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

Winnie Awuor Odhiambo
Master of Science (Microbiology), School of Pure and Applied Sciences, Kenyatta University, Kenya

Dr. Anthony Kebira N.
Department of Microbiology, Kenyatta University, Kenya

Dr. Andrew Nyerere
Department of Medical Microbiology, Jomo Kenyatta University of Agriculture and Technology, Kenya

International Academic Journal of Health, Medicine and Nursing (IAJHMN)

Received: 30th April 2017

Accepted: 12th May 2017

Full Length Research

Available Online at:

http://www.iajournals.org/articles/iajhmn_v1_i1_32_57.pdf

Citation: Odhiambo, W. A., Kebira, N. A. & Nyerere, A. (2017). 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. *International Academic Journal of Health, Medicine and Nursing, 1*(1), 32-57

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 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 (420C) generally seemed to have inhibitory effect on the population of the organism. Survival of Campylobacter was poor in water and chicken at room temperature (250C) while in chicken and milk stored at 40C, 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.

Key Words: *risk factors, microbial load, campylobacteriosis, campylobacter, pathogens*

INTRODUCTION

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).

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).

GENERAL OBJECTIVE

The main objective was to determine prevalence of *Campylobacter jejuni* and other food pathogens in food and drinks served in fast food kiosks in Ngara and Burma markets.

SPECIFIC OBJECTIVES

1. To determine the risk factors associated with campylobacteriosis.
2. To compare the levels of microbial load in the three foodstuffs; milk, chicken and water.
3. To determine the survival rates of *Campylobacter* in cooked chicken, milk and water samples.
4. To identify other pathogens found in cooked chicken, milk and water samples.

LITERATURE REVIEW

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).

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)

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.

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 (Yuki, 2007; WHO 2002). 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 & Kist, 2003).

MATERIALS AND METHODS

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

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

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).

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).

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).

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^{o}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^{o}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).

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).

Enumeration of pathogens in water 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).

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).

RESEARCH RESULTS

The study issued 70 questionnaires randomly where 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*.

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*. 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*. 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*. 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*. 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. This is consistent with previous studies in Uganda (Muyanja *et al*., 2011; Reij, 2004). 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*. 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*. 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*. 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. 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 handwashing 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. 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. 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.

Microbial Load

Microbial load in all the samples were found to exceed the acceptable limits set by FDA. 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).

Microbial Load in Chicken

Microbial load in chicken in this finding, was found to be much higher 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 $1.2x10^5$ (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).

Microbial Load in Milk

Microbial load in milk in this study was found to be higher than findings in Tanzania which had a total bacterial count of 3.3×10^3 CFU/ml (Kivaria, 2006). In another similar study in Tanzania, the microbial load was found to be higher, with a total bacterial count of $1.4x10^6$ CFU/ml (Swai

& 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).

Microbial load in water

Microbial load in water was found to be slightly higher 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.

Survival rates for *Campylobacter jejuni*

In this study, experiments were conducted on *Campylobacter 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, 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).

Survival rate in cooked chicken

In chicken stored at 25^0C, all samples showed declining levels of viable cells. 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 *Campylobacter jejuni*. In a similar study investigating survival in chicken meat, 70% of the chicken meat stored at ambient temperatures of 26-28^0C 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).

Survival rate in Sterile Milk

In this study, *Campylobacter jejun*i showed a 3-$\log_{10}$ reduction in population in milk, over a 14-day period at 4°C. 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)

Survival rate in sterile water

In the present finding, in water kept at 25°C, there was a 4$\log_{10}$ decrease in population for the 14-day storage period. In another related study in South Africa, it was found that *Campylobacter 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.

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. In the present finding, *Campylobacter* was not found in the water samples collected in the two markets. 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 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%, 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. 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%). These dicrepancies 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. 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, 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.

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, 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, 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. 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.

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 *Camplylobacter 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.

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

REFERENCES

Aboderin A.O, Smith S.I, Oyelese A.O, Onipede A.O, Zailani S.B, Coker A.O. (2002). Role of *Campylobacter jejuni/coli* in diarrhoea in Ill-Use, Nigeria. *East African Medical journal*, **79**: 423–426

Advisory Committee on Microbiological Safety of Food (2005). 2nd report on *Campylobacter. Food Standards Agency.*

Akanda A.S, Jutla A.S, Gute D.M, Evans T, Islam S. (2012). Reinforcing cholera intervention through prediction-aided prevention. *Bulletin of World Health Organization*, **90**: 243-244.

