THE PREVALENCE OF *CAMPYLOBACTER, SALMONELLA, SHIGELLA* AND *ESCHERICHIA COLI* SPECIES IN LIVE AND DRESSED POULTRY IN METROPOLITAN ACCRA

BY

BEN ARHIN SACKEY

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Philosophy in the Department of Nutrition and Food Science, University of Ghana.

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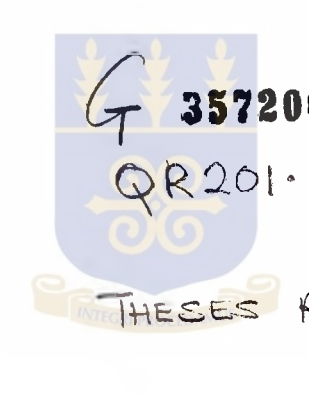
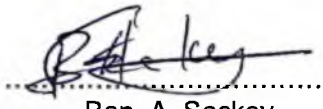


DECLARATION

I certify that this research was conducted by me under the supervision of Dr. Patience A. Akpedonu of the Noguchi Memorial Institute for Medical Research, University of Ghana, Legon.



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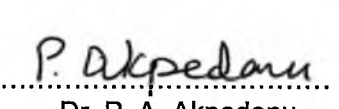

Dr. P. A. Akpedonu

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I dedicate this work to you lot and may God bless you all.

ABSTRACT

Qualitative studies on enteropathogenic bacteria in chicken on the Ghanaian market, using Accra Metropolitan Area, as a unit case, was assessed. A total of 184 samples, made up of 97 cloacal swabs from 3 different farms and 87 surface rinses of carcasses from different retail outlets were examined for the presence of *Campylobacter sp., Salmonella sp., Shigella sp.*, and *Escherichia coli.*

Out of the 97 cloacal swabs, 14 (14.4%) were positive for *Campylobacter*, 7 (7.2%) for *Salmonella*, 16 (16.5%) for *E. coli* and none for *Shigella*. For the 87 carcasses, none was positive for *Campylobacter*, 6 (6.8%) positive for *Salmonella*, 6 (6.8%) positive for *Shigella* and 5 (5.7%) positive for *E. coli*.

Four different *Campylobacter* serovars were isolated. These are *C. jejuni 1* (42.8%), *C. upsaliensis* (28.5%), *C. jejuni var doylei* (21.5%) and *C. jejuni 2* (7.2%). Four different serovars of *Salmonella* were also identified; they are Serovar 09 (28.4%), 021 (28.4%), 08 (15.3%) and 018 (7.6%). Two different *Shigella* serovars namely *Shigella boydii* (57.1%) and *Shigella flexneri* (42.9) were isolated. Twelve different serovars of *E. coli* were identified. These were *E. coli* 0158 (14.3%), 0125 (14.3%), 025 (9.5%), 028ac (9.5%), 0159 (9.5%), 015 (9.5%), 0126 (9.5%), 063 (4.8%), 0143 (4.8%), 026 (4.8%), 078 (4.8%), 0164 (4.8%). None of the *E. coli* strains isolated tested positive for ST-h, ST-p, and LT3 toxin production using DNA - DNA hybridization technique.

Antibiogram tests showed that fosfomycin, minocyclin, nalidixic acid, chloramphenicol, penicillin, amoxycillin, erythromycin and kanamycin were effective against the *Campylobacter* strains isolated. The strains of *Salmonella* showed varied reactions to the different antibiotics with fosfomycin being the most effective. Fosfomycin and chloramphenicol were effective against the *Shigella* strains. Nalidixic acid, chloramphenicol, kanamycin, ceftrioxone, amoxycillin and fosfomycin showed varied effectiveness against the *E. coli* strains. Tetracycline was ineffective against all the microbial strains isolated from the live birds. This could be attributed to its presence in the feed thereby exposing the birds constantly to it during feeding. This possibly may have resulted in the survival of only resistant strains.

Findings showed that chicken available on the market could be a source of bacterial enteropathogens. Also, there were no significant differences in the prevalence of the enteric pathogens isolated from the various retail outlets. Regular monitoring and proper handling practices are therefore needed to avert major outbreaks of food borne illnesses due to chicken and its products. The resistance of strains to some common antibiotics, such as tetracycline, is also disturbing. The withdrawal of such antibiotics, as constituents of poultry diets, is recommended.

