depends on the ingredients used and how they are prepared, stored and sold (Owusu-Darko and Ablordey, 2002).

2.2.2 Economic benefits of street food

The street food industry offers a significant amount of employment, often to persons with little education and training (Latham, 1997). Street food in Nairobi provides a substantial amount of income for most vendors, with most of them earning an income above the official minimum wage while some of them earn twice or more of this amount (Mwangi, 2002). Street food operations sometimes involve the entire family in the procurement of raw materials, preparation and cooking of the meals (Mensah et al. 2002). The role of women in the sector is significant, as they control a large share of market activity and commodity trading (Mensah et al. 2002). Street food vendors benefit from a positive cash flow, often evade taxation, and can determine their own working hours (Mensah et al. 2002). In selling snacks, complete meals, and refreshments at relatively low prices, they provide an essential service to workers, shoppers, travelers, and people on low incomes. However, the people who depend on such food are often more interested in its convenience than in questions of its safety, quality and hygiene (Mensah et al. 2002; Muinde and Kuria, 2005).

2.3 Preparation of street food

Street food is prepared by the vendors at home or at the road side stalls (Muinde and Kuria, 2005). Vendor's sites are mostly within five to metres radius of dusty roads and foot paths (Mwadime, 2001). The vending sites are self-allocated and not varnished with sanitary amenities (Mwangi, 2002). Foods are held in different ways before selling; fish are placed openly on the stalls and chips are held in cup boards next to the stalls while fruit salads are held in open bowls (Muinde and Kuria, 2005). After the food is prepared, it is not reheated to high temperatures before serving (Muinde and Kuria, 2005). The stalls are poorly constructed and

increase the exposure to contamination by dust and smoke on the road side (Muinde and Kuria, 2005). Street vendors use tap water supplied from the municipal council or buy from water kiosks (Mwangi, 2002; Mwadime, 2001). In other instances water is ferried from home of the food vendors because there is no portable water available in their area of operation. This water is not enough for dish washing and food preparation and vendors do not wash fresh foods properly (Muinde and Kuria, 2005). Mensah (1999) noted that without formal education, the street food vendors lack knowledge on proper food handling and may play a role in transmission of food borne pathogens.

2.4 Microbial safety of foods

2.4.1 Food borne diseases

The global incidence of food borne disease is difficult to estimate, but it has been reported that in the year 2000 alone 2.1 million people died from diarrhea diseases (WHO, 2011). Unsafe food causes many acute and life-long diseases, ranging from diarrhoeal diseases to various forms of cancer (WHO, 2011). WHO estimates that food borne and waterborne diarrhoeal diseases taken together kill about 2.2 million people annually, 1.9 million of them children (WHO, 2011). The risk of serious food poisoning outbreaks linked to street foods remains a threat in many parts of the world, with microbiological contamination being one of the most significant problems (FAO, 1998). Food-borne pathogens are recognized as a major health hazard associated with street foods, the risk being dependent primarily on the type of food and the method of preparation and conservation (FAO, 1998; FAO/WHO 2005). In Kenya, incidences of food borne disease outbreaks have been reported each year (MOH, 2003).

Pathogenicity and virulence of an organism are regulated by virulence coding genes present in the genomic regions known as pathogenicity islands (Hacker and Kaper, 2000). *Staphylococcus aureus* is one of the most prevalent pathogens causing several outbreaks (Veras et al. 2008). *Staphylococcus aureus* is a gram positive, catalase and coagulase positive microorganism (Veras et al. 2008). Contamination of food with enterotoxigenic *Staphylococcus aureus* causes staphylococcal enterotoxins (SEs) intoxication hence the associated symptoms like vomiting and diarrhea. Major serological enterotoxins that have been characterized are: SEA, SEB, SEC, SED, and SEE (Robbins et al. 1977) and recently SEG, SEH, SEI, SEJ, SEK, SEL, SEM, SEN, SEO, SEP, SEQ, and SEU (Letertre et al. 2003; Yarwood et al. 2002). SEA is the most common SE associated with food borne outbreaks followed by SED. However, the type of SE is not relevant because SEs are very similar in structure and function (Balaban and Rasooly, 2000). Shiga toxin-producing *Escherichia coli* are a group of bacteria strains capable of causing significant human disease (Richard, 1999). The pathogen is transmitted primarily by food (Richard, 1999). The subgroup enterohaemorrhagic *E. coli* includes the relatively important serotype O157:H7, and more than 100 other non-O157 strains (Richard, 1999). Infection is transmitted primarily by food and less commonly by direct contact or water (Richard, 1999). Shiga toxin is a family of toxins produced by a variety of organisms, including *Shigella dysenteriae* type I and Shiga toxin-producing *Escherichia coli*. These toxins have a cytotoxic effect on intestinal epithelial cells that probably causes the characteristic bloody diarrhea (Richard, 1999). Laboratory identification of *E. coli* O157:H7 is easily performed using specialized media but identification of non-O157 Shiga toxin-producing *Escherichia coli* strains requires detection of the Shiga toxin gene by polymerase chain reaction or DNA probe-for virulence genes stx_1, $stx2$ *and eae* (Richard, 1999).

In 2004, *Enterococcus* genus took the place of faecal coliforms as the new federal standard for water quality and public beaches in Hawaii USA. It provides a higher correlation than faecal coliforms with many of the human pathogen often found in city sewage (Jin et al. 2004). Enterococci however do not multiply in water especially in low organic matter. They are less numerous than *Escherichia coli* (James et al. 2005).

2.4.2 Microbial food safety of street foods

A lack of knowledge among street food vendors about the causes of food-borne disease is a major risk factor (FAO, 1998). Poor hygiene, inadequate access to potable water supply and garbage disposal, and unsanitary environmental conditions such as proximity to sewers and garbage dumps further exacerbate the public health risks associated with street foods (FAO, 1998). Traditional processing methods that are used in preparation, inappropriate holding temperatures and poor personal hygiene of food handlers are some of the main causes of contamination of street-vended food (Mensah et al. 2002; Barro *et al.* 2006). Recent studies have indicated that ready to eat foods and food preparation surfaces may be reservoirs for microbial contamination (Mankee *et al.* 2005; Ghosh *et al.* 2007; Christison *et al.* 2008). Street foods in some African countries have been tested for various microorganisms of public health concern, including feacal coliforms, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* species and *Bacillus cereus* (FAO/WHO 2005). *Escherichia coli* and *Staphylococcus aureus* were recovered in a significant proportion of the food, water, hand and surface swabs tested in Harare, Zimbambwe (FAO/WHO, 2005). Street foods can also be sources of several groups of enteropathogens (Mensah et al. 2002).

2.5 Epidemiological importance of microbial food borne disease in street foods

Despite the availability of food safety strategies for public health and economic development in many countries, food safety policies, plan of action and legislation have not been implemented especially in developing countries (Anonymous, 2001). In recent times, food safety issues have assumed a wider dimension because of the reliance on fast food whose preparation the consumer has no control over. In the busy way of life today, people eat more meals outside their homes. In developing countries a large portion of ready to eat is sold on the streets. If this food is not handled hygienically or not stored at the right temperature, food borne illnesses are bound to occur (Anonymous, 2001). All age groups consume street foods in Africa (FAO/WHO, 2005). However, there may be differences in the type of client depending on locality (Mensah *et al*. 2002). While it is often thought that children under five years of age are fed at home, Mensah *et al*. (2002) observed that many mothers working at the markets in Accra also bought some food items from vendors to feed their babies. This has serious implications on the health of the children (FAO/WHO, 2005). Mahale et al. (2008) cited documented outbreaks of illnesses in humans associated with the consumption of street vended foods.

2.6 Gaps in knowledge on street food safety

There is a lack of knowledge in microbial safety, pathogenicity, and strain distribution of street food microorganisms. The role of hygiene practices or their lack in occurrence of possible food borne pathogens in Nairobi street food is not clear. The possible impact of handling practices in street foods in Nairobi on human health remains unclear.

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3.0 Microbial safety of street food in Industrial area, Nairobi

Abstract

Street foods play a significant role in feeding the urban population with cheap, accessible and nutritious foods. Most street foods vendors are not trained on food hygiene and safety and operate in an unregulated business. Street food can lead to food poisoning and consequent food borne illnesses. Although studies on the safety of street foods have been carried out in most developing countries, not much has been done in Nairobi city in Kenya. This study was carried out to investigate microbial safety and hygiene practices in the vending of street foods in Nairobi city, Kenya. A total of 56 samples classified using seven modified FAO food groups from 29 vending stalls were evaluated. Standard microbiological methods were used for isolation, enumeration and identification of bacteria. No *salmonella* was detected per 25g in all food samples. *Escherichia coli* was qualitatively isolated in 3 food samples including mixed dish from Lunga lunga road and vegetables in Lunga lunga and Nanyuki roads. Vegetables from all locations had coliform levels (4.48 mean $\log_{10}$ cfu/g) that did not meet the quality standards (4.00 $\log_{10}$ cfu/g) of ready to eat food. The coliform counts ($\log_{10}$ cfu/g) were 3.84 in meats, 2.72 in mixed dishes;, 2.33 in legumes, 2.42 in starchy roots, 2.33 in cereals and below detection limits in beverages. *Enterococci* were detected ($\log_{10}$ cfu/g) in vegetables at 2.5, 2.40 in meats, , 2.04 in legumes, 2.44 in starchy roots 2.66 in cereals but below detection limit in mixed dishes and beverages. *Staphylococcus aureus* were detected ($\log_{10}$ cfu/g) in vegetables at 4.03, 3.45 in meats, 3.37 in legumes, 3.32 in mixed dishes and 3.27 in cereals. None were detected in starchy roots and beverages. Vegetable foods contained high microbial counts mostly greater than 4.0 $\log_{10}$ cfu/g) in all the food consumption food groups including Coliforms at 4.48 $\log_{10}$ cfu/g *Staphylococcus aureus* at 4.03 $\log_{10}$ cfu/g, total *Enterococci* at 2.50 $\log_{10}$ cfu/g. Vegetables however had acceptable total count of 4.71 $\log_{10}$ cfu/g against the

6.00 $\log_{10}$ cfu/g standard limits. Vegetables had unacceptable contamination levels of coliforms and *Staphylococcus aureus*, while meat (fish) from Nanyuki road had unacceptable levels of coliforms. The occurrence of indicator microorganisms in most of the foods indicated a need for improvement in the processing environment hygiene of street food. There is need to provide water, sanitary facilities and waste collection services, training programs and educating the street food vendors.

