PREVALENCE OF *Campylobacter jejuni* AND OTHER BACTERIAL PATHOGENS IN SELECTED FOODS AND DRINKS SERVED IN FAST FOOD KIOSKS IN NGARA AND BURMA MARKETS IN NAIROBI

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DECLARATION

This thesis is my original work and has not been submitted for a degree in any other University or for any other award.

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DEDICATION

I dedicate this thesis to my late dad Sylvan, my first love. You will not be there to see me get an accolade, but sure you will be smiling down on me.

To my loving mum Joyce, you have done more than I would have asked for. Your prayers, the reason I am breathing.

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ACRONYMS AND ABBREVIATIONS

ACMSF	Advisory Committee on Microbiological Safety of Food
AMAN	Acute Motor Axonal Neuropathy
AMR	Antimicrobial Resistance
CCDA	Charcoal Cefoperazone Desoxycholate Agar
CDC	Centers for Disease Control and Prevention
CDSC	Communicable Diseases Surveillance Centre
CFU	Colony forming Units
FQ	Fluoroquinoline
GBS	Guillain-Barré syndrome
KEBS	Kenya Bureau of Standards
MRVP	Methyl red Voges proskauer
NTS	Non typhoid Salmonella
SIM	Sulfide Indole Motility
SS	*Salmonella Shigella*
TCBS	Thiosulfate Citrate Bile Sucrose
TSI	Triple Sugar Iron
WHO	World Health Organization

ABSTRACT

Enteric pathogens such as *Salmonella, Shigella, E.coli, Vibrio and Campylobacter* are easily transmitted when contaminated food is consumed, ending up in outbreaks. *Campylobacter* is one of the most important pathogens as it can cause infections which may lead to health complications that could be fatal. Limited data exist on *Campylobacter* infections from fast food kiosks in Kenya. This study was aimed at determining the risk factors of campylobacteriosis, microbial load in water, milk and cooked chicken, survival rates and identify other pathogens in the food and water served at commercial catering points in Nairobi's Ngara and Burma markets. A questionnaire was administered to food vendors to obtain information on the risk factors associated with Campylobacteriosis. A total of 135 samples; chicken (45), water (45) and milk (45) were sampled and their microbial load determined by colony count. *Campylobacter* isolation was done using charcoal cefoperazone desoxycholate agar and confirmed by biochemical tests, while other enteric were isolated through standard bacterial culture and isolation techniques. Survival rates of these isolates in varied temperatures were evaluated. Age, education level, occupation, undercooking of chicken, site of processing chicken, source of milk, storage of milk, source of water and method of treating drinking water were found to influence transmission of *Campylobacter,* while hand washing and serving of food by chicken processors were not found to influence the transmission of *Campylobacter.* The microbial load in chicken was 111.738 x 10^{4} CFU/ml and 67.893 x 10^{4} CFU/ml in Burma and Ngara markets respectively, while in milk, it was 115.673 x10^{4} CFU/ml and 160.354 x10^{4} CFU/ml in Burma and Ngara market respectively. Microbial load in water was determined by the most probable number technique, the load was 3.08333/100 ml and 3.54167/100 ml in Burma and Ngara market respectively. There was no significant difference in the Microbial load across the samples; milk ($p<0.0396$), chicken ($p<0.0053$) and water ($p<0.3805$) in both Ngara and Burma markets. Temperatures below the optimum growth temperature for *C. jejuni* (42^{0}C) generally seemed to have inhibitory effect on the population of the organism. Survival of *Campylobacter* was poor in water and chicken at room temperature (25^{0}C) while in chicken and milk stored at 4^{0}C, *Campylobacter* had higher survival rates. Chicken in Burma market, 6 (13.3%) had the highest prevalence of the pathogens; *Salmonella*, *Shighella* and *Vibrio* isolates were detected with *E coli* as the most prevalent npathogen. This research has shown that the food eaten in most urban centres of Ngara and Burma market pose high risk to infection hence warranting consistent surveillance. Proper storage of food and drinks after they have been thoroughly boiled is recommended.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Campylobacter jejuni (C. jejuni) has been recognized as one of the most common causes of bacterial diarrheal infections (Gillespie, 2002). Infection by *C. jejuni* has been shown to lead to severe post-infectious sequelae, which could include post-infectious acute motor axonal neuropathy (AMAN), a subtype of Guillain-Barré syndrome (GBS) (Willison, 2002). This organism accounts for a large proportion of cases of human bacterial gastroenteritis especially in industrialized countries (Miller, 2005). The *C. jejuni* bacterium can be isolated from a range of sources, including surface and ground waters, both domestic and wild mammals, insects and wild birds (Jones, 2001).

The most common source of this infections is through consumption of contaminated chicken meat, cross-contaminated food amongst other food items (Newell *et al.*, 2003). Although this organism is a diverse multi-host pathogen, there are genotypes known to be restricted to certain hosts, or human disease than others (Dingle *et al.*, 2002). In the early years of bacterial surveillance, many hospital microbiology laboratories never used to screen for this organism when isolating enteric bacteria from stool. However studies have shown that diarrheal stool cultures for *Campylobacter* is isolated more than *Salmonella* or *Shigella* (Henao *et al.*, 2010). The number of registered cases of human Campylobacteriosis in Finland has ranged from 3,796 cases to 4231 cases in 2011.

The reported incidence in Finland in the last ten years is higher than the European Union average (WHO, 2011). It is conservatively estimated that Campylobacteriosis illness costs $1 billion annually for medical care, lost wages and other productivity losses (CDC, 2009). In developing countries, *Campylobacter* infection is very common in the first 5 years of life with asymptomatic infection being more common. In Bangladesh, up to 39% of all children aged less than 2 years have asymptomatic infection (CDC, 2011). In Kampala, Uganda, the prevalence of *Campylobacter* infection was estimated to be 9.3% among 226 children with acute watery diarrhea (Mshana *et al*., 2009). Another study done in Mwanza-Tanzania showed the prevalence of 18% and even a higher prevalence among those who are aged <18months. Among asymptomatic children aged <18months, the prevalence of 4% was reported in the same study (Lindblom *et al*., 1995). In Kenya, studies done in Nyanza province showed prevalence of 17% among children below 5 years in urban areas and 15% in the rural areas (Ochieng *et al*., 2009).

1.2 Problem statement

Majority of Nairobi residents who work away from their residence usually take their lunch in fast food restaurants. Majority of these residents are low income earners, who prefer taking their meals at low cost fast food kiosks. These kiosks hardly observe proper hygiene standards, therefore predisposing residents to the risk of contracting food borne infections. Many foods, particularly those of animal origin, have been identified as vehicles of transmission of these pathogens to human beings and spreading them to kitchen environment.

Although *Campylobacter* has been recognized as a major source of infection, No systematic studies have been carried out with respect to risk factors of campylobacteriosis, microbial load in cooked chicken, boiled milk and water, and survival rates of *Campylobacter* in foods and drinks served in Nairobi County. Although efforts have been made to educate food handlers on hygiene and standard operating procedures like Hazard analysis critical control points, the regulations are hardly observed.

The Centre for Disease Control (CDC) receives about 10,000 cases a year but it is estimated that between two and four million people in the world are infected annually. CDC monitoring shows that approximately 124 people die every year from campylobacteriosis. This morbidity is of significant economic impact. Moreover, recent studies have shown that infection by certain bacteria including *Campylobacter* significantly increases a victim's risk of developing ongoing, or permanent gastrointestinal infections, including Post-infectious or even Irritable Bowel Syndrome.

It is conservatively estimated that campylobacteriosis illness costs the world $1 billion annually for medical care, lost wages and other productivity losses (CDC, 2009).

1.3 Justification

Essentially, the presence of *C. jejuni* in chicken, milk and water served in these fast food kiosks would mean that the poor observance of the hygiene also poses the risk of spreading other food borne pathogens. This could include organisms such as *Escherichia* *coli*, *Shigella* spp., *Salmonella diarrhea* and *Clostridium botulinum.* These organisms are used as indicators of proper observance of high standards of hygiene. This research aimed to establish the prevalence of *C. jejuni* in cooked chicken, milk and water in order to establish whether preparations of these foods in the fast food kiosks around Ngara and Burma places in Nairobi meet minimum hygiene requirements for well prepared meals. The difference in survival of *C. jejuni* in the selected foods stuffs was examined to help determining which food type could warrant close observation during preparation.

1.4 Research questions

i) What are the risk factors for campylobacteriosis?

ii) Which of the three ; milk, chicken and water, has the highest level of *C. jejuni?*

iii) What is the survival rate of *C. jejuni* in chicken and boiled water and milk under different physiochemical conditions?

iv) Are there other pathogens isolated from the cooked chicken, boiled milk and water samples?

1.5 Hypotheses

i. There are no risk factors associated with campylobacteriosis

ii. Microbial load in cooked chicken and boiled water and milk samples is the same

iii. *C. jejuni* does not survive at the same rate in cooked chicken extracts, milk or water that is served in Nairobi restaurants.

iv. There are no other pathogens isolated from the cooked chicken and boiled milk and water

1.6 Objectives

1.6.1 General objective

To determine prevalence of *Campylobacter jejuni* and other food pathogens in food and drinks served in fast food kiosks in Ngara and Burma markets.

1.6.2 Specific objective

i) To determine the risk factors associated with campylobacteriosis.

ii) To compare the levels of microbial load in the three foodstuffs; milk, chicken and water.

iii) To determine the survival rates of *Campylobacter* in cooked chicken, milk and water samples.

iv) To identify other pathogens found in cooked chicken, milk and water samples.

1.7 Significance of the study

Campylobacter jejuni is an important pathogenic microorganism that naturally occurs in chicken intestines and is spread through contact with chicken intestinal contents, feces of dairy cows, ingestion of inadequately prepared chicken, consumption of untreated water, and ingestion of unpasteurized milk. Other sources of *C. jejuni* include contact with sheep, consumption of poorly cooked meat, and other inadequately cooked meat and dairy products. Infection of *C. jejuni* increases the risk of contracting campylobacteriosis and other diseases such as gastroenteritis, arthritis, meningitis, and Guillain-Barré syndrome.

Two markets were chosen as representatives, Ngara market, to represent a relatively hygienic environment and Burma market to represent an unhygienic environment, as indicated by availability of toilet facilities and their cleanliness, presence of garbage around the cooking points and predominance of flies around the cooking points. The publishing of the findings from this research will be useful for creating awareness on fast food kiosks that do not meet the established hygienic standards. The information will be used to enlighten food vendors on probable dangers of compromising hygiene. Additionally recommendations will be made so that relevant authorities concerned with health will provide legal requirements that regulate the opening and operation of such type of kiosks. The knowledge of survival rates of *Campylobacter* on the food will help to establish risk posed by samples to individuals.

CHAPTER TWO

LITERATURE REVIEW

2.1 Genus *Campylobacter*

Campylobacter is a genus of bacteria that are Gram-negative, spiral and microaerophilic, motile, with either unipolar or bipolar flagella. The organisms have a characteristic spiral or corkscrew appearance and are oxidase-positive (Ryan *et al.*, 2004).

In regard to the epidemiology of *Campylobacter* infections worldwide, several genetic typing methods have been developed in order to differentiate between isolates below species level (Wassenaar and Newell, 2000). The genus *Campylobacter* comprises of 17 species and 6 subspecies (Nachamkin 2007; Silva *et al.*, 2011). The two species most commonly associated with human disease are *C. jejuni* and *C. coli*. *C. jejuni* accounts for more than 80% of *Campylobacter*-related human illness, with *C. coli* accounting for up to 18.6% of human illness. *C. fetus* has also been associated with foodborne disease in humans (Gurtler *et al.*, 2005).