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I.O INTRODUCTION

1.1 BACKGROUND

Food-borne disease, that is illness caused by consuming contaminated food, is a widespread problem in every part of the world. Food contaminated with biological agents, for example, microorganisms are the major cause of food borne diseases. Other causes may be chemicals such as pesticide residues.

Recent developments in food production and processing techniques and the subsequent changing trends in food consumption have resulted in the emergence of new hazards. The increased contact among different nations has stimulated rapid global distribution of foods as well as food-borne pathogens. The number of individuals at risk due to these hazards may rise due to increase in life expectancy.

Data recorded in different countries have shown that the incidence of some of these food-borne diseases have increased dramatically over the past few years, but because of under-reporting the data are of limited value and cannot be compared between countries (Notermans and Hoogenboom-Verdegaal, 1992).

The number of episodes of disease caused by bacterial contamination of food has increased by about threefold in the United Kingdom (Lacey, 1993).

The numbers for 1992 were estimated to be 2 million and the causes were multi-factorial and complex. Four representative pathogens were reviewed: *Salmonella enteritidis, Listeria, Escherichia coli* and *Campylobacter.*

Bacillus, Yersinia, Campylobacter, Listeria, Sporothrix, Giardia, Anisakis and *Cryptosporidium* are the foci of new investigations as are the more familiar food-borne pathogens such as *Salmonella, Shigella, Clostridium, Staphylococcus, Entamoeba* and *Ascaris.*

Most of the above enteropathogens have been isolated in Ghanaian children and adults. Free range chickens, food and water have been shown to be some of the sources of infection (Abrahams *et al.*, 1985, Agbodaze *et al.,* 1990, Akpedonu, 1997).

The trade liberalization program of Ghana has resulted in an influx of foreign products on the Ghanaian market. These include imported chicken, which due to its relative cheaper price is enjoying a lot of patronage from the general public.

There appears not to be adequate microbiological monitoring of these foods, given the number of microbiology laboratories in the country and the facilities available to them. Chicken produced locally using modern methods, also lack microbiological monitoring. This study therefore seeks to assess the bacterial quality of chicken from some selected farms, wholesale and retail

outlets in the Accra Metropolitan Area. The following organisms known to be food borne pathogens were tested for: *Campylobacter, Salmonella, Shigella and Escherichia coli*. It is expected to provide insight into the health hazards associated with the consumption of chicken and the findings will be applied in the formulation of effective control strategies.

1.2 MAIN OBJECTIVE

The main objective of this study is to assess the role chicken, sold on the Ghanaian market, can play in the transmission of enteropathogenic bacteria namely: *Campylobacter, Salmonella, Shigella* and *Escherichia coli,* using the Accra Metropolitan Area as a unit case.

1.2.1 Specific objectives

The specific objectives include:

(i) to determine the prevalence of *Campylobacter, Salmonella, Shigella* and *Escherichia coli* species in chicken,

(ii) to assess the antibiotic sensitivity patterns of the serovars identified,

(iii) to determine the virulence mechanisms of pathogenic *E. coli* by DNA-DNA hybridization, and

(iv) to assess the microbial safety of chicken from farms, supermarkets and markets in Accra.

2.0 LITERATURE REVIEW

2.1 ETIOLOGY OF FOOD-BORNE DISEASE

Food may serve as a vehicle for the transmission of agents that could cause diseases. The agents may be intrinsic, including allergens and toxic compounds that occur naturally in food. It may also be extrinsic and these include microorganisms and toxic compounds, which may be present as contaminants. As have been stated earlier, microorganisms are a major cause and a number of bacteria **(Table 1)**, viruses and parasites have been shown to be etiological agents of food-borne disease.

Perry (1994) reported that the incidence of food-borne diseases in the United Kingdom has increased substantially, with about 50% arising in the home. Organisms implicated in the report included *Salmonella, Campylobacter* and *Shigella*.