3.1 Introduction

Street food is obtainable from a street side vendor, from a makeshift or portable stall (FAO, 2007). Street food feeds millions of people daily with a variety of foods that are cheap and easily accessible (Latham, 1997). Food safety is the assurance that food will not cause harm to the consumer when prepared and eaten or consumed according to its intended use (WHO, 2001) and is an increasingly important global public health issue (WHO, 2011). Millions of people fall ill and many suffer from serious disorders, long term complications or die as a result of eating unsafe food (FAO, 2007). Food borne and waterborne diarrhea diseases are leading causes of illness and globally kill an estimated 2.1 million people annually, most of whom are children in developing countries (WHO, 2001). Every person is at risk of food borne illness (WHO, 2011). Studies indicate that ready to eat foods and food preparation surfaces may be reservoirs for microbial contamination (Mankee *et al.* 2005; Ghosh *et al.* 2007; Christison *et al.* 2008). Street foods in some African countries have been tested for various microorganisms of public health concern including faecal coliforms,

Escherichia coli, Staphylococcus aureus, Salmonella species and *Bacillus cereus* were recovered in a significant proportion of the food, water, hands and surface swabs tested in Harare, Zimbabwe (FAO/WHO 2005). Cooked food should contain no more than 10^6 cfu/gm total counts, total coliforms should not exceed 100 cfu/g while *E. coli* should not exceed 10 cfu/g upon analysis (KEBS, 2003), 10^4 cfu/g of *Enterobacteriaceae* and greater than 10^4 cfu/g of *Staphylococcus aureus* (Gilbert et al. 2000). However there are limited studies on specific hazards posed by microorganisms of public health concern in street. This study was carried out to evaluate the microbial safety of street food prepared and vended on site in the streets of Industrial area, Nairobi with total viable microorganisms, total coliforms, total *Enterococci*, *Escherichia coli, Staphylococcus aureus* and *Salmonella* species as indicators.

3.2 Materials and methods

3.2.1 Study design

This was a cross sectional study carried out between March and August year 2011.

3.2.2 Study area

The study was carried out in Industrial area, Nairobi city (Coordinates: $1°17'S$ $36°49'E$). Nairobi is the largest city in East Africa with 3.1 million people and an annual population growth rate of 4 % (KNBS, 2010). The eating places with street food prepared on site in the major roads in Industrial area were selected to form the study area in Enterprise road, Lunga lunga road, Liken road, Nanyuki road and Ricky road.

3.2.3 Sampling procedure and sample size

The sampling was purposive based on availability of street vended food and prepared on site in the major roads of Industrial area of Nairobi city. Seven modified FAO food groups (FAO, 2011) were selected including cereals, mixed dishes, starchy roots, beverages, vegetables, meats and legumes were sampled (Table 1). Sample size was calculated as per Pfeiffer (2002), using a prevalence of 5 % in microbial contamination as reported on similar street foods (Mwadime, 2001). Therefore 56 samples were sampled and analyzed in this study. Food samples were collected in sterile polythene bags. All samples were transported to the University of Nairobi, Department of Food Science, Nutrition and Technology microbiology laboratory within three hours of collection in cool boxes which were cooled with ice.

Table 1 Sampled food according to FAO groupings.

Food consumption food group[a]	Food sampled
Cereal	ugali[b], chapati[c], rice, roast maize, mandazi[d]
Mixed dishes	boiled & stewed; muthokoi[e] , githeri[f]
Legume	boiled and stewed beans and green grams
Vegetables	cabbage, kales, salads/kachumbari[g]
Starchy root	boiled ; sweet potato & arrow roots, potato fries
Meat	stewed; beef, mutton, fried fish, roast mutura[h]
Beverages	fermented porridge, unfermented porridge, boiled milk, beef soup
	corriander, tomato, red onion, capsicum, red/green chillies,
Vegetables	cabbage, kales

[a] source: FAO and modified as per; FAO food consumption food group, 2011

[b] Thickened maize flour meal

[c] Dry baked unleavened wheat meal

[d] Deep fried leavened wheat meal

[e] Boiled decorticated maize and dry beans

[f] Boiled whole maize grain and dry beans

[g] Grated pepper, cabbages, capsicum, carrots, tomatoes and red onion

[h] Ruminants intestines casing stuffed with blood and minced meat

3.2.4 Microbial analysis

Samples were blended by use of a stomacher (400 laboratory blender type BA 7021 England, Senard Medical London SEI IPAGE UK). Serial dilutions were prepared, vortexed (Genie Patent # 3061280 for Bender & Hobein AG Zurich, Switzerland) and enumeration done a colony counter (Dr Gerber K. Schneider & co. AG Zurich, Suisse) as described by Harrigan (1976). Isolation was then done based on colony morphology, followed by purification by streaking three times of each colony on fresh medium. Baird Parker Agar (Himedia Laboratories pvt, India) was used for enumeration of presumptive *Staphylococcus aureus* (pour

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plate method). Gram reactions (3% KOH), catalase reaction (3% H_2O_2; VWR International), and coagulase test for *Staphylococcus aureus* were then performed.

Plate Count Agar (Oxoid Ltd, England) was used for total plate count and Violent Red Bile Agar (VRBA) (Himedia Laboratories pvt, India) for total coliforms. For total *Enterobacteriaceae*, further tests performed included verification of rod shaped cells by microscopy, catalase test (3 % H_2O_2 VWR International) and gram reactions (3 % KOH, Sigma-Aldrich). Specific coliform organisms were differentiated with IMViC tests (Harrigan, 1976). Tellurite agar (Harold) was used for enumeration of *Enterococci* species (pour plate method for 42°C for 24 hrs). Eosin Methyl Blue Agar Levine (Oxoid Ltd, England) was used for qualitative test of presence of *Escherichia coli*. For *Salmonella* species, pre-enrichment was done using sterile lactose broth. This was incubated at 37°C for 24 hrs. Ten ml of pre enrichment medium was aseptically pipetted and transferred into a jar with 100 ml sterile Tetrathionate broth (Himedia Laboratories pvt, India) and selenite cystine broth (Oxoid Ltd, England) and incubated at 37°C for 24 hrs. The resultant broth was streaked onto three *Salmonella* differential media namely Brilliant Green Phenol lactose Agar (Himedia Laboratories pvt, India), Bismuth sulphate Agar (Oxoid Ltd, England) and Desoxycholate citrate Agar (Oxoid Ltd, England).

3.2.5 Data analysis

Quantitative data on the microbial counts collected from the experiment was subjected to analysis of variance (ANOVA) using the Genstat 13th edition, VSN International. Difference among the microbial counts results was compared using the Fisher's protected LSD test at 5 % probability level only when the ANOVA Table indicates significant differences amongst the means.

3.3 Results

3.3.1 Microbial counts

Among the seven food categories, the street vegetable based foods had the highest total viable microorganisms (4.71 ± 0.3 $\log_{10}$ cfu/g) while beverages had the least (3.19 ± 0.2 $\log_{10}$ cfu/g) count. Total coliforms counts were 4.48 ± 0 and 3.84 ± 0 $\log_{10}$ cfu/g in vegetables and meats respectively and lowest in cereals and legumes at 2.33 ± 0.2 $\log_{10}$ cfu/g each. *Enterococcus* species were highest (2.66 ± 0 $\log_{10}$ cfu/g) in cereals, and lowest in legume foods (2.04 ± 0 $\log_{10}$ cfu/g). Counts of *Staphylococcus aureus* were highest in vegetables (4.03 ± 0 $\log_{10}$ cfu/g) (Table 2). Only vegetables had samples exceeding the recommended microbiological standards limits (Table 3).

Table 2: Microbial counts for street foods in industrial area, Nairobi

Bacteria group	Microbial Counts	Food group						
		Cereals n=12	Mixed dishes n=6	Legumes n=7	Vegetables n=11	Starchy roots n=5	Meats n=9	Beverages n=6
Total Viable Count	+ samples	5	4	7	11	5	8	5
	Log_{10} cfu/g	4.33 ± 0.5	4.14 ± 0.2	3.79 ± 0.4	4.71 ± 0.3	3.98 ± 0.4	4.21 ± 0.4	3.19 ± 0.2
	Range	3.70-5.08	3.78-4.30	3.00-4.08	4.20-5.18	3.70-3.65	3.30-4.56	3.00-3.25
Total coliforms	+ samples	1	4	2	7	2	4	0
	Log_{10} cfu/g	2.33 ± 0	2.72 ± 0	2.33 ± 0	4.48 ± 0	2.42 ± 0	3.84 ± 0	*
	Range	2.18-2.28	2.70-2.90	2.00-2.58	3.78-4.91	2.00-2.70	3.70-4.10	-
Enterococci	+ samples	4	0	1	2	1	2	0
	Log_{10} cfu/g	2.66 ± 0	*	2.04 ± 0	2.50 ± 0	2.44 ± 0	2.40 ± 0	*
	Range	2.58-2.70	-	2.00-2.08	2.18-2.78	2.40-2.48	2.00-2.56	-
Staphylococcus aureus	+ samples	1	1	1	6	0	6	0
	Log_{10} cfu/g	3.27 ± 0	3.32 ± 0	3.37 ± 0	4.03 ± 0	*	3.45 ± 0	*
	Range	3.26-3.28	3.30-3.34	3.34-3.40	3.00-4.75	-	3.00-3.78	-

*Not enumerated

+ Positive samples detected

Table 3: Samples exceeding the recommended microbial standards limits

Bacteria group	Counts	Food group						
		Cereals n=12	Mixed dishes 2=6	Legumes n=7	Vegetables n=11	Starchy roots n=5	Meats n=9	Beverages n=6
Total Viable Count	*	0	0	0	0	0	0	0
Total coliforms	*	0	0	0	10	0	0	0
Enterococci	*	0	0	0	0	0	0	0
Staphylococcus aureus	*	0	0	0	6	0	0	0

* Samples exceeding the recommended standards limit

There was a significant difference in the microorganisms evaluated in vegetables from all the five locations ($p < 0.05$). Ricky road had the highest mean *Staphylococcus aureus* (4.69 ± 0.05 $\log_{10}$ cfu/g) (Table 4). Ricky, Nanyuki and Likoni roads had similar counts of *Staphylococcus aureus* while enterprise road had the least mean counts (3.13 ± 0.15 $\log_{10}$ cfu/g). Counts of *Staphylococcus aureus* in Enterprise road were similar to that in Lunga lunga road. Ricky roads had the highest coliforms count (4.71 $\log_{10}$ cfu/g) but had similar levels of contamination as Nanyuki road. Total coliforms counts were not significantly different between Likoni and Lunga lunga road ($p < 0.05$). Contamination levels were significantly different among all the locations for *Enterococci and* TVC ($p < 0.05$) (Table 4).