2.2 Clinical presentation and diagnosis

Campylobacter infection frequently presents as self-limiting acute enteritis with diarrhea, malaise, fever and abdominal pain, sometimes with vomiting and the presence of blood in faeces (Allos, 2001). Disruption of epithelial cells and inflammation of the intestinal mucosa are hallmark features of severe cases (Beltinger *et al.*, 2008). Clinically, *Campylobacter* infection is indistinguishable from acute gastrointestinal infections produced by other bacterial pathogens, such as *Salmonella, Shigella*, and *Yersinia* species (ACMSF, 2005).

In most patients, the diarrhea is either loose and watery or grossly bloody; 8–10 bowel movements per day occur at the peak of illness (Blaser *et al.,* 2000). In some patients, the diarrhea is minimal and abdominal cramps and pains are the predominant features; this can lead to a mistaken diagnosis of acute abdomen and unnecessary laparotomy. Fever is reported by more than 90% of patients and can be low-grade or less than 40°C and persist for up to 1 week. By that time, the illness has usually resolved, even in the absence of specific antibiotic treatment. Occasionally, however, patients can develop a longer, relapsing diarrheal illness that lasts several weeks (Kapperud *et al.,* 2002).

Although *Campylobacter* is rarely identified in the stools of healthy persons, depending upon the population studied, as many as 50% of persons who are infected during outbreaks are usually asymptomatic (Kalva *et al.,* 1998). Fecal leukocytes and red blood cells are detected in the stools of 75% of infected persons (Blaser *et al.,* 2009). The peripheral white blood cell count may be mildly elevated. Other laboratory studies, including liver function, electrolytes, and hematocrit levels, are normal. Because diffuse colonic inflammation may be seen on sigmoidoscopic examination, *Campylobacter* enteritis may be confused with early inflammatory bowel disease (Blaser *et al*., 2009).

Diagnosis of *Campylobacter* enteritis is confirmed by obtaining cultures of the organism from stool samples. Some laboratories have begun performing PCR analysis on stool samples for *Campylobacter*, but this is not yet a standard practice (Parkhill *et al*., 2000). Species-specific assays, such as PCR-enzyme-linked immunosorbent assays to detect *Campylobacter* antigens in stool samples, have also been developed and these will be very useful in the diagnosis of *Campylobacter* infections (Lawson *et al.,* 1999; ACMSF, 2005)

2.3 Pathogenesis

Once ingested, the bacteria are able to adhere to intestinal epithelial cells or to the mucus overlying these cells. They then replicate in the intestine. *In vitro* and *in vivo* experiments have demonstrated that *C. jejuni* is capable of invading epithelial cells, although the invasive ability of strains differs (Everest *et al*., 1992). The organisms are attracted to mucus and fucose in bile, and the flagella is important in adherence to epithelial cells or mucus. *Campylobacter* motility is conferred by the polar flagella, and combined with their `cork-crew' form, it allows them to efficiently penetrate this mucus barrier (Newell *et al*., 1985; Lee *et al*.., 1986; Szymanski *et al*., 1995). Adherence may also involve lipopolysaccharides or other outer membrane components (Bessede *et al*., 2011). After moving into the intestinal epithelia, chemotaxis towards L-fucose, a component of both bile and mucin, which is important factor for affinity of *Campylobacter* for gall bladder and intestinal tract follows (Jones *et al*., 2002). Chemotaxis is the ability to detect and move up or down chemical gradients. Both motility and chemotaxis are essential for *C. jejuni* colonization.

In some experiments non-chemotactic mutants have been shown to be unable to colonize the intestine in animal models (Takata *et al.*, 1992). In infected individuals, this can result either in asymptomatic colonization status, that is, bacteria are present in the intestine but do not induce disease (Christenson *et al.*, 1983; Cawthraw *et al.*, 2002), or in diarrheal illness. After colonization of the intestine, clinical disease may occur. Based on clinical syndromes found in patients, two mechanisms by which *Campylobacter* can induce disease were postulated (Jones *et al.*, 2000).

The first mechanism involves adherence of *Campylobacter* to the intestine and the production of toxins (Wassennar *et al.*, 1997). One important mechanism by which bacterial enteropathogens induce diarrhea is through the production of potent toxins. *C. jejuni* is now known to produce at least two exotoxins: a heat-labile cytotonic or enterotoxin (CJT) and a cytotoxin (Johnson and Lior, 1984). These toxins alter the fluid resorption capacity of the intestine, resulting in secretory diarrhea. The second mechanism involves bacterial invasion and replication within the intestinal mucosa accompanied by an inflammatory response resulting in blood-containing, inflammatory diarrhea.

2.4 Epidemiology of *Campylobacter*

In developing and developed countries, continuous increases in the number of *C. jejuni* infections has been seen, with incidence rates as high as 73 cases per 100,000 population reported (WHO, 2007; Feierl *et al.,* 2009).

In the United States, 2 million symptomatic enteric *Campylobacter* infections are estimated per year (Alus, 2001). Incidence in the rural population is 5-6 times higher because of increased consumption of raw milk (CDC, 2011). In Africa, a few studies have indicated that campylobacteriosis is most common among children of young age. In Ile-Ife, Nigeria, *C. jejuni* was found to be an important agent of diarrhoea in children (Aboderin *et al*., 2002). In Durban, South Africa, *Campylobacter* were found in 21% of diarrhoeal cases among children aged less than five years (Mackenje *et al.,* 2000). Again in Venda, South Africa, *Campylobacter spp.* were also isolated from 20% of stool samples tested from HIV-positive individuals (Obi *et al*., 2002).

In Ethiopia, studies have revealed that diarrhoeal diseases are major causes of infant and child mortality and morbidity. About 39,000,000 episodes of diarrhoea per year were estimated to occur in Ethiopia; out of which 230,000 deaths occur in children below five years of age (Kaba *et al*., 2000). The pediatric admission review at a certain hospital in Ethiopia showed that diarrhoea was the second leading cause of admission and hospital deaths and *Campylobacter* is one cause of diarrhoea in the area (Mesoret *et al*., 2004).

In Tanzania, 8 out of 29 cases have been reported (Anna-Pendo *et al*., 2014). Studies done in Kenya show prevalence of *Campylobacter* infections at a rate of 4.9% (Saidi *et al*., 1997). Case fatality in Kenya stands at 8.8% (5/57) among children under five years in hospital with laboratory confirmed cases (O'Reilly *et al*., 2007). Overall, *Campylobacter* gastroenteritis is still common during the first 5 years of life (WHO, 2007). Isolation rates in children with acute diarrhea range from 10-46% (Lyang *et al.*, 2008; Soofi *et al.,* 2011).

2.5 Prevalence in different age groups.

Individuals of any age can be infected with *C. jejuni* enteritis (USA, Kansas 2007). The rate of infection differs between developed and developing countries. In developed countries, the peak attack rates are in infants younger than 1 year; a second, broader peak attack rate occurs in persons aged 20-29 years (Samuel *et al.*, 2008). In developing countries, symptomatic infection chiefly affects children younger than 5 years and declines with age, (Coker *et al.*, 2002; Sorokin *et al.,* 2002). This is likely due to the development of protective immunity secondary to a high level of exposure to the organism early in life.

2.6 Complications of Campylobacteriosis

One of the major complications of campylobacteriosis is the condition known as Guillain-Barré syndrome (GBS). GBS is a disorder of peripheral nerves and is characterized by ascending paralysis (ACSMF, 2005). Strong evidence suggests an association between preceding *C. jejuni* infection and GBS (Yuki, 2007). The antigenic similarity between specific regions that is terminal tetrasaccharide of lipopolysaccharide of *C. jejuni* and human gangliosides (GM1) led to the concept of molecular mimicry (WHO, 2002; Yuki, 2007). This concept implies the sharing of homologous epitopes between the bacterial lipopolysaccharide and ganglioside surface components of the peripheral nerve. Immune response from simple *C. jejuni* infection could induce antibodies that cross-react to the gangliosides and trigger GBS (Vunic *et al.*, 2009).

Other variants of GBS associated with *C. jejuni infection* include the following: Acute motor axonal neuropathy (AMAN), or Chinese paralytic syndrome, which is characterized by a rapid onset of paralysis. This may progress to tetraplegia and respiratory failure (Ritz *et al.*, 2007) It occurs in children in northern China during summer and fall (Mc Khann *et al.,* 1993). Fisher syndrome is characterized by ophthalmoplegia, areflexia, and cerebellar ataxia. Reactive arthritis is another complication whose incidence and prevalence varies among different reports, ranging from 0.6-24% (Mc Khann *et al.,* 1993).

Other infrequently reported complications are as follows: Reiter syndrome Erythema, nodosum Hepatitis Intestinal nephritis Hemolytic-uremic syndrome and Immunoglobulin A (IgA) nephropathy (Bereswill and Kist 2003).

2.7 Vehicle foods and drinks for *Campylobacter*

2.7.1 Cow milk

Consumption of unpasteurized milk is the most frequently reported case associated with outbreaks of infection (Nachamkin, 2000). Milk, being an animal product possesses the inherent risk of contamination from the source such as goat, sheep and cow. Contamination of milk is majorly through fecal contamination during milking and before the milk is pasteurized (Lira *et al.*, 2004; Tang *et al.*, 2011). Unpasteurized milk can become contaminated with the organism if the cow has a *campylobacte*r infection in the udder or if the milk is contaminated with cow manure (ACMSF, 2005). The dairy farm environment is an important reservoir for many food borne pathogens (Oliver *et al.*, 2005). Top of the diseases associated with consumption of raw milk include campylobacteriosis, yersiniosis and toxoplasmosis (Clark *et al.*, 2008).

Cow milk is an excellent growth medium for microorganisms since it contains most of the nutrients such as carbohydrates, proteins, fats, vitamins and minerals required for the growth of microorganisms (Jayarao and Henning, 2001; Oliver *et al.,* 2009). Outbreaks of Salmonellosis, Campylobacteriosis, and *E.coli* 0157: H7 have previously been linked to consumption of contaminated milk. In 2011, there was an outbreak of campylobacteriosis associated with consumption of raw milk in the American state of Alaska (State of Alaska, 2011). Although studies show that unpasteurized milk often leads to outbreaks of campylobacteriosis, little is documented about the load of *Campylobacter* in milk that leads to campylobacteriosis (Sandberg *et al.,* 2011).

2.7.2 Chicken

Chicken meat has been reported to be the most prominent source of infection, through consumption, either directly or through cross-contamination with other food items (Dingle *et al.,* 2002). Indeed, it has been estimated that just one drop of chicken juice may contain 500 infectious organisms (Riordan *et al.*, 199; Chen *et al.,* 2006). About 35 of 50 outbreaks reported to Communicable Diseases Surveillance Centre (CDSC) in Wales between 1995 and 1999 were food borne and in cases where a specific food was identified, poultry accounted for 68% of the cases (Frost *et al.*, 2002). A recent study involving animals and meat products in Kenya showed that *Campylobacte*r is a significant public health threat (Kariuki *et al.*, 2013). From the findings, *Campylobacter* had the highest prevalence of 12% compared to non-typhoid *salmonella* (NTS) (5%) and *Shigella* (8%) (Kariuki *et al*., 2013).

Even with strict adherence to good practice of hand washing and cleaning of cutting boards, simple errors in the handling of food might result in cross-contamination in the kitchen and, therefore resulting to human illness. Since heat kills viable *Campylobacter* species, thorough cooking of chicken should be emphasized as an important food safety measure.