Ala'a M.F. Eideh and Hamzah M. (2006). Al-Qadiri Effect of Refrigerated and Frozen Storage on the Survival of *Campylobacter jejuni* in Cooked Chicken Meat Breast. *Food Microbiology & Safety* **45**: 90-97.

Alam M.J, Rahman M.T, Siddique M.P, Khan R and Rahman M.B. (2010). Antibiogram and Plasmid Profiling Of *E. Coli* Isolates. *International Journal of Biology Research*, **1**(3): 01-07.

Bacon D.J, Alma R.A, Hu L, Hickey T.E, Ewing C.P, Batchelor R.A, Trust T.J, Guerry P. **(**2002**).** DNA sequence and mutational analyses of the pVir plasmid of *Campylobacter jejuni* 81-176. *Journal of Infection and Immunology*, **70:** 6242–6250.

Beltinger J, del Buono J, Skelly M.M, Thornley J, Spiller R.C, Stack W.A, Hawkey C.J. **(**2008**).** Disruption of colonic barrier function and induction of mediator release by strains of *Campylobacter jejuni* that invade epithelial cells. *World Journal of Gastroenterology*, **14**: 7345–7352.

Bereswill S, Kist M. (2003). Recent developments in *Campylobacter* pathogenesis, *Journal of Current Opinions on Infectious diseases*, **16** (5): 487–491.

Bessède E, Delcamp A, Sifré E, Buissonnière A, Mégraud F. (2011). New methods for detection of *Campylobacters* in stool samples in comparison to culture. *Journal of Clinical Microbiology*, **49(**3): 941-944.

Blaser M.J, Berkowitz I.D, LaForce F.M, Cravens J, Reller L.B, Wang W-LL. (2009). *Campylobacter* enteritis: clinical and epidemiologic features. *Annals of Internal Medicine*, **91**: 179-*1*85.

Blaser M.J, Engberg J. (2008). Clinical aspects of *Campylobacter jejuni* and *Campylobacter coli* infections. In: Nachamkin I, Szymanski C.M, Blaser M.J, *Campylobacter*. ASM Press; Washington DC, USA. **3**: 99–121.

Blaser M.J, Hardesty H.L, Powers B, Wang W.L. (2000). Survival of *Campylobacter* fetus subsp. *jejuni* in biological milieus. *Journal of Clinical Microbiology*, **11**: 309-313.

Buzby, Jean and Roberts Tonya (2009). "The Economics of Enteric Infections: Human Foodborne Disease Costs. *Journal of gastroenterology*, **136**(6): 1851-62.

Calva J.J, Ruiz-Palacioz G.M, Lopez-Vidal A.B, Ramos A, Bojalil R, Cohort study of intestinal infection with *Campylobacter* in Mexican children. *Lancet*, **1**: 503-506.

CDC (2009). *Campylobacter* Technical fact sheet http://www.cdc.gov/nczved/dfbmd/diseases-listing/*Campylobacter*-gi html.

CDC (2009). National Center for Zoonotic, Vector-Borne, and Enteric Diseases, "Shigellosis—General Information and Frequently Asked Questions,"

CDC (2011). Vital signs, incidence and trends of infections with pathogens transmitted commonly through food-borne diseases active surveillance network, 10 U.S sites, 1996-2010.MMWR morbidity **60**(22): 749-55.

CDC (2011). Vital signs: incidence and trends of infection with pathogens transmitted commonly through food--foodborne diseases active surveillance network, 10 U.S. sites, 1996-2010. Morbidity and mortality weekly report, **60**(22): 749-755.

CDC (2013), "*E. coli* (*Escherichia coli*)," National Center for Emerging and Zoonotic Infectious Diseases, Division of Foodborne, Waterborne, and Environmental Diseases.

CDC National Center for Emerging and Zoonotic Infectious Diseases. Retrieved 2012-10-02.

CDC. (2009) Surveillance for Foodborne Disease Outbreaks - United States, 2006. Morbidity and mortality weekly report, **58(**22): 609-636.

Chu Y.W, Chu M.Y, Luey K.Y, Ngan Y.W, Tsang K.L, Kam K.M. (2004). Genetic relatedness and quinolone resistance of *Campylobacter jejuni* strains isolated in 2002 in Hong Kong. *Journal of Clinical Microbiology*, **42**(7): 3321–3323.

Clark S, Costello M, Bodyfelt F, Drake M. (2008). The sensory Evaluation of Dairy Products. Springer 233-234.

Coker A.O, Isokpehi R.D, Thomas B.N, Amisu K.O, Obi C.L. (2002). Human campylobacteriosis in developing countries. *Journal of Emerging Infectious Diseases*, **8**(3): 237-44.