Table 4 Microbial counts of street food vegetables in five locations sampled in Industrial area of Nairobi city

| Microorganism | Location*,[†] | | | | | |
	Enterprise	Likoni	Lunga lunga	Nanyuki	Ricky	Mean
Staphylococcus aureus	$3.13 \pm$ 0.15^a	$4.51 \pm$ 0.06^b	$3.33 \pm$ 0.05^a	$4.45 \pm$ 0.05^b	$4.69 \pm$ 0.05^b	4.02
Total coliforms	$4.34 \pm$ 0.10^a	$4.53 \pm$ 0.04^b	$4.49 \pm$ 0.08^b	$4.65 \pm$ 0.02^c	$4.71 \pm$ 0.03^c	4.54
Total *Enterococci*	$2.04 \pm$ 0.09^b	$2.72 \pm$ 0.04^b	$2.04 \pm$ 0.14^c	$3.44 \pm$ 0.06^d	$2.27 \pm$ 0.02^e	2.67
Total Viable Counts	$4.61 \pm$ 0.04^b	$4.83 \pm$ 0.01^b	$4.75 \pm$ 0.07^c	$4.46 \pm$ 0.02^d	$5.03 \pm$ 0.03^e	4.74

*Values with same superscript in each row are not significantly different ($p > 0.05$); [†]Values represent log cfu/ml

There was significant difference ($p < 0.05$) among the microorganisms evaluated in meats from the four locations (Table 5); Enterprise road had the highest mean *Staphylococcus aureus* ($\log_{10}$ 3.67 ± 0.05 cfu/g) while Lunga lunga road had the least mean counts (3.07 ± 0.08 $\log_{10}$ cfu/g). Counts of *Staphylococcus aureus* and total coliforms were different among all the locations ($p < 0.05$). Nanyuki road had the highest coliforms contamination at 4.10 ± 0.02 $\log_{10}$ cfu/g. TVC

levels were significantly similar (p<0.05) in Likoni, Nanyuki and Lunga lunga roads and for Enterprise road, Nanyuki road and Ricky road.

Table 5 Microbial counts in street food meats in four locations sampled in Industrial area of Nairobi city

| Microorganism | Location*[†] | | | | |
	Enterprise	Lunga lunga	Nanyuki	Ricky	Mean
Staphylococcus aureus	3.67 ± 0.05[a]	3.07 ± 0.08[b]	3.22 ± 0.03[c]	3.33 ± 0.06[d]	3.30
Total coliforms	3.00 ± 0.02[a]	3.96 ± 0.03[b]	4.10 ± 0.02[c]	3.80 ± 0.02[d]	3.72
Total Viable Counts	3.85 ±0.13[b]	3.55 ± 0.17[a]	3.78 ± 0.13[ab]	3.77 ± 0.14[ab]	3.73

*Values with same superscript are similar; [†]Values represent log cfu/ml

Escherichia coli was detected in two food groups from two locations namely mixed dish collected from Lunga lunga road and two vegetable samples from Lunga lunga and Nanyuki roads as shown in Table 6.

Table 6 Presence of *Escherichia coli* in street food in Industrial area of Nairobi

| Food type | Location/road | | | | |
	Enterprise	Lunga Lunga	Nanyuki	Likoni	Ricky/ Falcom
Cereals	-	-	-	-	-
Mixed dishes	-	+	-	-	-
Legumes	-	-	-	-	-
Vegetables	-	+	-	+	-
Starchy roots	-	-	-	-	-
Meat	-	-	-	-	-
Beverage	-	-	-	-	-

Key + Present, - Absent

Salmonella species were not detected in any of the food groups in this study.

3.4 Discussion

Total viable microorganisms counts were not significantly different ($p > 0.05$) in all food consumption food groups among the locations. This may be attributed to the fact that most vegetables were served raw in salads or only partly cooked. Beverages had the least mean counts (3.19 ± 0.2 $\log_{10}$ cfu/g) of bacterial counts. This was expected from the fact all the beverages were subjected to heat treatment during their preparation. Cereals, mixed dishes, legumes, starchy roots and meats had varying levels of counts TVC while the highest contamination was in Ricky road vegetables at 5.03 ± 0.03 $\log_{10}$ cfu/g. Cereals, legumes, starchy roots, beverages and some meat were safe for consumption because they met the Kenya standards. By these standards, any cooked food should contain no more than 6.00 $\log_{10}$ cfu/g viable counts per gram upon analysis (KEBS, 2003; Gilbert et al. 2000). All vegetables had unacceptable levels of coliforms and *Staphylococcus aureus* at the unacceptable levels >4.00 $\log_{10}$ cfu/g each. Meat (fish) from Ricky road had unacceptable levels of *Staphylococcus aureus* at 4.10 $\log_{10}$ cfu/g (KEBS, 2003; Gilbert et al. 2000)

In terms of contamination with *Enterococcus* species, foods were safe at levels below 4.00 $\log_{10}$ cfu/g with the highest contamination was noted in Nanyuki road at 3.44 ± 0.06 $\log_{10}$ cfu/g. with other foods ranging 2.04 to 2.66 $\log_{10}$ cfu/g. *Enterococci* species were not isolated in mixed dishes and beverages. The levels enumerated may indicate a possible contamination during handling after cooking, and inadequate hygiene and sanitation.

Staphylococcus aureus was not isolated in starchy roots and beverages foods. The contamination in vegetables (4.03 $\log_{10}$ cfu/g) and meats (3.45 $\log_{10}$ cfu/g) suggest a contamination probably before serving.

Ricky road was noted to consistently have the highest contamination levels in vegetables with *Staphylococcus aureus* (4.69 $\log_{10}$ cfu/g), total coliforms (4.71 $\log_{10}$ cfu/g), and total viable

counts (5.03 $\log_{10}$ cfu/g) in vegetables which could be attributed to practices of food handling and the initial contamination of the raw material sourced in that area. *Staphylococcus aureus* suggests contamination emanating from handling of food preparation, processing or vending. *Staphylococcus aureus* being part of the micro flora present on/in several parts of the human body is a good indicator of contamination due to poor personnel hygiene practices (Nester et al. 2001).

Mensah et al (2002), Ghana found that foods were particularly heavily contaminated with *Staphylococcus aureus* on excessive handling and cross contamination after cooking. (KEBS, 2003; Gilbert et al. 2000) the meat (fish) from Nanyuki road were not acceptable for consumption (4.10 $\log_{10}$ cfu/g) of coliforms while those from Enterprise, Lunga lunga and Ricky roads are safe for consumption.

Enterobacteriaceae are useful indicators of hygiene and post processing contamination of heat-processed foods. Their presence in high numbers ($>10^4$/gram) in ready to eat foods indicated that an unacceptable level of contamination had occurred, or there had been under processing. Total coliform counts were high in vegetables at 4.48 $\log_{10}$ cfu/g, which may be attributed to the fact that most vegetables were served raw in salads or only partly cooked. This agrees with results from a study carried out by Amponsah-Doku et al (2010) in raw lettuce with range of 4.35 - 9.20 $\log_{10}$ cfu/g vended in the streets of Ghana as highlighted earlier by Donkor et al. 2008. The level of bacterial loading on lettuce and other raw vegetables may originate from the farm irrigation water (Mensah et al. 2001; Obiri-Danso et al. 2005). The contamination could also be attributed to substandard cutting and preparation practices, particularly poor hygienic conditions, of the premises that may result from, rubbish, sewage and other noxious substances present in the vicinity (WHO, 2011). Bhaskar *et al.* (2004) and Mosupye *et al.* (2000) had reported that bacteria from dirty dish washing waters and other sources on utensil surfaces and

constitute a risk for contamination during food vending. The contamination by coliforms was at unacceptable levels in ready to eat foods as enumerated in vegetables all >4.00 $\log_{10}$ cfu/g. The unacceptable counts of coliforms may be attributed to inadequate handling: where food like fried fish as in this case is displayed and sold in the open air and handled by vendors with bare hands. This agrees with Tambekar et al (2009) who found severe contamination of displayed food through handling. Only one cereal (rice) detected to be contaminated with coliforms (<2.00 $\log_{10}$ cfu/g). Mixed dishes and legumes coliforms contamination albeit at low levels could have emanated from post cooking handling. Presence of coliforms in street foods might be due to water used for cooking and serving which was contaminated with feacal coliforms as found by Khalil et al. (1994).

This study shows the foods were safe for human consumption which can be attributed to the fact that the foods were mostly served hot and only held for 1-3hrs before serving. Presence of coliforms and *Staphylococcus aureus* indicates a possibility of secondary contamination. One major source of contamination of foods sold by street vendors is the washing and processing water (Khalil et al. 1994). No coliform was detected in all the beverages probably since they were subjected to heat treatment during the preparation. This was expected owing the heat treatment during preparation. The study by Mwadime, 2001 indicated that majority of highly contaminated foods were prepared on site where holding time was 1.5-12 hours. Mensah et al. (2002) found that there was no *Bacillus cereus*, *Staphylococcus aureus*, or *Enterobacteriaceae* in breakfast, snack foods and porridge prepared and sold within 2–3 hours at 50–90 °C, a temperature range over which most vegetative bacteria do not survive. Manu et al. (2010) observed that foods generally boiled for long periods and not excessively handled have acceptable levels of microbial contamination.

Escherichia coli tested positive for mixed dish collected from Lunga lunga road (1/56) and vegetables in both Lunga lunga and Nanyuki roads (2/56). This was 5.3 % of all the samples and is slightly higher compared to a study that reported in 3 % of the examined samples by a study by (Joon-Il et al. 2010) in street foods of Korea. This disagrees with a study conducted in the street food of cape coast Ghana by Annan-prah et al. 2011 where all sampled food tested positive for *Escherichia coli*. However, *Escherichia coli* have been previously detected in 48% of foods sampled from Korogocho slums of Nairobi (Mwadime, 2001).

Salmonella species were not detected in any of the food group across all roads sampled in this study. As highlighted by Tambekar et al. 2009, the consumption of street food cannot be stopped on hygienic grounds. Hygienic practices can instead be improved to ensure more safety. Some street foods sampled and analyzed in this study including cereals, legumes, starchy roots, mixed dishes, legumes and meats from Enterprise, Ricky, Lunga lunga and Likoni roads were found to meet microbiological standards of ready to eat foods prepared on site. They were therefore safe for human consumption reference to the standards and guidelines by (KEBS, 2003; Gilbert et al. 2000). Annan Prah (2011) in Ghana also found khebabs, fried fish and beans with gari had acceptable levels of microbial contamination.