Human campylobacteriosis is the most common cause of food poisoning in much of the industrialized world. The reduction or elimination of *C. jejuni* in the food chain, particularly from chicken products, is a major strategy in efforts to control this disease (WHO, 2002). Reports also indicate that up to 98% of poultry are colonized by *C. jejuni* (Craven *et al.,* 2000), with the poultry's intestinal contents being the mostly infested part (Wedderkopp *et al.,* 2000). These scholarly works affirm that consuming inadequately prepared chicken increases the chance of contracting *Campylobacter.* Limited data exist on the prevalence of *Campylobacter*-positive poultry flocks in Nairobi area.

2.7.3 Untreated water

The ability of *C. jejuni* to survive in water under experimental conditions is well recognized (Cools *et al.,*2005). *Campylobacter* can be isolated from the water lines and reservoirs of broiler houses and with similar strains being isolated from chickens' feaces within the same house (Refregie *et al.,* 2001). Through contamination with feces, wild and domestic animals shed *Campylobacter* into lakes, rivers, streams and reservoirs, and so all water for human consumption must be properly treated. In most developing countries in Africa, Latin America, Caribbean and Asia, most of the waste water and sewage are discharged to the environment without treatment (WHO, 2002 a). Use of such water predisposes individuals to *Campylobacter* infection (WHO, 2002 b).

2.8 Survival rates of *Campylobacter* in the associated foods

Generally, *C. jejuni* is known to be a fastidious organism that is sensitive to environmental stress. *C. jejuni* survives better in poorly treated and untreated aquatic environments where it can cause human diseases (Jang *et al.,* 2007). *C. jejuni* can survive 2-4 weeks under moist, reduced-oxygen conditions at 4°C, often outlasting the shelf life of the product (except in raw milk products). They can also survive 2-5 months at -20°C, but only a few days at room temperature (Ritz *et al.,* 2007). *Campylobacter* is a generally thermophilic bacteria and it has been shown to produce at least 24 proteins after exposure to higher temperature than its optimal growth temperature (Worsfold and Griffith, 1997). However, increased heat inactivates the cells. Thus pasteurization and ordinary cooking procedures kill the cells (ACMSF, 2005). Environmental stresses, such as exposure to air, drying, low pH, heating, freezing, and prolonged storage, damage cells and hinder recovery to a greater degree than for most bacteria (Ala'a *et al.*, 2006). In related studies, many factors have been identified as affecting the survival of *C. jejuni* such as light levels, presence of other organisms, oxygen levels, and temperatures and for water, the source (Fernandez and Pison, 1996). However other studies have shown that it can mount adaptive responses to acidic and aerobic environments (Vandeplas *et al.*, 2008).

Relevant studies have documented that *Campylobacter* strains can survive overnight up to 16 hours at ambient temperatures of less than 30 ◦C, mainly in poultry slaughterhouse environments after cleaning and disinfection; thus indicating the ability of such pathogens to survive for a considerable period under adverse conditions such as nutrient depletion and reduced water activity (Karenlampi and H¨annin, 2004). In milk, *Campylobacter jejuni* has been shown to die at a faster rate in unpasteurized milk than in sterile milk (Doyle and Roman, 1980). *Campylobacter* has been shown to persist in water for 28 days at 30°C and for 42 days at 4°C (Buswell *et al.*, 1998). Generally, *Campylobacter* appears to lack many adaptive responses that are exhibited by other bacteria (Fernandez and Pison, 1996). There is still limited data on comparative studies on survival of *Campylobacter* on different foods, water and milk.

2.9 Food pathogens

Almost 25% of the food-borne outbreaks that occurred in Europe in 1995 were traced back to recontamination (WHO, 1995). The presence of these pathogens was associated with their preparation under poor hygiene (1.6%), cross-contamination (3.6%), processing or storage in unhygienic rooms (4.2%), contaminated equipment (5.7%) and contamination by personnel (9.2%). In Kenya the prevalence of food-borne diseases are not easy to estimate as most of them are lumped together as diarrhoeal diseases. Data from the health information system of the Ministry of Health reveals that between 1997 and 1999, there were 6,833 cases and 566 deaths from food-borne diseases (ROK, 2000) Quantifying the cross-contamination risk associated with various steps in the food preparation process is therefore important for evaluation of the risks for easy implementation of management efforts in both home and food service kitchens (Chen *et al.*, 2001). The other pathogens under this study includes: *Salmonella, E .coli, Vibrio and Shigella.*

2.9.1 *Escherichia coli*

Escherichia coli (*E. coli*) is a Gram-negative, facultative anaerobic, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms. Cells are typically rod-shaped, and are about 2.0 micrometers (μm) long and 0.25-1.0 μm in diameter, with a cell volume of 0.6–0.7 μm^3 (Kubitschek, 1990).

Most *E. coli* strains are not pathogenic, but some serotypes can cause serious food poisoning in humans, and are occasionally responsible for product recalls due to food contamination (Vogt and Dippoid, 2005; CDC, 2012). There are hundreds of known *E. coli* strains, with *E. coli* O157:H7 being the most recognized (CDC, 2013). This enterohemorrhagic *E. coli* (or EHEC) strain is responsible for an estimated 63,153 cases of infection and 20 deaths in the United States annually, and causes approximately $255 million in losses each year (Hoffman *et al.,* 2012).

In Kenya, Bacterial diarrhoea was diagnosed in 141/380 (37.1%) cases, of which enterotoxigenic *E. coli* (ETEC) compromised 29.8%, shigatoxigenic *E. coli* (STEC) 24.1%, enteroaggregative *E. coli* (EAEC) 14.2%, enteroinvasive *E. coli* (EIEC) 12.8% and enteropathogenic *E. coli* (EPEC) 3.5% (Sang *et al*., 2012).

E. coli are found everywhere in the environment but mostly occupy animal surfaces and digestive systems, making it important to thoroughly wash anything that comes into contact with these surfaces (Armstrong *et al*., 1996; Tuttle *et al*., 1999).

Sources of *E. coli* O157:H7 infections include undercooked or raw hamburgers, sheep, pigs, goats, poultry, game meat, alfalfa sprouts, unpasteurized fruit juices, dry-cured salami, lettuce, cheese curds, unpasteurized or raw milk, contaminated water and ice, and person-to-person transmission (Armstrong *et al*., 1996; Kessenborg *et al*., 2004; Tilden *et al*., 1996). Fruits and vegetables can cause infection from contact with contaminated water. The most common source of infection, however, is caused by consuming undercooked or raw meats (Armstrong *et al*., 1996; Tuttle *et al*., 1999). Because there appears to be a low infective dose for this organism (10–100 cells), adequate sanitation and/or proper processing of foods is critically important (Mathusa *et al*., 2010).

2.9.2 *Salmonella*

Salmonella is a genus of rod-shaped, Gram-negative, non-spore-forming, predominantly motile enterobacteria with diameters around 0.7 to 1.5 µm, lengths from 2 to 5 µm, and flagella that are attached to the cell at many locations. They are chemo-organotrophs, obtaining their energy from oxidation and reduction reactions using organic sources, and are facultative anaerobes.

Disease surveillance reports frequently identify poultry, meat and milk products as the main vehicles in salmonellosis outbreaks. *Salmonella* contamination is of animal origin.

Among livestock production systems, *Salmonella* is more frequently isolated from poultry (chicken, turkey, duck, and pheasants) than from other animals (Freitas *et al.*, 2010). Infected animals shed the microorganism in the feces from where it can spread into soil, water, crops and/or other animals.

All *Salmonella* serotypes can be harbored in the gastrointestinal tract of livestock. The most common chain of events leading to this food borne illness involves healthy carrier animals which subsequently transfer the pathogen to humans during production, handling and/or consumption. *Salmonella* transmission to food processing plants and food production equipment is a serious public health issue. *Salmonella* can enter the food chain at any point: crop, farm, livestock feed, food manufacturing, processing and retailing (Wong *et al.*, 2002). A number of workers handle animals during slaughter and processing, and contamination is possible when *Salmonella* or any other pathogen is present on the equipment or the workers' hands or clothing (Islam *et al.*, 2004).

Insects or birds may also transmit *Salmonella* to different foods. Flies are known *Salmonella* carriers (Greenberg and Klowden, 1972; Rice *et al.*, 2003), and can transmit various pathogenic microorganisms, as well as viruses such as polioviruses, coxsackie viruses, infectious hepatitis and anthrax (Ugbogu *et al.*, 2006). *Salmonella* species are the most common pathogenic bacteria associated with a variety of foods. Although myriad foods can serve as *Salmonella* sources, meat and meat products, poultry and poultry products, and dairy products are significant sources of food borne pathogen infections in humans. Presence of *Salmonella* spp. in fresh raw products can vary widely (Harris *et al.*, 2003). In fact, contaminated poultry, eggs and dairy products are probably the most common cause of human Salmonellosis worldwide (Herikstad *et al*., 2002).

2.9.3 *Shigella*

Shigella is a genus of Gram-negative, facultative anaerobic, nonspore forming, non-motile, rod-shaped bacteria closely related to *Salmonella. Shigella* is a species of enteric bacteria that causes disease in humans and other primates. The disease caused by the ingestion of *Shigella* bacteria is referred to as shigellosis, which is most typically associated with diarrhea and other gastrointestinal symptoms (CDC, 2009).

Shigella infection is the third most common cause of bacterial gastroenteritis in the United States, after *Salmonella* infection and *Campylobacter* infection and ahead of *E. coli* O157 infection (Gupta *et al*., 2004). The global burden of shigellosis has been estimated at 165 million cases per year, of which 163 million are in developing countries. More than one million deaths occur in the developing world yearly due to *Shigella* infection (Kotloff *et al.,* 1999). In Kenya *Shigella* species were isolated from 224 (23%) of 976 stool specimens. The overall adjusted incidence rate was 408/100,000 person years of observation (PYO) with highest rates among adults 34–49 years old (1,575/100,000 PYO) (Njuguna *et al*., 2013).

Shigella is easily spread person-to-person because of its relatively tiny infectious dose. Infection can occur after ingestion of fewer than 100 bacteria (Schmid and Frank, 2007). Another reason *Shigella* so easily cause infection is because the bacteria thrive in the human intestine and are commonly spread both by person-to-person contact and through the contamination of food. *Shigella* infections also may be acquired from eating contaminated food. In the United States, incidence of food borne illness is documented through FoodNet, a reporting system used by public health agencies that captures food borne illness in over 13% of the population (CDC, 2000; CDC, 2010). Of the 10 pathogens tracked by FoodNet, *Salmonella*, *Campylobacter*, and *Shigella* are responsible for most cases of food borne illness. An estimated 20% of the total number of cases of shigellosis involved food as the vehicle of transmission (Buztly *et al.,* 2009).

2.9.4 *Vibrio*

Vibrio is a genus of Gram-negative bacteria possessing a curved rod shape (Thompson *et al*., 2001) several species of which can cause food borne infection, usually associated with eating undercooked seafood. Several species of *Vibrio* are pathogens. Most disease-causing strains are associated with gastroenteritis, but can also infect open wounds and cause septicemia. Pathogenic *Vibrio* include *V. cholerae* (the causative agent of cholera), *V. parahaemolyticus,* and *V. vulnificus. Vibrio cholerae* is generally transmitted via contaminated water (O'Connor *et al*., 2010).

Vibrio cholerae has long been known to be responsible for the life threatening secretory diarrhea termed as Asiatic cholera or epidemic cholera (Ryan and Ray, 2004). The global disease burden has been estimated to be 3-5 million cases and accounts for a total of 100,000-130,000 death per year (WHO, 2010). In the year 2001 alone, cholera outbreaks were reported in nine (9) districts in Kenya with a total of 1001 cases and 55 deaths.