Cools I, Uyttendaele M, Caro C, D'Haese E, Nelis H.J, Dedevere J. (2003). Survival of *Campylobacter jejuni* strains of different origin in drinking water. *Journal of Applied Microbiology*, **94**(5): 886-892

Corry J.E, Allen V.M., Hudson W.R., Breslin M.F, Davies, R.H. (2002). Sources of *Salmonella* on broiler carcasses during transportation and processing, modes of contamination and methods of control, *Journal of Applied Microbiology*, **92**(3): 424-432

CPLS **(**2000**).** Common gastrointestinal infections. Communicable Diseases Report Weekly England and Wales, **10**: 9–12.

Craven S.E, Stern N.J, Line E, Bailey J.S, Cox N.A, Fedorka-Cray P. (2000). Determination of the incidence of *Salmonella* spp., *Campylobacter jejuni*, and Clostridium perfringens in wild birds near broiler chicken houses by sampling intestinal droppings. *Journal of Avian Diseases*, **44***:* 715-720.

Curtis V, Cairncross S. (2003). Effect of washing hands with soap on diarrhea risk in the community: a systematic review. *Lancet Infectious Diseases*, **3**(5): 275-281.

David O, Wandili S, Kakai R, Waindi E. (2009). Isolation of *Salmonella* and *Shigella* from fish harvested from the Winam Gulf of Lake Victoria, Kenya. *The Journal of Infection in Developing Countries* **3**: 2

Dingle K.E, Colles F.M, Ure R, Wagenaar J.A, Duim B. (2002). Molecular characterization of *Campylobacter jejuni* clones: a basis for epidemiologic investigation. *Journal of Emerging Infectious Diseases*, **8**: 949–955.

Eckburg P.B, Bik E.M, Bernstein C.N, Purdom E, Dethlefsen L, Sargent M, Gill S.R, Nelson K.E, Relman D.A. (2005). "Diversity of the human intestinal microbial flora". *Science* **308** (5728): 1635–1638.

Eman M.S and Sherifa M.S. (2012). Microbiological Loads for Some Types of Cooked Chicken Meat Products at Al-Taif Governorate, KSA. *World Applied Sciences Journal*, **17**(5): 593-597.

Engberg J, Aarestrup F.M, Taylor D.E, Gerner-Smidt P, Nachamkin I. (2001). Quinolone and macrolide resistance in *Campylobacter jejuni* and *C. coli*: resistance mechanisms and trends in human isolates. *Journal of Emerging Infectious Diseases*, **7**(1): 24–34.

Englen M.D, Hill A.E, Dargatz D.A, Ladely S.R, Fedorka-Cray P.J. (2007). Prevalence and antimicrobial resistance of *Campylobacter* in US dairy cattle. *Journal of Applied Microbiology*, **102**(6): 1570–157.

Feierl G, Jelovcan S. (2009). Campylobacteriosis in Austria: situation and trends. *Journal of Wiener Klinische Wochenschrift*, **12(**3-4): 103-7.

Feng P, Weagant S, Grant M. (2002). "Enumeration of *Escherichia coli* and the Coliform Bacteria". *Bacteriological Analytical Manual (8th ed.)*. FDA/Center for Food Safety & Applied Nutrition. Retrieved 2007-01-25.

Fluckey W.M, Sanchez M.X, McKee S.R., Smith D, Pendleton E. Brashears, M.M. (2003). Establishment of a microbiological profile for an air-chilling poultry operation in the United States. *Journal of Food Protection*, **66**(2): 272-279.

Foodborne Diseases Active Surveillance Network (FoodNet) (2013). Trends in Foodborne Illness in the United States, 1996–2011.

Fotadar U, Zaveloff P, Terracio L (2005). "Growth of *Escherichia coli* at elevated temperatures". *Journal of Basic Microbiology*, **45**(5): 403–404.

Freitas C.G, Santana A.P, Silva P.H, Goncalves V.S.P, Barros A.F, Torres F.A.G, Murata L.S. Perecmanis S. (2010). PCR multiplex for detection of *Salmonella* Enteritidis, Typhi and Typhimurium and occurrence in poultry meat. *International Journal of Food Microbiology*, **139**(1-2): 15-22.

Frost J.A, Gillespie I.A, O'Brien S.J. (2002). Public health implications of *Campylobacter* outbreaks in England and Wales, 1995-1999. Epidemiological and microbiological investigations. Epidemiology Infection, **128**(2): 111-118.

Gibreel A, Taylor D.E. (2006). Macrolide resistance in *Campylobacter jejuni* and *Campylobacter coli*. *Journal of Antimicrobials and Chemotherapy*, **58**(2): 243–255.