Vegetable foods in all locations road were classified as unacceptable as they could not meet the acceptable microbiological criteria for coliforms and *Staphylococcus aureus* and coliforms in meats from Nanyuki road.

3.5 Conclusion

Most of the foods sampled and analyzed in this study including cereals, legumes, starchy roots, mixed dishes, legumes and meats from Enterprise, Ricky, Lunga lunga and Likoni roads were found to meet *Staphylococcus aureus*, total counts and *Enterococci* standards of ready to eat foods. However, vegetable foods were contaminated. The presence of unacceptable levels of coliforms and *Staphylococcus aureus* in the vegetables, and coliforms in meat (fish) from Nanyuki road may suggest hygiene practices need be improved to assure food safety upon consumption. The vendors prepared and served their foods mostly hot and this factor combined with preparation on site, could have contributed to safe street food as revealed by this study.

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4.0 Pathogenicity of strains isolated from street foods from Industrial area Nairobi

Abstract

This study sought to characterize the strain distribution of *Enterobacter aerogenes, Enterococci* species and *Staphylococcus aureus* and further investigate the presence of enterotoxigenic genes in *staphylococcus strains* isolated from street foods of Industrial area, Nairobi. A rep PCR was performed using microsatellite primer GAC_5. A multiplex PCR was carried out to investigate the presence of staphylococcal enterotoxin genes (*se*); *sea, seb, sec, sed, see, seg, sei* and *sej*..

Rep PCR revealed a close relationship amongst the *Enterobacter aerogenes, Enterococci* species and *Staphylococcus aureus* isolates. None of the isolates from street food was found to possess genes coding for production of the staphylococcal enterotoxins as targeted in this study. The close strain relationships within each species imply possibility of a common source of contamination like contaminated processing water, or cross contamination of raw materials, cooked food by equipments or vendors. Further studies are required to establish the source of contamination with these related microorganisms.

4.1 Introduction

Although governments throughout the world are attempting to improve the safety of the food supply, the occurrence of food borne disease remains a significant health issue in both developed and developing countries (WHO, 2011).

Street foods in some African countries have been tested for various microorganisms of public health concern, including faecal coliforms, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* species and *Bacillus cereus*. *Escherichia coli* and *Staphylococcus aureus* were recovered in a significant proportion of the food, water, hands and surface swabs tested in Harare (FAO/WHO 2005). Street foods can also be sources of enteropathogens (Mensah et al. 2002). Pathogenicity and virulence of an organism are regulated by virulence coding genes present in the genomic regions known as pathogenicity islands (Hacker and Kaper, 2000). Contamination of food with enterotoxigenic *Staphylococcus aureus* causes staphylococcal enterotoxins (SEs) intoxication when growth and toxin production conditions are met which is associated with symptoms like vomiting and diarrhoea. Major serological enterotoxins (SE) that have been characterized are: SEA, SEB, SEC, SED, and SEE (Robbins et al. 1977) and recently SEG, SEH, SEI, SEJ, SEK, SEL, SEM, SEN, SEO, SEP, SEQ, and SEU (Letertre et al. 2003; Yarwood et al. No information is available on the virulence or pathogenicity of microorganisms isolated from street food matrices Studies on strain distribution reveals influence of hygiene and diversity or commonality of contamination sources.

There was need to study the strain distribution and pathogenicity of presumptive food pathogens in industrial area of Nairobi.

4.2 Materials and methods

4.2.1 Sampling and sample preparation

The study area was conducted between March and May 2011 in Industrial area, Nairobi (Coordinates: 1°17'S 36°49'E). Nairobi is the largest city in East Africa with 3.1 million people and an annual population growth rate of 4% (KNBS, 2010). The eating places with food prepared on site in the major roads with street food in the Industrial area were selected to form the study area as follows; Enterprise road, Lunga lunga road, Likoni road, Nanyuki road and Ricky road. Food samples from the menu were collected in sterile polythene bags. Seven (Modified FAO) food groups including cereals, mixed dishes, starchy roots, beverages, vegetables, meats and legumes were sampled and analyzed in this study. All samples were transported to the University of Nairobi Department of Food Science, Nutrition and Technology microbiology laboratory within three hours of collection on ice in cooler boxes and then refrigerated at $4°C$ until they were taken for analysis in 2 hours. Fifty six samples were sampled and analyzed in this study (Pfeiffer, 2002). Isolation was done as indicated in Chapter 3 section 3.2.4. The typical colonies were purified by streaking three times on selective media and stored 0.25 M sucrose solution at -44 °C until taken for PCR analysis.

4.2.2 DNA extraction

The preserved isolates were thawed and cultured on the respective selective media. Sixty four isolates of *Enterobacteriaceae*, 33 of *Staphylococcus aureus* and 23 of *Enterococci* species were isolated and analyzed in this study. Each colony was picked and suspended in 100 µl of sterile distilled water in 1.5 ml eppendorf tubes, heated to 95 °C for opening of DNA strands for 30 minutes and cooled immediately on ice water (0 °C). The solution was then be centrifuged at

15000 Rpm for 5 minutes at 4°C in (eppendorf centrifuge 5413 Germany). The resultant supernatant was drawn and preserved at -20°C as the template DNA.

4.2.3 Molecular typing

4.2.3.1 PCR analysis

A Rep-PCR for *Enterobacteriaceae, Staphylococcus aureus* and *Enterococci* species was done by the modified method described by (Walczak et al. 2007) with primer (GAC) $_5$. The primer pre-mix which consisted 2mM Mg^{2+} as $Mgcl_2$, 0.025 µl of Taq polymerase, 0.2mM dNTPs, distilled nuclease free water and the DNA template were added in a 200 µl eppendorf tube. This made a total reaction volume of 25µl with 1µl DNA template, 12.5 µl pre-mix, 3.25 µl GTG_5 and 8.25 µl distilled water. The primer used is (GAC)5- Oligonucleotide primer sequence, 5'-3' GACGACGACGACGAC (Walczak et al, 2007). The Rep PCR protocol used is presented in Table 7a while Table 7b indicates PCR for SEs.

Table 7: PCR protocol for (a) Rep PCR and (b) staphylococcal enterotoxins

Step	Temperature (°C)		Time		Number of cycles	
	a	b	a	b	a	b
Initial denaturation	94	95	3*	5*	1	1
Denaturation	94	94	20**	3*	35	35
Annealing	50	55	1*	3*		
Polymerization	72	72	20**	1		
Final polymerization	72	72	5*	5*	1	1
Hold	4	4	∞	∞	1	1

All PCR reactions were done with minicycler MJ Research Inc. USA.

*Time in minutes, **Time in seconds

Molecular typing was carried out in the Molecular laboratory of the Department of PHPT in University of Nairobi.

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Staphylococcus aureus strains isolated were typed for *sea, seb, sec, sed, see, seg, sei* and *sej*. Reference strains previously isolated and characterized by Stephan et al. (2001) were used as reference strains for enterotoxin gene typing (Table 8). These included *S. aureus* 463 (*seb, seg & sei*), 117 (*sea, seg, sej & sei*), 129 (*sea, seg, sei & sej*), 266 (*seb, seg, sei*), 216 (*sec, seg, sei*), 235 (*sec, seg, sei*), 243 (*sed, seg, sei, sej*) and 238 (*sed, seg, sei & sej*).

Table 8: Sequence of oligonucleotide primers used for staphylococcal enterotoxins and predicted lengths of PCR amplification products.

Target	Primer	oligonucleotide sequence, 5'-3'	product size	Reference
SEA	sea-1	AAAGTCCCGATCAATTTATGGCTA	219bp	Tsen & Chen, 1992
	sea-2	GTAATTAACCGAAGGTTCTGTAGA		
SEB	GSEBR-1	GTATGGTGGTGTAACTGAGC	164bp	Mehotra et al, 2000
	GSEBR-2	CCAAATAGTGACGAGTTAGG		
SEC	SEC-1	GACATAAAAGCTAGGAATTT	257bp	Johnson at al,1991
	SEC-2	AAATCGGATTAACATTATCC		
SED	sed-f	GTGGTGAAATAGATAGGAACTGC	385bp	Monday & Bohach,1999
	sed-r	ATATGAAGGTGCTCTGTGG		
SEE	SEE-1	TAGATAAGGTTAAAACAAGC	169bp	Johnson at al,1991
	SEE-2	TAACTTACCGTGGACCCTTC		
SEG	SEG-1	AATTATGTGAATGCTCAACCCGATC	642bp	Jarrand et al,1999
	SEG-2	AAACTTATATGGAACAAAAGGTACTAGTTC		
SEI	SEI-1	CTCAAGGTGATATTGGTGTAGG	577bp	Jarrand et al,1999
	SEI-2	AAAAAACTTACAGGCAGTCCATCTC		
SEJ	SEJ-1	CATCAGAACTGTTGTTCCGCTAG	192bp	Monday & Bohach,1999
	SEJ-2	CTGAATTTTACCATCAAAGGTAC		

4.2.4 Electrophoresis and visualization

PCR loading wells were made on 1.5% Agarose gel into which 7 µl EtBr (Carlsbad CA USA)/100 ml agarose had been added, solidified and the gel submerged into X1 TBE buffer. A total volume of 24 µl comprising of 20µl DNA template and 4 µl PCR loading buffer (Promega

Madison USA) were loaded per well. A 15 Milliampere current at a constant voltage was then applied and the amplified fragments were allowed to migrate until appropriate band separation was achieved. The DNA template were loaded against reference strains as controls including STM1 (*stx2*), STM2 (*stx1*), 4115/2 (*eae*) and 3750/2 (*stx1 & eae*) by Kohler et al. 2008. The separated bands were visualized with UV trans-illuminator (Vilber Laurmat-France). The results were preserved by photography. The visualized bands sequences were compared with those of the controls and 1 Kbp molecular marker.

4.3 Results

4.3.1 Strain distribution of microorganisms 4.3.1.1 *Enterobacteriaceae*

Sixty isolates of *Enterobacter aerogenes* were analyzed by a rep PCR. There were 17 isolates from vegetables, 7 from cereals, 18 from mixed dishes, 4 from starchy roots, 12 from meats and 2 from legumes. All samples had similar single band amplified (Figure 1).

Figure 1 Representative Rep PCR amplification gel electrophoresis picture for *Enterobacter aerogenes* isolates

4.3.1.2 *Enteroccocci* species

A total of 22 *Enterococci* species included 13 from vegetables, 3 from cereals, 3 from meats, 2 from starchy roots and 1 from legumes. All samples had same level of single bands amplified (Figure 2).