Three hundred and ninety six (396) of the cases and 27 deaths were from Wajir District and 291 of the cases and 2 deaths from Machakos District, (RoK, 2001).

Many studies have reported that foods including vegetables, fruits, sea foods, dairy products, poultry and meat products and others can become contaminated with *Vibrio* spp. through improper handling, undercooking, washing with unhygienic water and by the use of untreated night oil (Feachem, 1981; Huq *et al.*, 1983; Rabbani and Greenough, 1999). By far the most important source of transmission of *Vibrio cholerae* is contaminated drinking water followed by food (contaminated during or after preparation e.g. milk, cooked rice, eggs, chicken, potatoes etc) and fruits and vegetables (WHO, 2000).

CHAPTER 3

MATERIALS AND METHODS

3.1 Area of study

The study was conducted in Ngara area, and in Burma market located along Jogoo road in Nairobi County in Kenya Located 1 ° 17' 0" South, 36° 49' 0" East. Food samples were collected from fast food kiosks located in back streets of Nairobi in Ngara and Burma markets centers including eating places that serve foods under temporary structures. Fast food kiosks in these markets were targeted for this study.

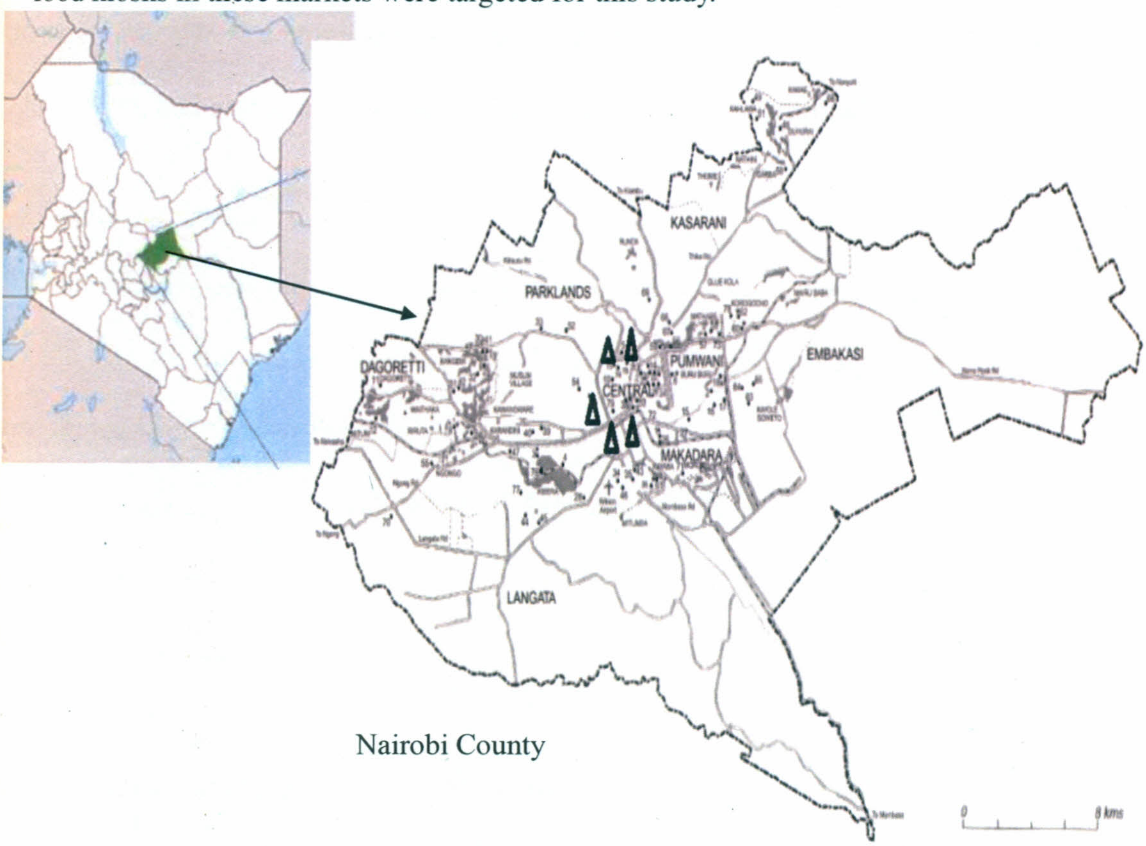

Figure 3. 1: Map of Kenya showing the sampling regions. The triangles indicate the specific sampling locations, while the arrow indicates position of Nairobi County.

3.2 Study design

This was a cross sectional study design. The sampling technique employed was stratified sampling in which the sampling zone was divided into two locations which were approximately 8 Kilometers apart and from each location, eight kiosks were identified. The samples were taken from every site six times. The sampling technique mainly targeted different kiosks with different type of settings. The sites were Ngara market and Burma market. The samples were collected between November 2012 and July 2013.

3.3 Administration of questionnaires

Questionnaires were administered to food vendors and consumers to collect demographic information, and also in order to determine the risk factors of Campylobacteriosis.

3.4 Sampling design

The number of food kiosks from which food samples were to be collected formed the sample size for this study. This was done by random sampling design. Sample size was determined by the Fisher formula:

$$n = \frac{Z^2 pqD}{d^2}$$ (Mugenda and Mugenda, 1999).

Where,

n= sample size

p= anticipated prevalence which is 3% in this study

q=failure which is calculated as 100-3 giving 97%

Z= is the appropriate value from the normal distribution for the desired confidence level

d= allowable error

D=design effect usually 1 where there are no replications

Based on 3% prevalence and Z value of 1.96 the sample size was determined as:

$$n = \frac{(0.03 \times 0.97)\ 1.96^2}{(0.05)2} = 44.71 = 45$$

A sample size of 45 was therefore used for each of the collected samples; cooked chicken, milk, and water, from each market.

3.5 Sample collection

Samples of chicken, water and milk were collected and placed in separate sterile bags, which were placed in a cooler box. They were then transported to the Kenyatta University laboratory in Nairobi, where they were analyzed within four hours.

3.6 Isolation of *Campylobacter*

3.6.1 Isolation of *Campylobacter* from chicken

For isolation of *Campylobacter*, 25 g of each chicken sample from Ngara and Burma markets was homogenized for 1 min in a stomacher (Stomacher 400 Lab Blender; Seward Medical, London, UK). A quantity of 0.5 g of the chicken extract was emulsified in 5 ml of sterile 0.1% peptone water to form a dilution of 1:10. This was followed by inoculation onto the selective medium Blood Free *Campylobacter* Selective Agar Base (CM0739, Oxoid) supplemented with Charcoal Cefoperazone Desoxycholate Agar Supplement (CCDA Selective Supplement), with sterile cotton tipped swabs, so that single isolated colonies could be formed. The plates were then incubated in an atmosphere consisting of approximately 5% Oxygen, 10% carbon dioxide and 85% Nitrogen, for 48 hrs at 42 °C (Baylis *et al*., 2002)

3.6.2 Isolation of *Campylobacter* from water and milk

The milk samples and water samples were collected and immediately transported to the laboratory in a cooler box with ice packs. The milk and water were processed using the aseptic technique. To isolate *Campylobacter* from milk and water, 0.1 ml of each sample was suspended in 5ml of sterile peptone water, followed by inoculation onto the selective media that is, Blood Free *Campylobacter* Selective Agar Base (CM0739, Oxoid) supplemented with Charcoal Cefoperazone Desoxycholate Agar Supplement (CCDA Selective Supplement), with sterile cotton tipped swabs. The samples were incubated in plates in an atmosphere consisting of approximately 5% Oxygen, 10% carbon dioxide and 85% Nitrogen, for 48 hrs at 42 °C (Baylis *et al.*, 2002).

3.7 Detection and Identification of *Campylobacter*

Presumptive identification of *Campylobacter* colonies was based on the colonial appearance and Gram-staining. *C. jejuni* grows to form grey, moist, glossy flat spreading colonies with or without a metallic sheen, while *C. coli* tends to be creamy-grey in color, moist with slightly raised shiny surface (Baylis *et al.*, 2000). Microscopic examination for Gram-negative rods with curved or spiral-shaped rods was done. Two presumptive *Campylobacter* colonies from each selective agar plate were sub cultured and confirmed to the species level by biochemical tests, such as motility, catalase and oxidase tests, oxidation/fermentation reactions, growth in 1% glycine, H_2S production, urease activity, and nitrate reduction, (Baylis *et al.,* 2000).

3.8 Detection and identification of other bacterial pathogens

These pathogens included: *Salmonella, Shigella, Vibrio and E. coli.* For *Salmonella* and *Shigella,* the samples were inoculated on Selenite F enrichment broth and incubated for 18 hours at 37°C. Using the aseptic techniques, a loopful of inoculum from the broth was then streaked onto plates of *Salmonella-Shigella* Agar and Deoxycholate Citrate agar (DCA) and incubated for 18 to 24 hrs at 37°C (Hyatt and weese, 2004). The suspected colonies, ones that formed transparent or transluscent colonies on SS, and those that were colourless were sub-cultured. The organisms were gram stained and confirmed by biochemical tests including: TSI, SIM, citrate, indole, oxidase, and urease tests (David *et al*., 2009).

To isolate *Vibrio,* the samples were inoculated in alkaline peptone water enrichment. The inoculated bottles were incubated for 18 hours at 37°C after which a loopful of inoculum from the peptone bottles was then spread on the surface of the Thiosulfate Citrate Bile Sucrose (TCBS) plates. The streaked plates were incubated for 18-24 hours at 37°C aerobically (Pfeffer *et al.,* 2003). The suspected colonies, ones that were large and yellow, or ones with blue to green centres, were sub-cultured, then subjected to the following biochemical tests for confirmation: TSI, oxidase test, motility, urease, MRVP, and citrate utilization test (Mariita and Okemo, 2009).

Isolation of *E. coli* was done by plating the samples on McConkey and on Eosin Methylene Blue (EMB) agars and incubating overnight at 37°C. The above isolations for various organisms were followed by purification of the cultures which was done by restreaking a single colony on another pure agar plate and incubating at 37°C for 18-24 hours. After isolation, the suspected colonies, ones that were dark blue-black with green metallic sheen on EMB, and those that were pink to red on McConkey were sub-cultured then confirmed using the following biochemical tests : TSI, gram stain, SIM, urease, citrate, indole and oxidase (Tasnim *et al.,* 2012) (Appendix 111).

3.9 Enumeration of micro-organisms in milk and chicken samples by spread plate technique

The microorganisms were enumerated by spread plate technique. The enumeration targeted the total bacterial counts in the food samples. Nutrient agar to be used was sterilized by autoclaving after which it was dispensed into agar plates to make a thickness of approximately 7 mm. The plates were then left to cool. The chicken and milk samples were serially diluted from 10^{-1} to 10^{-6} and appropriately labeled. A volume of 0.1 ml of each of the dilutions was pipetted into the base of correctly labeled agar plates using separate sterile pipettes to avoid carryover errors. After this step, the inocula were spread using sterile glass spreaders. The inoculated plates were then incubated at 37°C for 24 hours (Clesceri *et al.*, 1998).