Gillespie I.A, O'Brien S.J, Frost J.A, Adak G.K, Horby P, Swan A.V, Painter M.J, Neal K.R. (2002). *Campylobacter* Sentinel Surveillance Scheme Collaborators. A case-case

comparison of *Campylobacter coli* and *Campylobacter jejuni* infection: a tool for generating hypotheses. *Journal of Emerging Infectious Diseases*, **8**(9): 937-939.

Gillespie I.A, O'Brien S.J, Penman C, Tompkins D, Cowden J, Humphrey T.J. (2008). Demographic determinants for *Campylobacter* infection in England and Wales: implications for future epidemiological studies. *Journal of Epidemiology and Infection*, **136**(12): 1717-25.

Journal of Clinical Nutrition, **25**: 1459-1466.

Grimaud P, Sserunjogi M.L, Grillet N. (2007). An evaluation of milk quality in Uganda: value chain assessment and recommendations. *African Journal of Food Agriculture Nutrition and Development*, **7**(3): 5-18.

Hakkinen M, Heiska H, Hanninen M.L. (2007). Prevalence of *Campylobacter* spp in cattle in Finland and antimicrobial susceptibilities of bovine *Campylobacter jejuni* strains. *Journal of Applied Environmental Microbiology*, **3**(10): 3232–3238.

Hald T, Brondsted T. (2000). Annual report on zoonoses in Denmark 2000. *Ministry of Food, Agriculture and Fisheries, Copenhagen, Denmark.B.*

Hannu T, Mattila L, Rautelin H, Pelkonen P, Lahdenne P, Siitonen A.(2004). *Campylobacter*-triggered reactive arthritis: a population-based study. *Rheumatology Oxford*, **41**(3): 312-318.

Harris L.J, Farber J.N, Beuchat L.R, Parish M.E, Suslow T.V, Garrett E.H, Busta.F.F. (2003). Outbreaks Associated with Fresh Produce: Incidence, Growth, and Survival of Pathogens in Fresh and Fresh Cut Produce. *Comprehensive Reviews in Food Science and Food Safety*, **2**: 78-141.

Hazegeler W.C, Wouters J.A, Rombouts F.M, Abee T. (2008). Physiological activities of *campylobacter jejuni* far below the minimal growth temperature. *Journal of Applied and Environmental microbiology*, **64**(10): 3917-3922.

Herikstad H, Motarjemi Y, Tauxe, R.V. (2002). *Salmonella* surveillance, a global survey of public health serotyping, *Journal of Epidemiology and Infection* **129**(1): 1-8.

Hoffmann S, Batz M.B, Morris Jr J.G. (2012). "Annual Cost of Illness and Quality-Adjusted Life Year Losses in the United States due to 14 Foodborne Pathogens," *Journal of Food Protection*, **75**(7): 1292–1302.

Ishii S, Sadowsky M.J. (2008). "*Escherichia coli* in the Environment: Implications for Water Quality and Human Health". *Journal of Microbes Environment*, **23**(2): 101–108.

Islam M, Morgan J, Doyle M.P, Phatak S.C, Millner P. Jiang X. (2004). Persistence of *Salmonella enteric* serovar Typhimurium on lettuce and parsley and in soils on which they were grown in fields treated with contaminated manure composts or irrigation water. *Foodborne Pathogens and Disease*, **1**(1): 27-35.

Jacob P, Mdegela R.H, Nonga H.E. (2011). Comparison of Cape Town and Skirrow's *Campylobacter* isolation protocols in humans and broilers in Morogoro, Tanzania. *Tropical*

Jafari F, Garcia-Gil L.J, Salmanzadeh-Ahrabi S, Shokrzadeh L, Aslani M.M, Pourhoseingholi M.A. (2009). Diagnosis and prevalence of enteropathogenic bacteria in children less than 5 years of age with acute diarrhea in Tehran children's hospitals. *Journal of infectious diseases*, **58(**1): 21-27.

Jain D, Sinha S, Prasad K.N, Pandey C.M. (2005). *Campylobacter* species and drug resistance in a north Indian rural community. *Transaction of the royal Society Tropical Medicine and Hygiene,* **99**: 207-214.

Jang K.I, Kim M.G, Ha S.D, Kim K.S, Lee K.H, Chung D.H, Kim C.H, Kim K.Y. (2007). Morphology and adhesion of *Campylobacter jejuni* to chicken skin under varying conditions. *Journal of Microbiology and Biotechnology*, **17**: 202– 206.

Jayarao B.M, Henning D.R. (2001). Prevalence of food borne pathogens in bulk tank milk. *Journal of Dairy Science,* **84**: 2157.