Figure 2 Representative Rep PCR amplification gel electrophoresis picture for *Enterococcus* isolates

4.3.1.3 *Staphylococcus aureus*

A total of 33 isolates of *Staphylococcus aureus* were obtained from street foods including 17 from vegetables, 2 from cereals, 10 from meats, 2 from starchy roots, 1 from legumes and 1 from mixed dishes. All samples had similar single band amplified (Figure 3).

Figure 3 Representative Rep PCR amplification gel electrophoresis picture for *Staphylococcus aureus* isolates

4.3.1.4 Presence of *Staphylococcal* enterotoxins in the street foods of Industrial area of Nairobi

The seven categories of foods collected from streets of Industrial area of Nairobi were evaluated for the possession of staphylococcal enterotoxins. All the isolates were negative for the *sed* and *seg*. A Simplex PCR amplification gel electrophoresis picture (Figure 4) showing *Staphylococcus aureus* reference isolate positive for *sed*.

Figure 4 PCR gel picture with positive identification of sed in *Staphylococcus aureus*.

4.4 Discussion

According to the reviewed literature, this is the first study evaluating the strain distribution of microorganisms isolated from street food in Kenya. From the PCR analysis, the isolates of *Staphylococcus aureus, Enterococci* species and *Enterobacter aerogenes* from street food were similar as shown by Rep PCR. This is also the first study to evaluate the pathogenicity of *Staphylococcus aureus* strains in street food in Kenya. However, none of the isolates from the street food was seen to possess genes coding for production of staphylococcal enterotoxins *sea* and *seg*. Kenyan camel milk samples were positive for *seb*, *sed*, *seg* and *sej* in a study carried out in 2010 by Njage et al (2010). Prevalence of 22 % in *Staphylococcus aureus* containing enterotoxin genes *seb*, *sec*, *sed* and *seg* were also detected in mastitis isolates in Turkey by Murat et al. (2009) The absence of genes coding for production of staphylococcal enterotoxins implies that *Staphylococcus aureus* from street foods are not be likely to threaten human health. The similar bands from Rep PCR analysis indicate that all isolates were similar strains. They could therefore have originated from a similar source such as processing water, raw material and cooked food cross contamination or contamination from the body of food handlers.

4.5 Conclusion

From the results *Staphylococcus aureus* isolated from street food did not possess enterotoxigenic genes that code for production of *sed* and *seg*. Street foods in Nairobi are not likely to pose a public health risk through staphylococcal food borne illness. The isolated microbial strains were found to be similar at molecular level, suggesting a similar source of contamination. This indicates the need to improve hygiene practices in street food handling despite lack of safety hazards as judged by *S. aureus*. The microorganisms signify gaps in hygiene and sanitation and the need for improvement and not a direct threat to human health

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upon consumption. More studies are required to assess the status of virulence factors both enterotoxigenic and enteropathogenic food borne microorganisms in different street foods. This would allow a clear conclusion on safety of the street foods.

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5.0 Hygienic practices in handling of street foods in Industrial area, Nairobi

Abstract

Street food plays a significant role in feeding the urban population with cheap, accessible and nutritious foods. Street food can lead to food poisoning and food borne illnesses resulting mainly from poor hygienic practices. There are limited studies on the hygienic practices and food safety for street food production in Nairobi. This study was conducted to evaluate hygienic practices in preparation of street food in Nairobi, Kenya. Twenty nine street foods vending stalls from five roads including Enterprise road, Lunga lunga road, Ricky, Likoni and Nanyuki road were evaluated. Seventy six percent (22/29) of vendors did not hold food handlers medical certificate. Eighty eight percent of the sampled sites were clean while 79 % of the stalls were constructed using polythene bags. In terms of protective clothing 66 %, (19/29) of vendors spread across all studied locations did not have protective clothing. Seventy nine percent (23/29) of vendors had no training on food hygiene, although vendors in Lunga lunga road reported having received some training through a Non Governmental Organisation and a women group. Majority of vendors, 87 % used polythene bags for packaging take away rations. Another 79 % of the vendors have not received any customer complaints. Sixty nine percent of vendors dumped their wastes into Nairobi city council waste bins, while 79 % (23/29) stated using the Nairobi city council sanitary facilities. There ought to be adequate provision of water, sanitary facilities and waste collection services, and training programs for the street food vendors to preempt possibility of food poisoning outbreaks from weaknesses in hygienic practices in these areas.

5.1 Introduction

Nairobi is the biggest city in East Africa with a population of 3.1 million residents (KNBS, 2010). The street food industry in Industrial area of Nairobi plays an important role in meeting the dietary needs of the Industry workers owing to its accessibility, low cost (Latham, 1997) and provision of employment and nutritious diets to the urban populations (Mwangi, 2002). FAO stipulates that street foods raise concern with respect to their potential for serious food poisoning outbreaks due to improper use of additives, the presence of adulterants, environmental contaminants and improper food handling practices amongst street food vendors (FAO, 1997). Hilda (2002) noted that the demand and popularity of street food will continue to increase. Street food vendors are often unlicensed, untrained in food hygiene and sanitation, and work under unsanitary conditions (FAO, 1997). This study was conducted to evaluate the status of hygiene practices of street foods prepared on site in Nairobi. The aim of the study was to advise on the appropriate measures which could be employed to avoid potential cases of food borne diseases.

5.2 Materials and methods

A descriptive survey was conducted in Industrial area, Nairobi through structured questionnaire and observation. Twenty nine sites were purposively selected to include vendors who prepare and sell food on site and formed for the study area. The study sites included 13 stalls from Enterprise road, 5 from Lunga lunga road, 6 from Ricky road, 3 from Likoni road and 2 from Nanyuki road. It covered areas on the stalls status, raw materials, work methodology, food handlers, utensils, sanitary facilities, customer complaints and pests. The questionnaire used in this study is attached as appendix1.

5.2.1 Data analysis

Descriptive analysis involving frequencies and percentages on the qualitative data from the hygiene questionnaire partitioned as per the locations was carried out using SPSS version 17.

5.3 Results

5.3.1 Hygienic and sanitary status of food stalls and its environs

5.3.1.1 Potential contaminants sources

The surrounding environment of the food vending facilities was assessed by observation for cobwebs, soot and dust.

All locations were exposed to at least one potential source of contaminants. Waste water drainage tunnels were 24 %, (7/29) in the proximity stalls to roads. Vehicles passed within ten metres in 27 %, 8/29 of the street food vending stalls Six percent (2/29) of the sites were considered safe from contaminants (Table 9) at the time of assessment. Other potential sources of contaminants included dusty within five metres, and mud and sludge within two metres. High frequency of stalls (85 %) in Enterprise road had food preparation and serving areas with drainage tunnels and passing vehicles as potential contaminants. The potential contaminants included houseflies with the highest occurrence at 55 % (16/29) of all stalls and 77 % (10/13) of stalls in Enterprise road and soot in 21 %, 6/29 of all stalls. Dust and cobwebs were among the least common sources of contaminants besides raw material peelings in one stall of Lunga lunga road (Table 9).

Table 9 Potential contaminants sources in five roads in Industrial area, Nairobi

	Enterprise n=13	Ricky n=6	Lunga lunga n=5	Likoni n=3	Nanyuki n=2
Dusty road within two metres	7 %	0 %	0 %	0 %	0 %
Drainage tunnel within five metres	11 %	0 %	30 %	33 %	0 %
Dust	7 %	7 %	10 %	33 %	0 %
Dusty road within five metres	3 %	0 %	0 %	0 %	0 %
Garbage site within fifteen meters	3 %	7 %	0 %	0 %	0 %
Mud and sludge within two meters	3 %	0 %	10 %	0 %	0 %
Vehicles passing within ten meters	11 %	17 %	0 %	33 %	50 %
None	3 %	9 %	0 %	0 %	0 %
House fries	33 %	14 %	30 %	0 %	25 %
Dust	0 %	7 %	0 %	0 %	0 %
Soot	7 %	21 %	0 %	0 %	25 %
Cobwebs	3 %	7 %	0 %	0 %	0 %
Open air no risk	7 %	7 %	10 %	3 %	0 %
Raw material peelings	0 %	0 %	10 %	0 %	0 %

5.3.1.2 Cleanliness of vending stalls

Majority of sampled stalls were found to be clean (Figure 5). Highest levels of cleanliness were observed in Enterprise road, (85 %) 11/13 stalls were clean. Likoni road had 100 % unclean stalls with dust, soot, cobwebs, houseflies and mud recorded at the time of assessment. Also, Majorities consisting of 83 % (24/29) of the stalls sampled were found to have clean equipments/utensils and vendors used soap in cleaning.
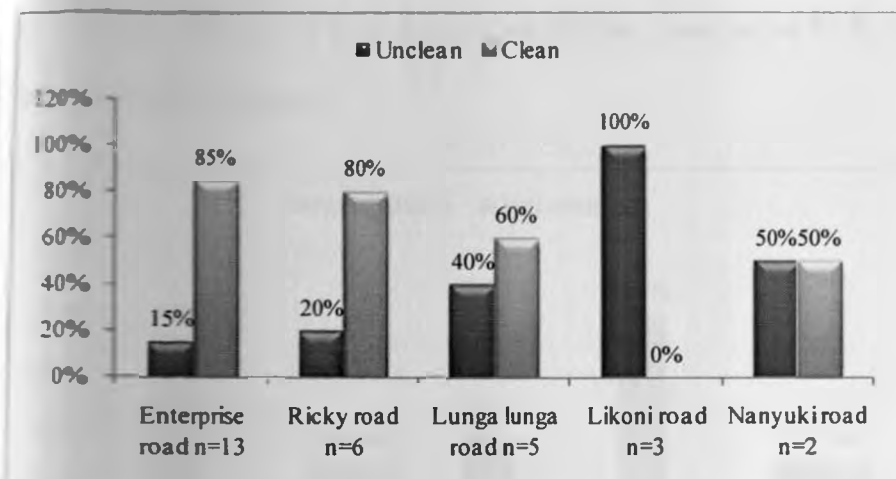
Figure 5: The percentage of street food stalls which were clean at the time of the study in 5 road of Industrial area, Nairobi

5.3.1.3 Construction materials for the street food stalls

An array of materials were used to construct the make shift stalls. Majority of stalls in all locations were constructed using polythene bags, while hard board and iron sheet were less commonly used Table 10). One vendor operated in open air in Likoni road.