3.10 Enumeration of pathogens in water

This was done by the Most Probable Number (MPN) technique (Krewski *et al.*, 2004). One set of double strength Lactose F broth was prepared coupled with two sets of single strength Lactose F broth. The broth was dispensed into tubes and Durham tubes inserted to trap any gas formed. The broth was sterilized at 121°C for 15 minutes and left to cool. To 10ml of the double strength Lactose F broth, 10ml of the water sample was added. To the second and third sets of 5ml single strength Lactose broth, 1ml and 0.1ml of the water sample was added respectively. The tubes were then incubated at 35°C for 48 hours under aerobic conditions. Upon incubation the tubes were inspected for growth (indicated by turbidity) and presence of gas. The number of positive tubes from each set was recorded to form a three number code that was compared to the standard table and recorded as MPN index/ 100ml of water. This formed the presumptive test.

Sterile loop transfers were made from all tubes showing acid and gas production to tryptose bile broth (EC Broth) and incubated at 44 °C for 24 hours. Gas production in a fermentation tube within 24 hours or less was considered as a positive reaction. For confirmation, samples that were considered to have a positive reaction from the tryptose broth were streaked on a plate of Eosin Methyl Blue (EMB) agar. The plates were incubated at 44°C for 24 hours and inspected for shiny green colonies with a metallic sheen. Gram stain and Biochemical tests, that is,urease, oxidase, indole,citrate,TSI and SIM, were then performed. This formed the confirmed test. For the complete test, the identified *E. coli* were inoculated into Lactose broth containing Durham tubes and incubated for 48 hours at 44°C. If growth and gas production occurred the sample was verified as positive for contamination by coliforms (Tortorello, 2004).

3.11 Plate count

After incubation period of 24 hours, the plates were examined for growth and morphological characteristics. Plates that had between 30-300 colonies were selected for counting. Using a colony counter and a marker pen, the colonies in each of the dilutions having between 30 - 300 colonies were counted by putting a dot above each colony after it has been counted. Those with above 300 colonies were reported as "too numerous to count" (TNTC) while those with less than 30 were not counted. After counting the duplicate recordings were averaged and multiplied by the dilution factor and amount of initial inoculum to get the number of colonies per milliliter of the sample. This was recorded as colony forming units per milliliter of the sample (CFU/ml).

3.12 Survival rates of *Campylobacter jejuni*

3.12.1 Survival rate in milk

The aim of the survival experiments was to evaluate the impact of temperature variation on the survival of *Campylobacter*. Fresh raw milk was obtained and a quantity of 25 ml of milk was pasteurized to eliminate any contaminating microorganisms. The preparation of *C. jejuni* to be inoculated was done by suspending a small amount of freshly cultured *C. jejuni* in a tube containing sterile normal saline to match the 0.5 McFarland's standard of turbidity. This was done in order to start with a uniform number of cells i.e. 8 $\log_{10}$ CFU/ml. A quantity of 1ml of *C. jejuni* suspension was inoculated into 25 ml of pasteurized milk. The milk was kept at a temperature of 4°C and sampled at 2 day intervals for 14 days. At each sampling, the milk was assayed for *C. jejuni*.

This was done by drawing 1ml of the milk and serially diluting it before spreading in duplicate on to plates of *Campylobacter* agar supplemented with CCDA. Incubation was then done at 42°C for 48 hours under anaerobic conditions. The plates were then counted and the organisms were enumerated and tabulated. The assays were continued until the 14th day according to the method of Baylis *et al.* (2000).

3.12.2 Survival in water

Bottles containing 25ml of the water samples to be used were first sterilized by autoclaving at 121°C for 15 minutes. After autoclaving, the bottles were allowed to cool to room temperature. A small amount of freshly grown cultures of *C. jejuni* was suspended in sterile normal saline to make a suspension matching the 0.5 McFarland's turbidity standard. This ensured the starting cell density was approximately 8 $\log_{10}$ CFU/ml. 1ml of the *C. jejuni* inoculum was aseptically transferred into the bottles. The bottles were then placed at a temperature of 4°c and conditions of oxygen tension, then sampled at 2-day intervals for 14 days. At each sampling, the water was assayed for *Campylobacter.*

This was done by drawing 1ml of the water and serially diluting it before spreading in duplicate on to plates of *Campylobacter* agar supplemented with CCDA. Incubation was then done at 42°C for 48 hours under anaerobic conditions. The plates were then counted and the organisms were enumerated and tabulated. The assays were continued until the 14th day according to the method of Baylis *et al.* (2000).

3.12.3 Survival in cooked chicken

For chicken, 10g portions of cooked chicken meat were suspended in sterile water and blended to make a uniform suspension. The blended chicken meat was then dispensed into bottles and autoclaved at 121°C for 15mins in order to eliminate any microorganism. Upon autoclaving, the bottles having the chicken meat were allowed to cool. A small amount of freshly grown cultures of *C. jejuni* was suspended in sterile normal saline to make a suspension matching the 0.5 McFarland's turbidity standard (approximately 8 $\log_{10}$ CFU/ml).

An inoculum of 1ml of *C. jejuni* was introduced into the bottles using the aseptic technique. One set of the inoculated chicken meat bottles was kept at 4°C while the other was kept at room temperature (25°C). As in milk and water above, sampling was done after 2-day intervals for 14 days. At each sampling, 1ml of the sample was drawn aseptically, serially diluted and spread in duplicate on plates of *Campylobacter* agar media supplemented with CCDA and incubated at 42°C for 48hrs (Baylis *et al.*, 2000).

3.13 Data analysis

One way Anova was used to determine the significant difference at P –value ≤ 0.05. P-value of <0.05 was considered as significant. For significant difference, Tukey's Honest significant difference (HSD) test was used to separate the means. Prevalence of *Campylobacter* was determined by calculating the percentage frequency of isolation.

CHAPTER 4

RESULTS

4.1 Risk factors associated with Campylobacteriosis

In this study, 70 respondents were randomly sampled. Among this 35 were from Burma market and the other 35 were from Ngara market. Age, gender, education level, occupation, undercooking of chicken, site of processing chicken, source of milk, storage of milk, source of water and method of treating drinking water were found to be risk factors for transmission of *Campylobacter,* while hand washing and serving of food by chicken processors were not found to be risk factors for transmission of *Campylobacter* (Table 4.1).

Table 4.1: Demographic characteristics of respondents and risk factors of campylobacteriosis

Demographics	Cluster	Frequency (n)	Percent (%)	p- Value
Gender	Male	23	32.9	0.0654
	Female	47	67.1	
Age	18-20	3	4.3	0.0453
	20-29	10	14.3	
	30-39	41	58.6	
	40-49	16	22.9	
	50 and above	0	0	
Level of Education	Primary	26	37.1	0.0034
	Secondary	23	32.9	
	Diploma	12	17.1	
	Degree and above	9	12.9	
Current Profession	Employed	5	7.1	0.043
	Business	7	10.0	
	Skilled worker	18	25.7	
	Self employed	17	24.3	
	Retired	6	8.6	
	Student	17	24.3	
Using Soap	No	18	25.7	0.0672
	Yes	52	74.3	
Chicken served cooked well	Agree	25	35.70	0.045
	Strongly Agree	12	17.14	
	Disagree	23	32.90	
	Strongly disagree	10	14.26	
Source of milk	Packeted	2	2.8	0.0452
	Homestead	42	60	
	Dairies	26	37.2	
Treating water	No	28	40	0.0416
	Yes	42	60	
Water treatment n=42	Boiling	26	61.90	0.00892
	Chlorination	8	19.05	
	Filtration	8	19.05	
Storage of milk	refrigerated	34	48.6	0.00054
	Boiled uncovered	2	2.8	
	Boiled covered	34	48.6	
Where chicken is process	In hotel	51	72.9	0.03754
	Outside hotel	10	14.3	
	Others	9	12.8	
Chicken handler serve food	Yes	14	20	0.0754
	No	56	80	
Source of water	Bore holes	14	20	
	Tap water	48	68.6	0.00453
	Rain water	5	7.1	
	Others	3	4.3	

4.2 Microbial load

4.2.1 Microbial load in chicken

The average microbial load in chicken was 111.738×10^4 CFU/ml in Burma market as compared to Ngara market which had an average of 67.893×10^4 CFU/ml.(Table 4.2). There was a significant difference in the microbial load in chicken from Burma market and Ngara market ($p=0.0053$).

4.2.2 Microbial load in milk

The average microbial load in milk was 115.673×10^4 CFU/ml in Burma market while in Ngara market, the average microbial load was 160.354×10^4 CFU/ml (Table 4.2). There was a significant difference in the microbial load in milk between the two markets ($p=0.0396$).

4.2.3 Microbial load in water

The microbial load in water was determined by MPN and the population was determined to be 3.08333/100ml in Burma market and 3.54167 in Ngara market (Table 4.2). There was no significant variation in the population between the two markets ($p= 0.3803$).

Table 4. 2: Mean population of pathogens in cooked chicken and boiled Milk and water

Sample type	Chicken ($x10^4$ CFU/g)	Milk ($x10^4$ CFU/g)	Water index per 100ml
Burma market	111.738±11	67.893±9.8	3.0833±0.41
Ngara market	67.893±9.8	160.354±16.1	3.54167±0.31
Acceptable level By FDA	10^3 CFU/g	10^3 CFU/g	2.2/100ml
p-value	0.0053	0.0396	0.3803

4.3 Survival rates of *Campylobacter jejuni*, in chicken, water and milk

This was established by counting viable cells over 14 days period. This was done in a 2 day interval (Table 4.3). There was significant difference in survival rates between the three samples ($P<0.05$). Generally, chicken stored at 4^0c showed the greatest survival rate, while chicken stored at 25^0c showed poorest survival (Table 4.3).

Table 4. 3: Survival rates of *Campylobacter jejuni* in chicken, water and milk

Mean pop x10 ^{6}

Date	Chicken at 4 °C	CHICKEN AT 25°C	WATER AT 25 °C	MILKAT 4 °C
Day 0	150.00 ±0.00a	150.000±0.00a	150.000 ±0.00a	150.000 ±0.00a
Day 2	19.500 ±0.87b	12.300±0.58b	29.000 ±4.04b	72.000 ±1.73b
Day 4	13.500 ±1.44c	10.800 ±0.17bc	13.100 ±1.15c	31.733 ±0.70c
Day 6	12.000 ±0.29c	8.700 ±0.46cd	1.010 ±0.01d	1.420 ±0.03d
Day 8	0.850 ±0.38d	4.240 ±1.78e	0.095 ±0.02d	0.650 ± 0.01d
Day 10	0.980 ±0.01d*	6.100 ±0.11ed	0.080 ± 0.00d	0.160 ± 0.02d
Day 12	0.763 ±0.17d	5.100 ±0.17e	0.053± 0.01d	0.140 ±0.01d
Day 14	0.900 ±0.11d	4.900 ±0.06e	0.047 ±0.01d	0.121 ± 0.01d
P value	0.0001	0.0001	0.0001	0.0001

Note* Values followed by the same letter along the columns are not significantly ($p<0.05$) different

4.4 Detection of other bacterial pathogens

In addition to *Campylobacter*, (plate 1) other pathogens were also detected in the samples. They included *Vibrio* (plate 2), *E. coli*, *Salmonella* and *Shigella*. They were confirmed by the following biochemical tests: TSI, SIM, urease, citrate (plate 3), indole and oxidase tests (Appendix iii and iv).Their frequency of occurrence in the 45 samples was also established. Milk from both markets were assayed for *E. coli, Salmonella*, *Shigella*, *Vibrio* and *Campylobacter.* Of the 45 samples from Ngara market, *E coli* (Plate 4) was the most frequently isolated, 16 (37.6%) while *Campylobacter* had the least frequency of isolation 4 (8.9%). *Salmonella*, *Shigella*, and *Vibrio* had a frequency of 9 (20%), 8 (17.7%) and 9 (20%) respectively. (Table 4.4) Similarly, in Burma market. *E. coli* had the highest frequency of isolation 14 (31.1%). *Shigella* had the least frequency of isolation 4 (8.9%). Both *Salmonella* and *Vibrio* had a frequency of isolation 6 (13.3%). No *Campylobacter* was isolated in the milk samples from Burma market (Table 4.4).