Jones F.R., Baqar S, Gozalo A, Nunez G, . Espinoza N, Reyes S.M, Salazar M, Meza R.C. Porter K, and Walz S.E. (2006). New World monkey *Aotus nancymae* as a model for *Campylobacter jejuni* infection and immunity. *Journal of Infections and Immunuology*, **74:**790-799.

Kapperud G, Lassen J, Ostroff S.M, Aasen S. (2002). Clinical features of sporadic *Campylobacter* infections in Norway. *Scandinavian Journal of Infectious Diseases*, **24**: 741-749.

Kariuki S. R, Onsare J, Mwituria R, Ngetich C, Nafula S. (2013). Improving food safety in meat value chains in Kenya. *Journal of Tropical health production*, **89**: 123-220

Kassenborg H.D, Hedberg C.W, Hoekstra M, Evans M.C, Chin A.E, Marcus R, Vugia K, Smith D.J, Ahuja S.D, Slutsker L, Griffin P.M, (2004). Emerging Infections Programs FoodNet Working Group, "Farm Visits and Undercooked Hamburgers as Major Risk Factors for Sporadic *Escherichia coli* O157:H7 Infection: Data from a Case-Control Study in 5 FoodNet Sites," *Journal of Clinical Infectious Diseases*, **38**(3): 271–278.

KEBS (2003). Standard microbial limits of food. K.S.05-220, Government press Nairobi Kenya 1-7

Kelly A.F, Kelly, Park S.F, Bovill R, Mackey B.M. (2001). Survival of *Campylobacter jejuni* during stationary phase: evidence for the absence of a phenotypic stationary-phase response. *Journal of Applied and Environmental Microbiology*, **67**: 2248–2254.

Kivaria, F. M., Noordhuizen, J. P. T. M. and Kapanga, A. M. (2006). Evaluation of the hygienic quality and associated public health hazards of raw milk marketed by smallholder dairy producers in the Dar es Salaam region, Tanzania. *Journal of Tropical Animal Health Production*, **38**: 185 – 194.

Konkel M.E, Klena J.D, Rivera-Amill V, Monteville M.R., Biswas D, Raphael B, Mickelson J. **(**2004**).** Secretion of virulence proteins from *Campylobacter jejuni* is dependent on a functional flagellar export apparatus. *Journal of Bacteriology*, **186**: 3296–3303.

Kotloff S. (1999). "Global burden of *Shigella* infections: implications for vaccine development and implementation of control strategies," *Bulletin of the world health organization*, **77**: 8

Lawson A.J, Logan A.M, O'Neill G.L, Desai M, Stanley J. (1999). Large-scale survey of *Campylobacter* species in human gastroenteritis by PCR and PCR-enzyme-linked immunosorbent assay. *Journal of Clinical Microbiology*, **37**: 3860-*3864*.

Lira W.M, Macedo C, Marin J.M. (2004). The incidence of Shiga toxin-producing Escherichia coli in cattle with mastitis in Brazil. *Journal of Applied Microbiology*, **97**: 861-866.

Mackenjee M.K, Coovadia Y. M, Coovadia H.M, Hewitt J, Robins-Browne R.M. (2004). Etiology of diarrhoea in adequately nourished young African children in Durban, South Africa. *Annals of Tropical Paediatrics.* **4**:183–187.

Mariita, M. R. and Okemo, O. P. (2009). Usefulness of fecal streptococci as indicator of presence of *Salmonella sp.* and *Vibrio cholerae* sewage effluents. *Journal of Microbiology*, **5**(1): 19-24.

Mathusa E.C, Chen Y, Enache E.L. Hontz L. (2010). "Non-O157 Shiga Toxin-Producing *Escherichia coli* in Foods, *Journal of Food Protection*, **73**(9): 1721–1736.

McGhie E.J, Brawn L.C, Hume P.J, Humphreys D, Koronakis V. (2009). "Salmonella takes control: effector-driven manipulation of the host". *Current Opinions in Microbioogy*, **12** (1): 117–24.

Meseret E. (2004). Analysis of pediatric admission to Jimma Hospital pediatric ward: A three year retrospective study. Bull JIHS, **4**:1–11.

Miflin J.K, Templeton J.M, Blackall P.J. (2007). Antibiotic resistance in *Campylobacter jejuni* and *Campylobacter coli* isolated from poultry in the South-East Queensland region. *Journal of Antimicrobial Chemotherapy*, **59**(4): 775–778.