Table 10: Construction materials used in the street food stalls from five selected locations in industrial area, Nairobi

	Enterprise n=13	Ricky n=6	Lunga lunga n=5	Likoni n=3	Nanyuki n=2
Polythene bags temporary roof	42 %	40 %	40 %	50 %	100 %
Polythene roof and wall	33 %	60 %	40 %	0 %	0 %
Hardboard wall and polythene roof	8 %	0 %	0 %	0 %	0 %
Iron sheet roof and polythene wall	8 %	0 %	0 %	0 %	0 %
Iron sheet roof and wall	8 %	0 %	20 %	0 %	0 %
Open air no roof or wall	0 %	0 %	0 %	50 %	0 %

5.3.2 Food handlers/vendors practices

5.3.2.1 Possession of medical health certification by food handler

Seventy six percent, (22/29) of vendors did not have a food handlers' medical certificate in all

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the locations. Majority of those without certificates consisted of 92 %, (12/13) of vendors from
Enterprise road (Figure 6).
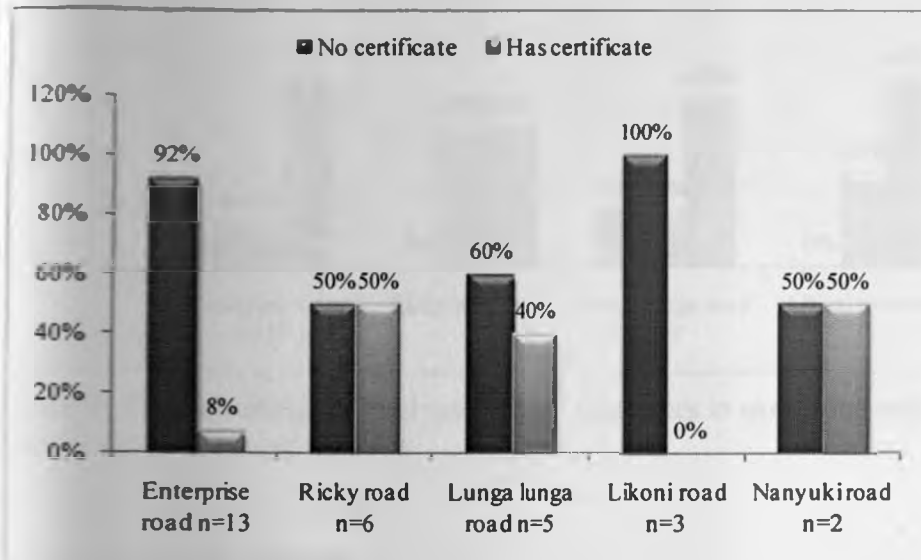
Figure 6: The percentage of street food vendors with food handlers' medical certification in five roads of Industrial area, Nairobi

3.2.2 Hand washing

Majority (66 %) 19/29 of vendors from the sampled stalls did not facilitate washing hands of by customers (Figure 7). Among those who washed the customers' hands, 24 % (7/29) use a jug and a basin to aid in hand washing while 10 % (3/29) had a hand wash station with water and soap. No hand washing was done in Nanyuki road (Figure 7). Vendors in all the locations reported that most of the customers preferred partial packaging of the foods with polythene bags or use of cutlery altogether. Also, Use polythene bags to package take away rations was spread in all locations, while use of personal containers and maize leaves was used in one stall.
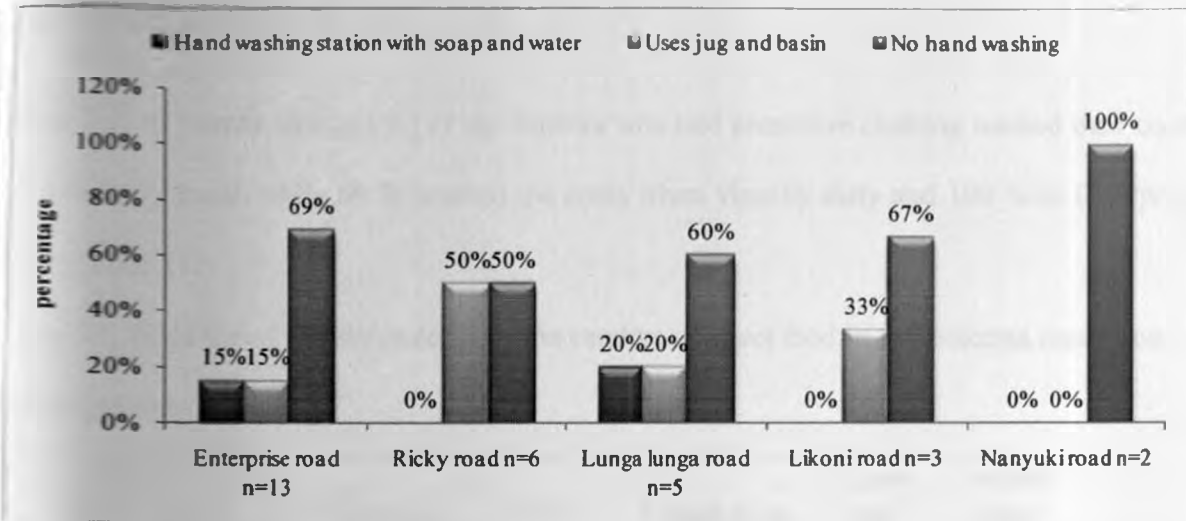
Figure 7: The percentage of hand washing by customers in street food stalls of Industrial area, Nairobi from five locations with

5.3.2.3 Protective clothing

Thirty four percent (10/29) of the vendors used protective garment (Figure 8). However, only 10 % (3/29) had complete coats while 24 % (7/29) had half coats which could not offer protection. The lack of protective coat was noted in all the locations but high levels in Enterprise 85 % (11/13) and all 3 (100 %) stalls in Likoni roads (Figure 8).
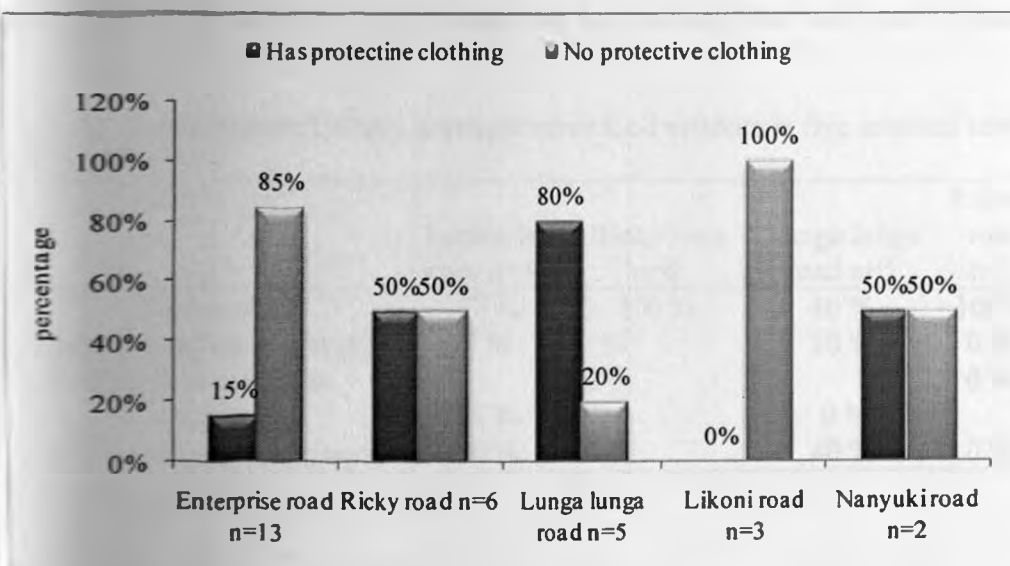
Figure 8: The percentage of vendors wearing protective coats in the street food stalls of five

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roads in Industrial area, Nairobi

Seven out of twenty nine (24 %) of the vendors who had protective clothing washed their coats on a weekly basis, while 69 % washed the coats when visually dirty and 100 % in Enterprise road (Table 11).

Table 11: Washing of protective coats by the vendors of street food in five selected roads from Industrial area, Nairobi

	Enterprise road n=13	Ricky road n=6	Lunga lunga road n=5	Likoni road n=3	Nanyuki road n=2
Weekly	100 %	67 %	75 %	0	0
When visually dirty	0	0	25 %	0	50 %
After 3 days	0	33 %	0	0	0

5.3.2.4 Training of street food vendors on food hygiene and safety

Only 6/29 (21 %) have had training on food hygiene and food safety (Table 12). Another 7 % (2/29) relied on basic primary education, 2 vendors in Enterprise road had secondary school training while 2 vendors in Lunga lunga road had training from NGO and women groups.

Table 12: Food hygiene training amongst street food vendors in five selected roads in Industrial area, Nairobi

	Enterprise road n=13	Ricky road n=6	Lunga lunga road n=5	Likoni road n=3	Nanyuki road n=2
No training	77 %	100 %	40 %	100 %	100 %
Basic education in primary	7 %	0 %	20 %	0 %	0 %
Basin education in		0 %		0 %	0 %
secondary	15 %		0 %		
NGO and women groups	0 %	0 %	40 %	0 %	0 %

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5.3.3 Food handling practices before serving

5.3.3.1 Holding food after cooking and before serving

Majority consisting of 82 %, (23/28) of respondents let the food cool naturally while they wait for the customers. All vendors in Lunga lunga and Nanyuki road reported to let the food cool naturally. Others 8 % in Enterprise road and 17 % in Ricky road held their food on hot surface while some held the food warm (Table 13).

Table 13: Method of food holding after cooking and prior to serving by street food vendors in five elected roads from Industrial area, Nairobi

	Enterprise road n=13	Ricky road n=6	Lunga lunga road n=5	Likoni road n=3	Nanyuki road n=2
Left to cool naturally	92 %	50 %	100 %	67 %	100 %
Held on hot surface	8 %	17 %	0 %	0 %	0 %
Held warm	0 %	33 %	0 %	33 %	0 %

5.3.3.2 Water quality and handling of raw materials

All the vendors do not treat or boil their drinking water. Majority 62 % (18/29) of vendors obtain their water from street water vendors and water kiosks 34 % (10/29) while a vendor in Enterprise road carries water from home. Some vendors in Enterprise road obtain water from both water kiosks and water vendors while all vendors in Likoni and Nanyuki roads obtain from water vendors (Table 14)

Table 14: Sources of water for street food vendors from locations in Industrial area, Nairobi

	Enterprise road n=13	Ricky road n=6	Lunga lunga road n=5	Likoni road n=3	Nanyuki road n=2
Water kiosk	54 %	40 %	20 %	0	0
Water vendors	54 %	60 %	80 %	100 %	100 %
Home	1	0	0	0	0

Majority 52 % (15/29) of the vendors obtained their raw materials from local groceries,

followed by Wakulima market 38 % (11/29), local shops 31 % (9/29), local butcheries, Kia

Michael market and raw food vendors/suppliers. Local sourcing was widely practiced (Table

15). Most vendors had multiple sources of food raw materials.