The frequency of isolation of the microorganism in chicken samples was also calculated. This revealed that *E. coli* was the most frequently isolated microorganism in Ngara market 20 (44.5%) while *Campylobacter* and *Shigella* had the least frequency of isolation 4 (8.9%) each. *Salmonella* and *Vibrio* had a frequency of 8 (17.7%), and 9 (20.%) respectively. (Table 4.6). In Burma market, *E. coli* again was the most frequently isolated microorgnanism 28 (62.2%), while *Campylobacter* and *Shigella* had the least frequency 6 (13.3%). *Salmonella* and *Vibrio* and had a frequency of 15 (33.3%) and 12 (26.7%) respectively (Table 4.4).

In this study, the frequency of isolation of micro-organism in water revealed that in Ngara market, 9 (20%) of the samples had *E. coli*. This was the highest, while *Salmonella* had the least frequency of isolation 2 (4.4%). *Vibrio* was isolated in 6 (13.3%) of the samples, similarly, 6 (13.3%) of the samples had *Shigella.* No *Campylobacter* was isolated from any of the samples (Table 4.7). In Burma market, *E. coli* was found in 13 (28.9%) samples, *Shigella* had the least frequency of occurrence 4 (8.9%), *Vibrio* was detected in 9 (20%) samples. No *Salmonella* and *Campylobacter* was detected in in any of the 45 samples of water collected (Table 4.4).

Table 4. 4 Detection of pathogens and their percentage frequency of isolation

	NGARA MARKET Frequency (n =45)			**BURMA MARKET** Frequency (n =45)		
Organism	Chicken	Milk	Water	Chicken	Milk	Water
E.coli	20 (44.5%)	16 (37%)	9 (20%)	28 (62.2%)	14 (31.1%)	13 (28.9%)
Salmonella	8 (17.7%)	9 (20%)m	2 (4.4%)	15 (33.3%)	6 (13.3%)	0
Shigella	4 (8.9%)	8 (17%)	6 (13.3%)	6 (13.3%)	4 (8.9%)	4 (8.9%)
Vibrio	9 (20%)	9 (20%)	6 (13.3%)	12 (26.7%)	6 (13.3%)	9 (20.9%)
Campylobacter	4(8.9%)	4 (8.9%)	0	6 (13.3%)	0	0

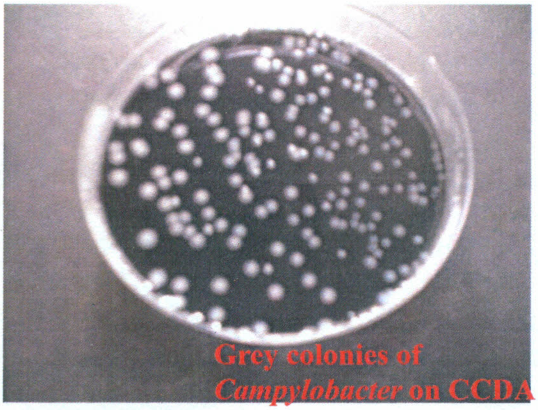

Plate 1: Grey colonies of *Campylobacter* from chicken on CCDA.

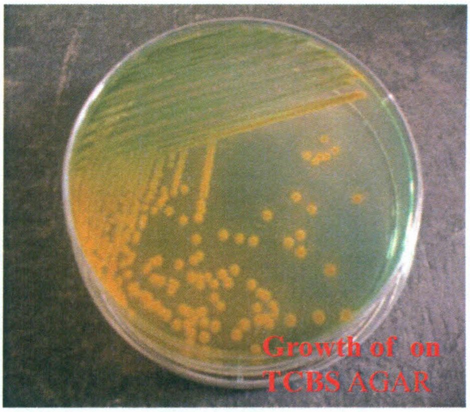

Plate 2: *Vibrio* species from water samples on TCBS

Plate 3: Biochemical test: TSI,SIM, urease and citrate.

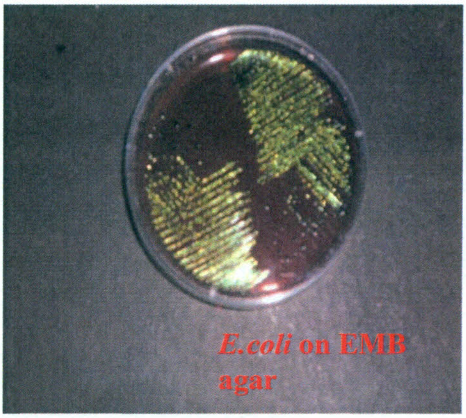

Plate 4: *E.coli* on EMB from milk sample

CHAPTER 5

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 DISCUSSION

5.1.1 Risk factors of *Campylobacter* infections.

This study sought to determine whether age, sex, level of education, hand washing, undercooking of chicken, handling of raw chicken while cooking, place of processing and slaughtering of chicken, source of water, method of water treatment, source of milk, and method of storage of milk had any risk associated with *Campylobacter* infections. Findings from this study indicated that age was a risk factor to transmission of *Campylobacter* (Table 4.1). These findings were similar to those obtained in Burkina Faso where young adults were at a greater risk of contracting campylobacter (Sangare *et al*., 2011). This could be associated with a high number of young adults who constitutes majority of workers who eat in the streets outside their homes (Mukhola, 2000).

Level of education was found in this study, to influence the transmission of *Campylobacter* (Table 4.1). Those who were well educated had increased knowledge on importance of hygiene and safer food handling practices. This was similar to findings in Jordan where schooling and the level of education influence acquiring enteritis due to *Campylobacter* (Nimri and Meqdam, 2004). The education of the consumers of vector products of campylobacteriosis is important in the implementation of national programs of surveillance and fight against *Campylobacter*. This finding was contrary to findings of previous studies in Burkina Faso, where the level of education was not found to be a risk factor for campylobacteriosis (Sangare *et al*., 2011).

Profession of an individual was found to be a risk factor for transmission of *Campylobacter* (Table 4.1). Majority of the individuals who were interviewed were skilled workers, those who are self employed on small scale jobs and students who could only afford to buy food at low costs. This finding was consistent with findings in a similar study in Kenya (Jagals and Jagals, 2004), where profession of an individual had a high influence on infection. This relationship could be as a result of the fact that, majority of the kiosks where food is sold at low cost, are generally located in open environments where hygiene is poor.

Undercooking of chicken in this study was found to be a risk factor for transmission of *Campylobacter* (Table 4.1). Data on risk factors for foodborne disease in Kenya indicate that the majority of outbreaks result from inappropriate food handling practices and methods of cooking (Jones and Angulo, 2006).This was also consistent with findings in Tanzania (Eric *et al.*, 2013), In UK (Rodrigues *et al.*, 2001) and in Ireland (Renzi *et al.*, 2009), where food that is not properly cooked at the right temperatures could lead to infections by *Campylobacter*. However, differences between risk factors across studies may reflect either different study methodologies or variations in the sources of infection across different countries (Carrique-Mas *et al.*, 2005). The place of processing and slaughtering chicken was found to be a risk factor of Campylobacteriosis. If processing of the chicken is done in the same environment where cooking is done, it increases the risk of contracting *Campylobacter* infections (Table 4.1). This is consistent with previous studies in Uganda (Reij, 2004; Muyanja *et al.*, 2011). This could be as a result of direct contact or cross contamination, of already prepared food, from equipment, utensils, cutting boards, knives and surfaces. This is possible when the chicken is slaughtered within the cooking environment (WHO, 2006).

Source of water was found to be a risk factor for transmission of *Campylobacter* (Table 4.1). This finding is in agreement with those in Kenya (Iijima *et al.*, 2001) and In Zimbabwe (Maponga *et al.*, 2013). Research in developed and developing countries have also shown that water is risk factor for endemic diseases and implicated in many outbreaks of human diseases (Baldursson and Karanis, 2011). This result could be due to pollution of water by garbage, which is not properly disposed. Method of water treatment was found to be a risk factor for transmission of *Campylobacter* (Table 4.1). The results found in this study were in agreement with those obtained in South Africa (Iijima *et al.*, 2001; Liang *et al.*, 2008), which showed that different methods of water treatment differs in their efficacy, with some methods being better than others. This is also similar to previous studies that stipulates that exposure to inadequately treated water is assumed to be an important risk factor for acquiring *Campylobacter* infection (Newell *et al.*, 2003).

Source of milk was found to be a risk factor for transmission of *Campylobacter* (Table 4.1). This finding was the same to those obtained in Tanzania and Ethiopia (Swayi *et al.*, 2000; Zelalem and Faye, 2006), where it was found that milk from homesteads and dairies posed a greater risk of contracting *Campylobacter*. This similarity could be due to inappropriate boiling of milk to the right temperatures. It could also be associated with numerous outlets for the purchase of milk which operate under unhygienic conditions without adequate monitor or regulation by authority (FAO, 2003). The Findings of this study shows that method of storage of milk could predispose one to campylobacteriosis. This is consistent with previous findings in Kenya (Muinde and Kuria, 2005) and in Ethiopia (Mekennen *et al.*, 2012), where poor storage methods of food were found to be a risk factor for campylobacteriosis. This similarity could be associated with improper food storage and prolonged time lapse between preparing and consuming food items which could lead to cross contaminations (Linda du and Irma, 2005).

Hand washing practices was not found to be a risk factor of Campylobacteriosis (Table 4.1). These findings are in contrast to studies in Kenya (Muhonja and Kimathi, 2014) and in Ghana (Isaack *et al.*, 2014), where it was found that lack of adherence to proper hand washing practices could lead to contracting bacterial infections. This difference could be as a result of increased knowledge on the importance of hand washing amongst the population that was interviewed. Accumulative evidence has shown that appropriate hand washing with soap, reduce the risks of intestinal infections (Curtis *et al.*, 2003). The general role of hand washing in preventing disease is well known, especially in the catering industry, and positive attitudes to hand washing among caterers have been reported (Clayton and Griffifth, 2004) However, observational studies suggest that knowledge is not always put into practice as those who report the importance of hand washing actually don't often wash their hands before handling food (Ombui *et al.*, 2001). Proper hand washing can reduce the risk of diarrheal and respiratory diseases (Luby *et al.*, 2005).

Handling raw chicken while cooking was not found to be a risk factor in this study (Table 4.1). This is in contrary to previous studies where it has been demonstrated that handling raw chicken while cooking could lead to cross contamination (Altekruse *et al.*, 1999). Food handlers play an important role in food safety and in the occurrence of food poisoning because they may introduce pathogens into food during production, processing, distribution and/or preparation (Green *et al.*, 2005). These associations are expected. Since most data shows that most chicken in stores is contaminated with *C jejuni*, (Zhao *et al.*, 2001). However, this contradiction may have been brought about as result of difference in the sources from which the chickens are obtained.

The findings of this study showed that gender is not a risk factor for Campylobacteriosis (Table 4.1). This is contrary to those obtained from previous studies where it has been demonstrated that the rate of *Campylobacter* enteritis was higher in males than in females (Galanis, 2007; Ruiz *et al.*, 2007). This difference could be explained by the fact that, the association between gender and campylobacteriosis may vary according to geographical area and the ratio of males and females within the population. The difference may have also been as a result of difference in the feeding habits of males and females.