Mosyupe M.F, Alexander V. (2000). Microbiological hazard identification and exposure assessment of street food vending in Johannesburg, South Africa. *International Journal of Food Microbiology*, **61**: 137–145

Mshana S.E, Joloba M, Kakooza A, Kaddu- Mulindwa D. (2009). *Campylobacter* spp among children with acute diarrhea attending Mulago hospital in Kampala-Uganda, *Journal of African Health Science.* **9**: 201-205.

Nachamkin I, Ung H, Li M. (2002). Increasing fluoroquinolone resistance in *Campylobacter jejuni*, Pennsylvania, USA, 1982–2001. *Journal of Emerging Infectious Diseases*, **8**(12): 1501–1503.

Nakari U.M, Puhakka A, Siitonen A. (2008). Correct identification and discrimination between *Campylobacter jejuni* and *C. coli* by a standardized hippurate test and species-specific polymerase chain reaction. *Journal of European Clinical Microbiology of Infectious Diseases*, **27**(7): 513-518.

Nichols G.L, Richardson J.F, Sheppard S.K, Lane C, Sarran C. (2012). *Campylobacter* epidemiology: a descriptive study reviewing 1 million cases in England and Wales between 1989 and 2011. *BMJ Open*, **2**(4): 331-335.

Njuguna H.N, Cosmas L , Wiliamson J, Nyachiewo D, Williamson J, Nyachieo D, Olack B, Ochieng J.B, Wamola N, . Oundo J.O, .Feikin.R, Mintz E.D, Breiman R.F (2013) Use of Population-based Surveillance to Define the High Incidence of Shigellosis in an Urban Slum in Nairobi, Kenya.Plos ONE **8**(3): e58437.doi:10.1371.

Northcutt J.K, Berrang M.E, Dickens J.A, Fletcher D.L, Cox N.A. (2003). Effect of broiler age, feed withdrawal, and transportation on levels of coliforms, *Campylobacter, Escherichia coli* and *Salmonella* on carcasses before and after immersion chilling. *Journal of Poultry Science*, **82**(1): 169–173.

O'Reilly C.E, Jaron P, Ochieng B, Nyaquara A.J, Tate E, Parsons M.B, Bopp C.A, Williams K.A, Vinje J. (2007). Risk factors for death among children less than 5 years old hospitalized with diarrhoea in rural Western Kenya. A cohort study. PloS Medicine 9: e1001256.

Obi C.L, Bessong P.O. (2002). Diarrheagenic bacterial pathogens in HIV-positive patients with diarrhoea in rural communities of Limpopo province, South Africa. *Journal of Health Population and Nutrition, **20**:230–234*.

Obiri-Danso, Paul K.N, Jones K**.** (2001). The effect of UVB and temperature on the survival of natural populations and pure cultures of *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter lari* and urease-positive thermophilic *campylobacters* (UPTC) in surface waters. *Journal of Applied. Microbiology*, **90**: 256-267.

Ochieng J.B, Beatty M.E, Chege W, Kumar L, Okoth G, Shapiro R.L, Wells J.G, Parsons M.B, Bopp C, Chiller T, Vulule J, Mintz E, Slutsker L, Brooks J.T (2009). "Sporadic paediatric diarrhoeal illness in urban and rural sites in Nyanza Province, Kenya." *East African Medical Journal*, **86**(8): 387–398.

Oliver S.P, Boor K.J, Murphy S.C, Shin S.J. (2009). Food safety hazards associated with consumption of raw milk. *Journal of Foodborne Pathogens and Disease*, **6**: 793-806.

Onyango D.F, Machoni R, Kakai G, Waindi E.N. (2008). Multi drug resistance of *Salmonella enterica* serovars *Typhi* and *Typhimirium* isolated from clinical samples in two rural hospitals in Western Kenya. *Journal of Infection in Developing countries* **2**: 106-111.

Pacanowski J, Lalande V, Lacombe K, Boudraa C, Lesprit P, Legrand P. (2008). *Campylobacter* bacteremia: clinical features and factors associated with fatal outcome. *Journal of Clinical Infectious Diseases*, **47**(6): 790-796.

Papavasileiou E, Voyatzi A, Papavasileiou K, Makri A, Andrianopoulou I, Chatzipanagiotou S. (2007). Antimicrobial susceptibilities of *Campylobacter jejuni* isolates from

hospitalized children in Athens, Greece, collected during 2004–2005. *European Journal of Epidemiology*, **22**(1): 77–78.

Parkhill J, Park S.F, (2002). The physiology of *Campylobacter* species and its relevance to their role as foodborne pathogens. *International Journal of Food Microbiology*, **74**: 177–188.

Pfeffer C, Oliver J.D. (2003). A comparison of thiosulphate-citrate bile salts-sucrose (TCBS) and thiosulphate-chloride-iodide (TCI) agar for isolation of *Vibrio* species. *Letters in applied Microbiology* **36**(3): 150-151.