Table 15: Sources of raw material for the street food vendors in five locations in Industrial area, Nairobi

	Enterprise road n=13	Ricky road n=6	Lunga lunga road n=5	Likoni road n=3	Nanyuki road n=2
Wakulima	18 %	30 %	43 %	33 %	0 %
Raw food vendors	5 %	0 %	0 %	0 %	0 %
Local groceries grain vegetables vendors	36 %	20 %	43 %	33 %	25 %
Local butcheries	9 %	10 %	0 %	0 %	50 %
Kia michael	9 %	20 %	0 %	0 %	0 %
Local shops	18 %	20 %	14 %	33 %	25 %
Some food precooked at home	5 %	0 %	0 %	0 %	0 %

5.3.4 Pests

None of the respondents reported having encountered any pest or rodent. No control measure

was noted to have been used because they encountered none.

5.3.5 Customer complaints

Only 21 % (6/29) of vendors had received customer complains. (Table 16), including; food

quality consistency, flavour, taste and texture in Enterprise road, Ricky and Nanyuki roads and

poor ration mixture in Lunga lunga.

Table 16: Customer complains in street food stalls from five locations in Industrial area, Nairobi

	Enterprise road n=13	Ricky road n=6	Lunga lunga road n=5	Likoni road n=3	Nanyuki road n=2
Food quality consistency flavour taste and texture	31 %	17 %	0 %	0 %	50 %
Ration mixing	0 %	0 %	20 %	0 %	0 %
None	69 %	83 %	80 %	100 %	50 %

5.3.6 Waste disposal

Seventy two percent (21/29) of vendors dump wastes from their stalls into Nairobi city council waste bins (Table 17). Only 31 % (9/29) sell the vegetable wastes. A vendor in Likoni road drains the waste water into the drainage nearby.

Table 17: Methods of waste disposal by the vendors of street foods from five locations in Industrial area, Nairobi

	Enterprise road n=13	Ricky road n=6	Lunga lunga road n=5	Likoni road n=3	Nanyuki road n=2
NCC bins	85 %	67 %	60 %	33 %	100 %
Sell vegetable wastes		50 %	80 %	33 %	0 %
Wash water to drain	0 %	0 %	0	33 %	0 %

5.3.7 Access to sanitary facilities

Seventy nine percent, (23/29) vendors use the Nairobi City Council (NCC) sanitary facilities (toilets) while 21 % (6/29) use sanitary facilities in the nearby slums (Figure 9). All vendors in Lunga lunga, Likoni and Nanyuki roads used NCC toilets.
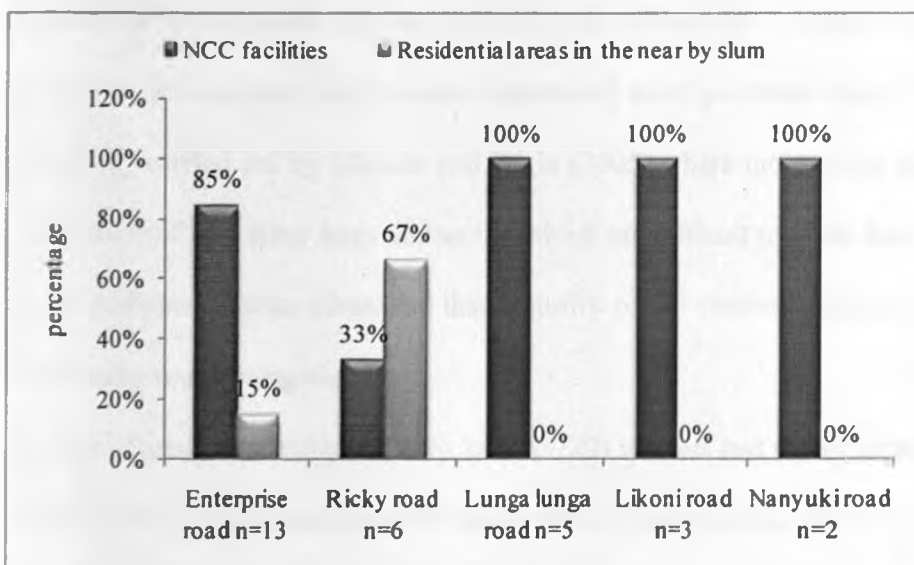

Figure 9: Sanitary facilities used by street food vendors in five locations in Industrial area, Nairobi

5.4 Discussion

Majority of street food vending stalls (22/29) were clean at the time of the survey signifying an effort by the vendors to keep their area of work clean. This was despite some stalls being exposed to potential contaminants and houseflies in all the locations except Likoni road stalls. The presence of houseflies implies probable lack of adequate sanitation. This agreed with (Muinde and Kuria (2005) who found houseflies in most of the street food stalls in Nairobi. Mwadime, 2001 noted house flies in 54.8 % of the vending stalls. This implies that food contamination is most likely to occur despite efforts to keep the stalls clean. 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. In some of the stalls vehicles passed by within ten meters while 97.6 % stalls were situated where vehicles passed within 20 meters radius. The maintenance of clean stalls is made difficult by the nature of construction material given the fact that most stalls where constructed using polythene bags. This was also observed in a study carried out by Muinde and Kuria (2005) where most of the stalls in Nairobi (23/29) were made of polythene bags and wood, which are difficult to clean and sanitize. In the present study equipments were clean and that majority of the vendors cleaned the utensils after every meal using soap during cleaning.

I terms of medical certificates, only 24 % (7/29) vendors had food handlers' medical certificate. This is lower than levels noted in the streets of Accra Ghana (40 %) by Ackah et al. (2011). Annan-prah et al. (2011) observed that 45 % of street food vendors in Cape coast Ghana were not certified medically to handle food. As highlighted in the standard news paper, Kenya of

September 13 2011, there is a need to ensure food handlers are immunized or treated against typhoid and other food borne illnesses.

The equipments could be contaminated during the drying step where the vendors overturned the utensils on a basin and on a make shift rack uncovered and could not protect the utensils from possible contamination from the environment.

Only 34 %, 10/29 of the vendors provide equipments for hand washing. Some use a jug and a basin to aid in hand washing, while others had a hand wash station with water and soap. This differs from a study by Mwadime (2001) where it was revealed that 50 % of vendors had hand washing vessels. It has been established that ineffective personal hygiene can facilitate the transmission of these pathogenic bacteria found in environment and on people's hands through food to humans (Tambekar *et al*. 2008; Mensah *et al*. 2002). Atbara city, Nahr Elneel, Sudan where 98% of the respondents agreed the hand must be washed before eating meal (Abdalla et al. 2009).

Sixty six percent (66 %), (19/29) of the vendors did not have protective clothing and could not protect the foods they handle from any contamination from their bodies and clothing. Muinde and Kuria (2005) also reported that 81.3 % of the vendors did not use aprons.

There was a lack of training of the 79 % (23/29 of the vendors were not trained suggesting lack of control in food protection from contaminants. This was also as noted in India by Tambekar et al (2011) where food vendors were unaware of food regulations and were untrained on food hygiene.

Majority of the vendors79 % (23/29) left the food cool naturally which could lead to multiplication of microorganisms present in the food at the time of storage. Eighty seven per cent (26/29) of the vendors use polythene bags to wrap take away rations. Mwadime (2001) in contrast reported that printed papers were the major packaging media in street foods in Nairobi.

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This could have resulted from the revolution into use of plastics recently to replace most other packaging material. Annan-prah et al. (2011) also had observed that 6 % of street food vendors in Cape coast Ghana use newsprints, and 20 % polythene bags to package food. The increase in the usage of polythene bags in Nairobi suggest measures are required to ensure these packaging forms are free of any potential food contaminant.

All vendors have not received any customer complaints related to food safety while some 21 % (6/29) stated to have received complaints on the texture and consistency of the foods Three per cent of vendors have received complains on the varying quantities of the rations/menus. Annan-prah et al. (2011) observed that only 4 % of street food clients in Cape coast Ghana were concerned with hygienic considerations of street food. This could be the case in Kenya where street food customers may have not reported food borne disease. Majority of the vendors obtained water from water vendors (62 %; 18/29) and water kiosks (34 %; 10/29) indicating dependence on water supply the vendors have no control over. This agreed to a study by Mwadime in 2001 where majority of vendors obtained water from kiosks, suggesting more people engage in water vending business in the streets. Muinde and Kuria (2005) observed that water was ferried from homes of the street food vendors because no potable water was available at their areas of operation. However, this water may not be enough for dish washing and food preparation.

Water in all the stalls is not boiled before serving for drinking suggesting the need for assurance that the water must be safe for human consumption from the source if the water is not obtained from treated sources of the city council. Some vendors (31 %) did not wash their raw food before cooking. These results agree with those of Muinde and Kuria (2005) who found that vendors did not wash fresh foods properly. Pathogens may enter the food system during preparation, cooking, packaging or marketing (Barro et al. 2007). Vendors obtained their raw

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materials from multiple sources indicating the need for a wider approach in addressing the safety of street food.

Majority (69 %) of vendors dump their waste into Nairobi City Council waste bins. Some vendors (21 %) sell the vegetable wastes while 33 % 0f vendors in Likoni road drain the waste water into the drainage nearby. Muinde and Kuria (2005) reported that 92.5 % of street food vendors in which Nairobi did not have garbage receptacles; hence they disposed their garbage near the stalls. This suggests waste bins may have been introduced in which Nairobi city after the study by Muinde and Kuria (2005).

A total of 79 % (23/29) vendors sampled use the NCC sanitary facilities while 21 % use the nearby slums which indicate the need to improve access of these facilities in the streets where the food vending business is prevalent. The NCC has created access for this. There however need to be awareness creation for people to use more of the existing facilities.

5.5 Conclusion

The findings of this study reveal areas of improvement which would translate into positive change towards attaining safe street food.

Majority 76 % of vending stalls were clean which indicates an effort by the vendors to keep their premises clean. However, there is a need to ensure the stalls are properly located to prevent the food contamination from the potential contaminants. Construction materials should be designed for easier cleaning. Seventy six per cent of vendors did not have food handlers' medical certificates. It is recommended that medical screening be carried as a requirement as any carrier of a food pathogen can contaminate food. Only 34 % of the vendors hand wash their customers but customers were reported to prefer polythene bags and cutlery instead of washing hands. Stalls owners and the food handlers should be trained to embrace a hand washing culture

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to avoid any food contamination. Only 34 % vendors wore protective clothing and majority could not protect the food from contamination emanating from their bodies. The wearing of protective clothing will need to be enforced to ensure potential of food contamination is reduced.