5.1.2 Microbial load

Microbial load in all the samples were found to exceed the acceptable limits set by FDA (Table 4.2). According to WHO, water suitable for drinking should show undetectable limits of feacal coliforms (WHO, 2008), as opposed to the finding of this study. The microbial load in milk and chicken also exceeded the limits set by KEBS, where the acceptable limit is 10^4 CFU/g (KEBS, 2003).

5.1.2.1 Microbial load in chicken.

Microbial load in chicken in this finding, was found to be much higher (Table 4.2) than in a study in Pakistan, where chicken sample had a load of 2.85×10^4 CFU/g (Tavakoli and Riazipour, 2008) and in Argentina where the load was 3.63×10^4 CFU/g (Tessi *et al.*, 2002). In a similar study in Saudi Arabia, the microbial load in coocked chicken was found to be higher, that is $1.2\text{x}10^5$ (Eman and Sherifa, 2012). This differences could have been brought about by lengthy gaps between preparation and consumption of foodstuffs, and lack of attention to the essential temperature required for cooking foods, which are among the most important reasons of food contamination (Reglier, 2005).

5.1.2.2 Microbial load in milk

Microbial load in milk in this study was found to be higher (Table 4.2) than findings in Tanzania which had a total bacterial count of 3.3×10^3 CFU/ml (Kivaria *et al.*, 2006). In another similar study in Tanzania, the microbial load was found to be higher, with a total bacterial count of $1.4\text{x}10^6$ CFU/ml (Swai and Schoonman, 2011). This differences could be as a result of the fact that milk passes through increasing numbers of intermediaries thereby increasing bacterial count (Omore *et al.*, 2005). The organism usually gains access to foods from food handlers or other surfaces like the processing equipment (Leenalitha and Peter, 2007). Consistent with studies in Kenya, the finding of this study reveals higher bacterial counts as the milk moves up the market chain, suggesting poor handling along the process (Omore *et al*., 2001).

5.1.2.3 Microbial load in water

Microbial load in water was found to be slightly higher (Table 4.2) than those found in treated water samples from Dunga beach in Kenya, which was contaminated with total viable counts of 2.5 CFU/ml, and water samples from Kisumu market with 2.45 CFU/ml and Luanda market 2.3 CFU/ml, (Onyuka *et al*., 2011). This difference may have resulted from methods of water treatment and water storage facilities.

5.1.3 Survival rates for *Campylobacter jejuni*

In this study, experiments were conducted on *C. jejuni* to establish its response to environmental conditions vis-à-vis temperature variation. Storage temperatures were chosen to reflect what occurs on a daily basis at homes and commercial food service points regarding handling and storage of raw and/or cooked chicken, before, during, or after preparation. Generally, the population of cells decreased with time in all the three food types, (Table 4.3) that is, chicken, milk and water. This is because ambient temperature, refrigeration, and freezing are considered as stress factors for *C. jejuni* (Ala'a *et al*., 2006).

5.1.3.1 Survival rate in cooked chicken

In chicken stored at 25^{0}C, all samples showed declining levels of viable cells (Table 4.4). The decline increased with increase in the storage time. For the 14 day period, there was a 5-$\log_{10}$ decrease in the numbers of *C. jejuni*. In a similar study investigating survival in chicken meat, 70% of the chicken meat stored at ambient temperatures of 26-28^{0}C showed decline of 4-$\log_{10}$ in the total viable cells. (Ala'a *et al*., 2006). This could be due to the fact that *Campylobacter* appears to lack many adaptive responses that are exhibited by other bacteria (Fernandez and Pison, 1996). At 4°C, there was only a 2-$\log_{10}$ decrease in population over a 14-day storage period. Previous studies carried out in order to study the survival of *C. jejuni* during refrigerated storage (4°C) on various chicken meat cuts and preparations have reported a decrease of 2.2-$\log_{10}$ over a period of 2weeks. (Ala'a *et al*., 2006). In another related study, reduction in cell counts of 1.38 to 3.39 $\log_{10}$ CFU/g on chicken over a 2-week period was reported (Bhaduri and Cottrell, 2004).

These variable results could be attributed to the fact that other environmental factors such as UVB light level, the level of oxygenation, water source and the presence of other microorganisms may have influenced *C. jejuni* survival (Obiri-Danso *et al*.,. 2001). Chicken meat as a highly perishable food must be stored refrigerated during processing throughout the food chain in order to prevent and reduce microbial contamination and growth. Survival at such storage temperatures means the organism can still persist in foods stored at ambient temperatures and hence caution should always be taken in food handling (Vandeplas *et al*., 2008).

5.1.3.2 Survival rate in sterile milk

In this study, *C. jejuni* showed a 3-$\log_{10}$ reduction in population in milk, over a 14-day period at 4°C (Table 4.4). In a related study, (Blaser *et al*., 2000) have reported that *C.*

jejuni may survive in sterile milk initially containing >107 cells per ml for up to 22 days at 4°C. Similar observations have been reported, that is, approximately 4-log_{10} decrease in cells in sterile skim milk over a period of 14 days, (Christopher *et al*., 2002). The ability of *C. jejuni* to survive at such low temperatures is as a result of active transcriptional machinery resulting in protein synthesis, motility and oxygen consumption, which allows the organisms to continue surviving (Hazegeler *et al*., 2008).

5.1.3.3 Survival rate in sterile water

In the present finding, in water kept at 25°C, there was a 4log_{10} decrease in population for the 14-day storage period (Table 4.3). In another related study in South Africa, it was found that *C. jejuni* showed a decline by 6 to 7 log_{10} units when in water at 25°C for a similar interval (Jacob *et al.,* 1998). This difference may have been as a result of type of media used, which may have determined the number of surviving organisms depending on the components of the media, and difference in dissolved oxygen tension, which also a factor that would affect survival of the organisms.

5.1.4 Detection of Other bacterial pathogens

In addition to *Campylobacter*, other pathogens, that is, *E coli*, *Salmonella*, *Shigella* and *Vibrio*, were also found in the milk, water and cooked chicken samples (Table 4.4). In the present finding, *Campylobacter* was not found in the water samples collected in the two markets (Table 4.4). This result is in contrast with those found in Nigeria and South Africa where *Campylobacter* was isolated in 39(52.7%) and in 2% water samples respectively (Porgieter *et al*., 2005; Ugboma *et al*., 2013). This difference could be attributed to difference in water sources.

In this study, the chicken samples were positive for *Campylobacter.* These results were lower (Table 4.4) than findings obtained from previous studies in Kenya and China, where thermophilic *Campylobacter* spp. have been isolated from 77 and 76% of chicken samples, respectively (Osano and Arimi 1999; Shih 2000), while in this study, the prevalence was 8.9% and 13.3% in Ngara and Burma market respectively. This difference may have as a result of difference in the source from which the poultry was obtained and the cooking method used.

In these finding the prevalence of *Campylobacter* in milk was higher, 8.9%, (Table 4.4) than in other previous studies in Iran where (3.0%) dairy product samples were positive for *Campylobacter* (*Rahimi et al.,* 2013). This result is also in contrast with the results reported by (Salihu *et al.,* 2010) from Nigeria, (El-Sharoud, 2009) from Egypt and (Whyte *et al.,* 2004) from Ireland who got lower prevalence of 4.4%, 3.7% and 6.5% respectively. Variation in the prevalence of *Campylobacter* isolates from raw milk and traditional dairy product samples reported in other studies may be a result of different sampling techniques employed, seasonal effects and/or laboratory methodologies employed in different studies (Soapwith *et al*., 2003). The variation may have also been brought about by levels of hygiene in the different places.

In the present finding, *E coli* was the most prevalent in the water samples, 5 (20.9%) of water samples in Ngara and 7(29.2%) in Burma market (Table 4.4). This is contrary to findings in Kisumu, where *Salmonella* had the highest prevalence that is, 162 (49.6%), while *E. coli* had a prevalence of 162 (46.6%.) (Onyuka *et al.*, 2011). The prevalence of *E. coli* in studies in Kisumu was higher than in this study. This difference may have arisen due to the difference in sample size. Müller *et al.* (2003) in a bid to assess the prevalence of microorganisms in environmental waters in South Africa reported a 20% isolation rate for *E. coli.* This is in agreement with the findings in this study where the isolation rate was found to be 20.9%. This could be attributed to the similarity in methods of water treatment and storage facilities.

In the present finding the prevalence of *E. coli* in chicken was found to be lower than a finding in Kenya, where *E. coli* was isolated in 4 (67%) of cooked chicken samples (Maina *et al.*, 2013). Burma market, had a prevalence of 28 (62.2%), which was consistent with a finding in Kenya, of 4 (67%) (Table 4.4). These discrepancies may have arisen because of difference in food handling techniques.

In milk, *E. coli* had the highest prevalence of 9 (37.5%) in Ngara market and 8 (33.3%) in Burma market (Table 4.4). This finding is in contrast with finding in a similar study in Kenya (Omore *et al.*, 2001) who isolated *E. coli* in only 1% of the samples. This difference may have been due to method of pasteurization and storage of milk. In another study in Tanga city, 100% of the milk samples had *E. coli* (Swai and Schoonman, 2011). This could be due to adulteration of milk by addition of water which may introduce chemical or microbial health hazards as well as reducing the nutritional and processing quality, palatability and marketing value of the milk (Giangiacoma, 2001).

Salmonella and *Shigella* in chicken was found in this study to have a higher prevalence, 20% and 13.3% in Ngara and Burma market respectively, (Table 4.4) than those obtained from a previous study in Kenya, where the prevalence of *Salmonella* and *Shigella* was 5% and 8% respectively (Kariuki *et al.*, 2013). This could be attributed to poor waste disposal and inadequate toilet facilities in these markets.

The frequency of contamination in pooled farm milk has been reported to be <1% to 8.9% for *Salmonella* and *Shigella* in other studies (Oliver *et al.*, 2005), contrary to these finding where the isolation rate was higher (Table 4.4).

In this study, *Vibrio* was isolated in the milk, chicken and water samples. The frequency of isolation of *Vibrio* in water was much lower, 20% and 26.7% in Ngara and Burma market respectively, (Table 4.4) than that obtained in Bangladesh where 53.33% of the milk samples tested positive (Nawas *et al.*, 2012). This contrast could have resulted from methods of water treatment and the source of water.

In this finding, the frequency of isolation of *Vibrio* in chicken, 20% and 13.3% in Ngara and Burma market respectively, (Table 4.4), was found to be higher that found in a similar study in South Africa, where the prevalence was found to be at 0 % (Mosupye and Von holy 2000). The detection of *Vibrio* may have been as a result of improper handling, undercooking, and washing with unhygienic water (Sack *et al.*, 2003).

The prevalence of *Vibrio* in milk from Ngara and Burma markets in this study were 9 (20%) and 6 (13.3%) respectively (Table 4.4). The finding in Ngara market was similar to that obtained in Uganda while that from Burma Market was found to be lower 7 (9.3%) (Grimaud *et al.*, 2007). Poor hygiene practices especially milking using bare hands and poor farm management practices could have contributed to detected *Vibrio* in milk.

5.2 Conclusions

Age, education level, occupation, undercooking of chicken, site of processing chicken, source of milk method of storage of milk, source of water and method of treating drinking water were found to be associated with transmission of *Campylobacter.*

Microbial load in all the samples exceeded the acceptable total viable counts of 10^3 CFU/g in milk, 10^4 CFU/g in chicken and 2.2/100ml in water as the limits set by FDA. It also exceeded limits set by WHO and KEBS, thus rendering the foods and drinks unfit for consumption.

There was a general decrease in the number of *C. jejuni* in all the samples within the 14 days period of study, hence survival rates decreased as the days go by. Generally *C. jejuni* can survive and outlast the shelf life of cooked chicken, boiled milk and water.