Pope J.E, Krizova A, Garg A.X, Thiessen-Philbrook H, Ouimet J.M. (2007). *Campylobacter* reactive arthritis: a systematic review. *Seminars in Arthritis and Rheumatology*, **37**(1): 48-55.

Potgieter H, Natasha O, Chikwelu L, Bessong N, Pascal O, Igumbor B, Eunice O, Samie A, Nengobela M. (2005). Bacterial Contamination of Vhuswa - A Local Weaning Food and Stored Drinking-water in Impoverished Households in the Venda Region of South Africa. *Journal of health, population and nutrition*, **23**(2): 150-155.

Republic of Kenya (2001). Report on disease out breaks in Kenya, Ministry ofHealth, Health Information System, Nairobi, Kenya.

Republic of Kenya (2001). Report on Typhoid outbreak in Embu District. Ministry of Health. Nairobi,Kenya.

Ritz M, Nauta M.J, Teunis P.F, van Leusden, Federighi M, Havelaar A.H. (2007). Modelling of *Campylobacter* survival in frozen chicken meat. *Journal of Applied Microbiology*, **103**: 594–600.

Sack D.A, Lyke C, Mclaughlin C, Suwanvanichkij V. (2001). Antibacterial resistance in shigellosis, cholera and campylobacrteriosis, p. 8-10. Geneva, Switzerland: WHO press, World Health Organization.

Sack R.B, Siddique A.K, Longin I.M, Nizam A.J, Yunus M, Islam M.S. (2003). A 4-year study of the epidemiology of *Vibrio cholerae* in four rural areas of Bangladesh. *Journal of Infectious Diseases*, **187**(1): 96-101.

Samuel M.C, Vugia D.J, Shallow S, (2004). Epidemiology of sporadic *Campylobacter* infection in the United States and declining trend in incidence, FoodNet 1996-1999. *Journal of Clinical Infectious Diseases*, **38** (Supplemented) 3: S165-174.

Samuel S.O, Aboderin A.O, Akanbi II A.A, Adegboro B, Smith S.I, Coker A.O.(2006). *Campylobacter* enteritis in Ilorin, Nigeria. *East Africam Medical Journal*, **83**: 478-48.

Sandberg M, Nygard K, Meldal H, Valle P, Kruse H, Skjerve E. (2011). Incidence trend and risk factors for *Campylobacter* infections in humans in Norway. BMC Public Health **6**,179. Doi:10.1186/1471-2458-6-179.

Sang W. K, Boga H.I, Peter G. Waiyaki P.G, David S, Njeri C.W. Kariuki S.M (2012). Prevalence and genetic characteristics of Shigatoxigenic Escherichia coli from

patients with diarrhoea in Maasailand, Kenya. *Journal of Infection on Developing Countries*, **6**(2): 102-108.

Saumya Bhaduri and Bryan Cottrell (2004). Survival of Cold-Stressed *Campylobacter jejuni* on Ground Chicken and Chicken Skin during Frozen Storage. *Journal of Applied and Environmental Microbiology*, **70**(12): 7103–7109.

Schmid-Hempel P, Frank S.A (2007). Pathogenesis, Virulence, and Infective Dose. PLoS Pathogen **3**(10): e147. doi:10.1371

Serichantalergs O, Dalsgaard O, Bodhidatta L, Krasaesub S, Pitarangsi C, Srijan A, Mason C.J.(2007). Emerging fluoroquinolone and macrolide resistance of *Campylobacter jejuni* and *Campylobacter coli* isolates and their serotypes in Thai children from 1991 to 2000. *Journal of Epidemiology and Infection*, **135**: 1299-1306.

Sharma H, Unicomb L, Forbes W. (2003). Antibiotic resistance in *Campylobacter jejuni* isolated from humans in the Hunter Region, New South Wales . Commun Dis Intell. **27**: S80–S88.

Soofi S.B, Habib M.A, von Seidlein L. (2011). A comparison of disease caused by *Shigella* and *Campylobacter species*: 24 months community based surveillance in 4 slums of Karachi, Pakistan. *Journal of Infectious Public Health*, **4**(1): 2-21.

Sorokin M, Usein C.R, Irimia M, Damian M. (2007). A laboratory-based survey of *Campylobacter* infections in Prahov Yuki N. *Campylobacter* stransferase gene polymorphism directs clinical features of Guillain-Barré syndrome. *Journal of Neurochemicals*, **103**, (Supplemented) 1: 150-158.