There were no customer complaints regarding food safety signifying satisfaction by consumers on the services obtained from these stalls or ignorance altogether. It could also mean under reporting of the complaints.

Training on food hygiene and food safety was lacking in 79 % of the vendors. All vendors were women and consideration can be enhanced by food hygiene training programs that can be correlated to women participation in the society.

A total of 79 % vendors could let the food cool naturally and if the food is not purchased and served when hot, there is potential microbial multiplication if contamination occurs.

A total of 90 % vendors use polythene bags to pack take away rations. The usage of polythene bags to package food required these material evaluated for potential source of contamination.

Seventy nine percent; (79 %), (23/29) of the vendors used NCC sanitary facilities. The high level of dependence of NCC facilities indicates a need to harmonize and provide services at close proximity to the food vendors and their clients.

The water is not boiled before serving to customers. Since some (7 %) of this water is not obtained from treated sources of the NCC, then it should be evaluated as a potential source of food contamination.

The outcome of this study can serve as a baseline data for management and improvement of the street food safety.

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6. General conclusion

Studies have indicated that ready to eat foods and food preparation surfaces may be reservoirs for microbial contamination (Mankee *et al.* 2005; Ghosh *et al.* 2007; Christison *et al.* 2008). Street foods can also be sources of enteropathogens (Mensah et al. 2002). Food borne microorganisms cause disease through infection or intoxication. There was need to study the strain distribution and pathogenicity of presumptive food pathogens and the relationship between their occurrence and relate to the hygienic practices in Nairobi. This could reveal potential of food poisoning outbreaks relating to street food consumption. All food sampled were prepared, vended and consumed on site. The foods sampled were served within three hours of preparation which could explain the non occurrence of microbiological contamination at levels likely to threaten human health.

The vegetable foods had the highest total viable counts at $4.71 \pm 0.3 \log_{10}$ cfu/g, highest total coliforms counts at $4.48 \log_{10}$ cfu/g and highest *Staphylococcus aureus* at $4.03 \log_{10}$ cfu/g. All vegetables were served raw. They were not heat treated and were held at ambient temperature between preparation and service. There were a significant difference ($p>0.05$) in the microbial counts in vegetable foods in all locations which could be attributed to the different sources of raw materials, their processing and service in raw form. It could also be as a result of the holding time at ambient temperature between preparation and serving unlike the cooked dishes. Vegetables from Ricky road were sourced from Wakulima market and local groceries and had the highest *Staphylococcus aureus* counts of $4.69 \pm 0.05 \log_{10}$ cfu/g. There was significantly similar ($p<0.05$) counts ($\log_{10}$ cfu/g) of *Staphylococcus aureus* in vegetables from Nanyuki road (4.45), Ricky road (4.60) and Likoni (4.51). Enterprise road and Likoni road whose raw material vegetables were obtained from local groceries and Wakulima market had significantly similar counts ($p<0.05$) of *Staphylococcus aureus* in vegetables suggesting contamination may

have originated from the raw materials. The wearing of protective coats by personnel was 50 % in Ricky road and 15 % in Enterprise road. Majority of the food vendors in Enterprise road, 92 % did not have protective coats while prominent contaminants were dust, houseflies, and vehicles passing by. Protocarrero et al (2002) pointed out that staphylococcal food poisoning can occur if food is handled by persons, who carry the pathogen in their skin.

Total coliform counts from vegetables in Likoni and Lunga lunga road were significantly similar ($p < 0.05$). Fifty percent of vegetables in lunga lunga road and likoni road had been obtained from Wakulima market and local groceries each. Sixty percent of raw materials used for preparation of street foods from were from Wakulima market while 40 % were from local groceries. Fifty percent of raw materials for preparation of street foods in Nanyuki road were sourced from local groceries and all had significantly similar contamination levels of coliforms counts in vegetables ($p < 0.05$). These suggest coliforms could have emanated from several sources as reported including the raw materials or wash water. All vendors in these locations obtained water from vendors and could have resulted in the microbial counts level.

Enterococci counts in vegetables were highest in Nanyuki road with 3.44 $\log_{10}$ cfu/g. All vegetables were obtained from local groceries. Vendors in this location were not trained on food safety and hygiene, 50 % of the vendors had no food handlers' medical certificate and protective coat and no hand washing was of clients. There were 77 % of the stalls with houseflies as a potential source of contamination. In the same location, 69 % of the vendors did not hand wash their customers, 92 % did not have food handlers' medical certificate. This indicates a potential sanitation problem despite the fact that 85 % of stalls accessed were clean. Also 85 % of the vendors in this location used Nairobi City Council waste bins to dispose off their wastes.

Staphylococcus aureus and coliforms contamination in the meats were significantly different (p

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< 0.05) in all the locations. These were sourced from Kia Michael meat market and local butcheries for all the street food vendors. Contamination with *Staphylococcus. aureus* have resulted from post cooking handling of the foods. Meats in Nanyuki road had unacceptable counts of coliforms of 4.10 $\log_{10}$ cfu/g; levels above the limits of 4.00 $\log_{10}$ cfu/g (KEBS, 2003). It was noted in Nanyuki road that 50 % vendors had no food handlers' medical certificate and protective coat and additionally no food hygiene training and no hand washing of clients.

The presence of unacceptable levels of coliforms in meat (fish) from Nanyuki road may suggest inadequate handling during the display of fish before sale by the vendors. In this location 50 % of stalls were dusty and had houseflies suggesting inadequate sanitation.

Enterococci species counts were lowest in legumes (2.04 ± 0.06 $\log_{10}$ cfu/g). Coliform counts were 2.33 $\log_{10}$ cfu/g and 3.37 $\log_{10}$ cfu/g for *Staphylococcus aureus.* All legumes met microbial safety standards (KEBS, 2003; Gilbert et al. 2000).

Microbial counts in cereal based street foods were 2.42 $\log_{10}$ cfu/g for coliform and 2.44 $\log_{10}$ cfu/g for *Enterococci*. No microorganisms were detected in starchy roots from Ricky and Lunga lunga. The absence was expected from the fact that these foods are extensively boiled on site and peeled before serving.

Microbial counts in cereal were within the standards or limits for acceptable street foods (KEBS, 2003; Gilbert et al. 2000).

Escherichia coli was qualitatively detected from only one sample. These foods are extensively boiled. This may be why microbial count was low at 2.70-2.90 $\log_{10}$ cfu/g for coliforms and 3.30 - 3.34 $\log_{10}$ cfu/g for *Staphylococcus aureus.*

The beverage had the lowest total viable counts at 3.19 ± 0.2 $\log_{10}$ cfu/g. Coliforms, *Staphylococcus aureus* and *Enterococci* species were not detected in the beverage foods. This was expected since these foods were heated to boiling during cooking and were subsequently

served hot.

It was noted that all of microbial groups in this study had intra specific similarities between *Staphylococcus aureus, Enterococci* and *Enterobacter aeorogenes*. This could imply a similar source of contamination. None of the *Staphylococcus aureus* isolates from the street food was seen to possess genes coding for production of staphylococcal enterotoxins *sed* and *seg*. The microbial contaminations detected in this study could be indicators pointing to the need to improve the hygiene and handling of food but not a direct threat to food safety. *Staphylococcus aureus* are capable of picking and loosing virulence traits from unstable genetic material like plasmids.

Strains of *Escherichia coli* detected in vegetables from Lunga lunga and Likoni road and mixed dish (githeri from Likoni road) were confirmed to be different through Rep PCR analysis against reference strains previously reported to contain virulence genes *stx2*, *stx1* and *eae* by Kohler et al. (2008). More studies should however be carried out on street foods to determine the presence of specific genes coding for pathogenicity. Conclusion cannot be reached by the fact that they differed from pathogenic strains by Rep PCR alone

Recommendations

Most of the foods sampled and analyzed in this study were found to meet microbiological standards of ready to eat foods prepared on site including; cereals, legumes, starchy roots, mixed dishes, legumes and meats from Enterprise, Ricky, Lunga lunga and Likoni roads and are acceptable for human consumption with reference to the standards and guidelines (KEBS, 2003; Gilbert et al. 2000). However, vegetable foods preparation chain and holding require more studies to explain the source of high microbial counts.

More studies are required to certainly recommend the street foods as safe from the virulent microorganisms' perspective. This would establish the likelihood of the food pathogens' ability

to pick virulent plasmids which could result to their pathogenicity. These are enteropathogenic traits including capsular polysaccharides 5 and 8 in *Staphylococcus aureus* and intimin and shigatoxigenic genes in *Escherichia coli*.

Though utensils were physically clean, Bhaskar et al. (2004) and Mosupye et al. (2000) noted that dirty dish washing water and other sources can adhere to utensil surfaces and constitute a risk for contamination during food vending. Studies are also required to establish the safety of water used in the street food preparation and the efficacy of the hygienic practices in the street food stalls of Nairobi, Kenya.

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Appendices

Appendix 1: Hygiene practices survey questionnaire

	HH MM		HH MM
Time interview started......	☐ ☐	Time interview ended....	☐ ☐

Date [＿＿＿＿＿＿＿＿＿]   Location [＿＿＿＿＿＿＿＿＿]

Interviewer's

name...

Respondent's name

(optional)...

1. Is the surrounding environment free of potential contaminants?

If no, list

them..

2. Is the establishment interior free of potential contaminants?

     Yes ☐

     No ☐

List them..

3. a) Is the vending facility clean? Floor, roof and walls.   Yes ☐   No ☐

b) What are the construction material(s) used?

Do personnel have food handlers' medical certificates?

Yes ☐

No ☐

How is hand washing done?

a) Are protective clothing worn? Yes ☐ No ☐

b) Are protective clothing clean? Yes ☐ No ☐

c) How often are they washed..

Do the vendors have any training on food hygiene? Yes ☐ No ☐

If yes specify.

a) Are the equipments/utensils clean? Yes ☐ No ☐

b) How often are they cleaned? ....

c) Do you use soap? Yes ☐ No ☐

How is food held after cooking and before serving? ....

Which method(s) of packaging do you use for take away rations

Is the drinking water boiled or treated before serving for drinking?   Yes [ ]   No [ ]

Do you wash the food before cooking? State the number /times of rinsing

a) Do you encounter pests and rodents?   Yes [ ]   No [ ]

b) If any, which pests are frequently encountered? ....

How are these pests controlled?

....

Which customer complains do you get?

....

Where do you obtain water for cleaning and other activities?

....

Where do you obtain your raw material?

....

How do you dispose-off the wastes generated during food preparation?

....

Where do you access the sanitary facilities?

....

Thank you.