Other than *Camplyobacter*, *Salmonella* spp, *Shighella* spp, *Vibrio* spp and *E. coli* were also detected in the cooked chicken, water and milk samples, indicating poor microbial quality. The *E. coli* detected were found to be pathogenic.

5.3 Recommendations

This study recommends the following measures in order to prevent food borne outbreaks related to the foods investigated

- Thorough cooking of poultry and other meat products to the required temperatures to kill *Campylobacter* and other harmful bacteria.
- Proper boiling of milk and treatment of water before consumption
- Regular washing of hands with soap by food handlers before preparation of food and after handling raw chicken meat.
- Screening other food products for the same pathogens
- Molecular identifications of the pathogens

5.4 Limitations of the study

This study did not include performance of antimicrobial susceptibility testing on the isolates to determine the resistance patterns of the pathogenic microorganisms. The research was conducted on only two market centers with Nairobi city and thus did not cover a larger area of the city. Molecular work was not done on the isolated organisms to establish the exact strains of the organisms.

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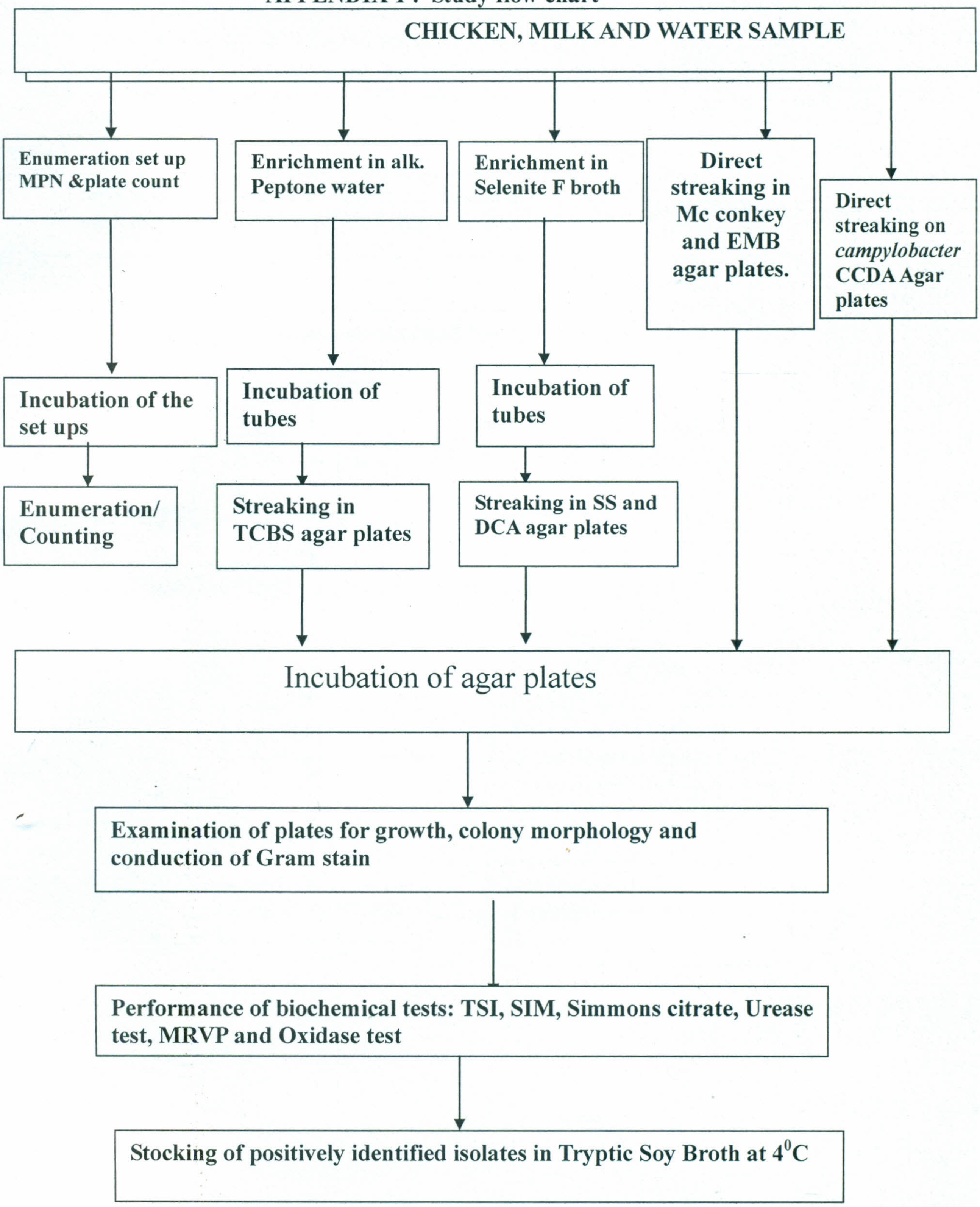
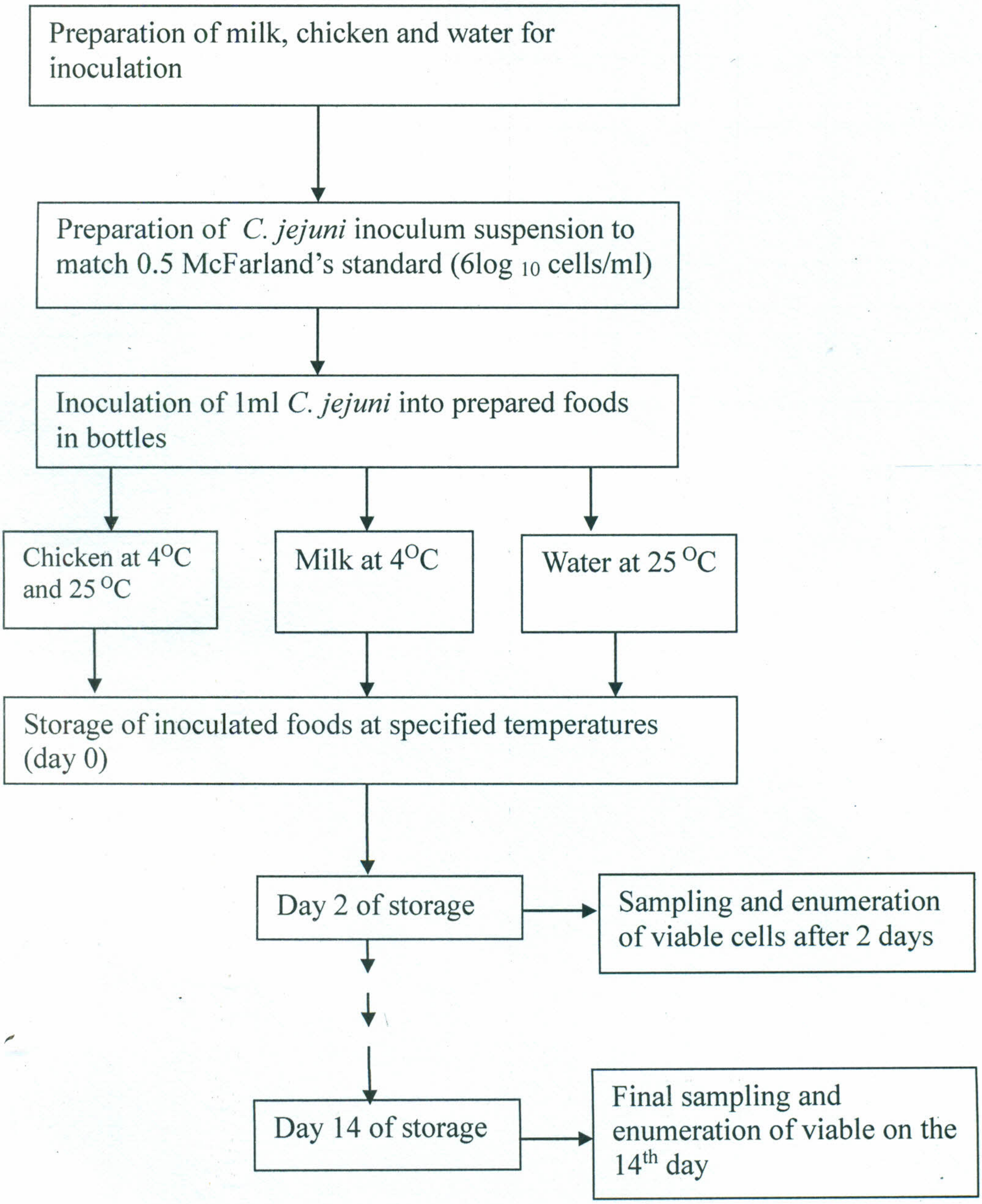
APPENDICES

APPENDIX 1 : Study flow chart



APPENDIX 11: Survival experiments flow chart



Appendix 111: Biochemical tests for other bacterial pathogens

Test	***Salmonella spp***	***Shigella spp***	***Vibrio spp***	***E. coli***
Urease	–	–	–	–
Simmon's citrate	+	–	+	–
Motility	+	–	+	+
Indole	+	–	+	+
Oxidase	–		+/_	+
TSI	+	–	–	–

Appendix 1V: Biochemical test for *Campylobacter*

Test	***Campylobacter jejuni***
Nitrate reduction	+
Catalase	+
Oxidase	+
Motility	+
Hippurate hydrolysis	+

APPENDIX 1V: Questionnaire

PART A: DEMOGRAPHIC INFORMATION

1. Gender of the respondent

[ ] 1. Male [ ] 2. Female

2. What is your highest level of education

[ ] 1.Standard Eight [ ] 2.High School Graduate [ ] 3.College Credit

[ ] 4.Bachelors Degree [ ] 5.Postgraduate Degree

3. What is your current profession?

[ ] 1.Professional [ ] 2.Employee [ ] 3.Skilled worker

[ ] 4.Self employed [ ] 5.Farmer [ ] 6.Retired

[ ] 7.Student [ ] 8.Others specify.................................

4. What is your age? (Please give age bracket).

[ ] 1. 18- 25years [ ] 2.26-30 years [ ] 3.31-35 years

[ ] 4. 36-45 years [ ] 5. Above 45 years

PART B (CONSUMERS)

1. How many times do you eat your meals in a restaurant on a weekly basis?

1) Always [ ] 2) Four times [ ] 3) Thrice [ ] 4) Rarely [ ]

2. Are you provided with soap for washing your hands?

1) Yes [ ]

2) No [ ]

3. Do you take chicken from the restaurant?

1) Yes [ ]

2) No [ ]

4. The chicken served is well cooked.

1)Agree [ ] 2)Strongly agree [ ] 3)Disagree [ ] 4) Strongly disagree [ ]

5. How frequently do you take milk in a restaurant on a weekly basis?

1) Always [ ] 2) Four times [ ] 3) Thrice [ ] 4) Rarely [ ]

6. How is the milk served?

1) In a cup [ ] 2) In a packet 3) Others specify.........................

7. Do you drink water from the restaurant?

1) Yes [ ] 2) No [ ]

PART C (HOTEL EMPLOYEES)

1. Do you serve milk in your restaurant?

1) Yes [ ] 2) No [ ]

2. From which source do you get milk?

1) Packeted [ ] 2) Homesteads [ ] 3) Dairies [ ]

4) Other (specify) ....

3. How do you store your milk once it is in the restaurant?

1) In a refrigerator [ ] 2) Boiled then left uncovered [ ] 3) Boiled and covered [ ]

4. Where do you process your chicken?

1) In the hotel kitchen [ ] 2) Outside the hotel kitchen [ ]

3) Others (specify) ....