Swai E, Schoonman L. (2011). Microbial quality and associated health risks of raw milk marketed in the Tanga region of Tanzania. *Asian Pacific Journal of Tropical Biomedicine*. **1**(3): 217-222. doi:10.1016/S2221-1691(11)60030-0.

Tam C.C. (2001) *Campylobacter* reporting at its peak year of 1998: don't count your chickens yet, *Journal of Community Public Health*, **4**(3): 94-199.

Tang J.Y, Nishibuchi M, Nakaguchi Y, Ghazali F.M, Saleha A.A, Son R. (2011). Transfer of *Campylobacter jejuni* from raw to coocked contaminated through fecal contamination during milking and before milk is pasteurized.,*Letters in Applied Microbiology*, **52**: 581-588.

Tasnim F, Zubia B, Fakhar-un-Nisa Y. (2012) Isolation and biochemical identification of Escherichia coli from wastewater effluents of food and beverage industry, *Journal of Cell and Molecular Biology*, **10**(1): 13-18.

Thompson F.L, Gevers D, Thompson C.C, Dawyndt, P,Naser S, Hoste B, Munn C.B, Swings J. (2005). "Phylogeny and Molecular Identification of Vibrios on the Basis of Multilocus Sequence Analysis".*Journal of Applied and Environmental Microbiology*, **71**(9): 5107–5115.

Thompson, Andrea (2007). "E. coli Thrives in Beach Sands". Live Science. Retrieved 2007-12-03.

Ugbogu O.C, Nwachukwu N.C, Ogbuagu U.N. (2006). Isolation of *Salmonella* and *Shigella* species from house flies (*Musca domestica* l.) in Uturu, Nigeria. *African Journal of Biotechnology*, **5**(11): 1090-1091.

Ugboma A.N, Salihu M.D, Magaji A.A, Abubakar M.B, (2013). Prevalence of *Campylobacter* species in ground water in Sokoto state, Nigeria, *Veterinary World Journal*, **6**(6): 285-287.

USA Kansas (2007). Campylobacteriosis, unpasteurized milk. International Society for Infectious Diseases.

Van Kessel J.A, Karns J.S, Lombard J.E, Kopral C.A. (2011). Prevalence of *Salmonella enterica*, *Listeria monocytogene*s, and *Escherichia coli* Virulence Factors in Bulk Tank Milk and In-Line Filters from U.S. Dairies, *Journal of Food Protection*, **74**(5): 759-768.

Vucic S, Kiernan M.C, Cornblath D.R. (2009) "Guillain-Barré syndrome: an update," *Journal of Clinical Neuroscience*, **16** (6): 733–741.

Wang S.C, Chang L.Y, Hsueh P.R. (2008). *Campylobacter* enteritis in children in northern Taiwan--a 7-year experience. *Journal of Microbioliology, Immunology and Infections*, **41(**5): 408-413.

WHO. (2007). Food safety and foodborne illness. Fact sheet No 237. World Health Organisation

Willison H.J, Yuki N. (2002). Peripheral neuropathies and anti-glycolipid antibodies. **125**: 2591-2625.

Wong T.L, Nicol C, Cook R, MacDiarmid S. (2007). *Salmonella* in uncooked retail meats in New Zealand, *Journal of Food Protection*, **70**(6): 1360-1365, ISSN 0362-028X.

World Health Organisation (2008). Guidelines for drinking water quality: incorporating 1st and 2nd addenda vol 1.

World Health Organization (2002). The increasing incidence of human campylobacteriosis. Report and proceedings of a W.H.O. consultation of experts, Copenhagen, Denmark, 21-25 November 2000.

World Health Organization (2002a) Water, Sanitation and Health Report. Water and Sanitation Programme. WHO/WSH/WWD/TA.8

World Health Organization (2002b) World Health Report; Reducing Risks, Promoting HealthyLife.Geneva, Switzerland.

Yang J.R, Wu H.S, Chiang C.S, Mu J.J. (2008). Pediatric campylobacteriosis in northern Taiwan from 2003 to 2005. *Journal of BioMedaicCentral Infectious Diseases*, **8**: 151.

Zilbauer M, Dorrell N, Wren B.W, Bajaj-Elliott M. (2008). *Campylobacter jejuni*-mediated disease pathogenesis: an update. *Transactions of Royal Society of Tropical Medicine and Hygiene*, **102(**2): 123-129.

Ziprin R.L, Young C.R, Byrd J.A, Stanker L.H, Hume M.E, Gray S.A, Kim B.J, Konkel M.E. (2001**)**. Role of *Campylobacter jejuni* potential virulence genes in cecal colonization. *Journal of Avian Diseases*, **4**: 549–557.