Swaddiwudhipong *et al.* (1990) reported on an epidemiological study performed to identify the source of infection and the mode of transmission of an outbreak of cholera in a village in Thailand during October 1987. The study showed the source of transmission to be an infected butcher who had acquired cholera from eating uncooked pork in a village where cholera epidemic had occurred.

Table 1: Bacteria causes and clinical features of classical food borne diseases (Varnam and Evans, 1991).

Organism	Clinical features
Salmonella	Diarrhea, vomiting, fever and malaise, usually 12 to 16 hours after ingestion
Shigella	Diarrhea with mucous, bloody stools, 12 to 50 hours after ingestion
Campylobacter	Prodomal fever and malaise, 2 to 11 days after Sp. Ingestion followed by abdominal pains
E. coli	Diarrhea, meningitis, septicemia, arteriosclerosis wound infections, hemolytic uremic syndrome and immunological diseases such as reactive and rheumatoid arthritis.
Yersinia	Abdominal pain, fever, headache, diarrhea enterocolitica *and* possibly vomiting and malaise 24 to 48 hours after ingestion.
Vibrio parahaemolyticus	Profuse diarrhea which leads to dehydration, Vomiting and fever, 2-48 hours after ingestion.
Aeromonas	Diarrhea in normal patients, disseminating infections in the immunocompromised
Pleisiomonas shigelloides	Predominantly diarrhea accompanied possibly by abdominal pain, nausea and vomiting. Symptoms appear within 48 hours of ingestion.
Staphylococcus	Vomiting, abdominal pain and diarrhea on some occasions, 2-6 hours after ingestion. Severe dehydration may result in collapse
Clostridium botulinum	Fatigue lassitude, dizziness and effects on the central nervous system including speech difficulties and visual disturbances. Onset is 24-72 hours after ingestion
Clostridum perfringes	Abdominal pain, nausea and vomiting (occasional diarrhea), 8-22 hours after ingestion.
Listeria monocytogens	Meningitis in neonates, abortion in pregnant females, septicemia. Usually extended between ingestion and appearance of symptoms.

Food-borne outbreaks are reported regularly from correctional facilities in the United States. In a study by Cieslak and Curtis (1996), eighty-eight food-borne outbreaks involving 14,307 cases of illness were reported from 31 states and territories. *Salmonella* species accounted for 15 (37%) of 41 outbreaks of known cause from 1974 to 1991, *Clostridium perferingens* for 14 (34%), and *Staphylococcus aureus* for 9 (22%).

Morton (1993) reported that gastrointestinal disease, traced to bacterial contamination of foods, have recently increased in number and scope. *Escherichia coli*, a variety of *Salmonella* species and other bacterial pathogens have been isolated. Food trace-backs have implicated a variety of uncooked, cooked and under-cooked food sources, including cantaloupes, apple cider, ground beef, cow's milk, poultry products and even fresh, shell-intact table eggs.

Layton *et al.* (1997) investigated a mixed *Salmonella heidelberg* and *Campylobacter jejuni* food-borne outbreak in a nursing home in Brooklyn, New York. Among the 42 pureed foods served during the five days before the outbreak, five meat or poultry items were associated most strongly with culture positivity. Of these five meat items, only a chopped-liver salad was implicated by the two employees reporting illness. A reported food-handling error had occurred when ground cooked chicken livers were placed in a bowl containing raw chicken-liver juices. It was recommended that proper cleaning

and sanitizing of kitchen equipment should be encouraged to prevent cross-contamination between raw and cooked foods.

Lee *et al.* (1996) undertook a study involving a comparative epidemiological observation on outbreaks of food-borne diseases between Korea and Japan during the period from 1971 to 1990. The study reported the following organisms; *Vibrio* sp. (37.6%), *Salmonella* sp. (23.1%), Staphylococcus sp. (14.9%), pathogenic *E. coli* (6.8%), *Clostridium* sp. (0.5%) and other species (17.1%) in Korea. In Japan, the majority of bacterial causes were *Vibrio* sp. (47.3%), *Staphylococcus* sp. (24.6%), *Salmonell*a sp. (14.8%), *E. c*oli (3.5%), *Clostridium* sp. (0.2%) and other species (9.6%).

Mahon *et al.* (1997) reported an outbreak of *Salmonella stanley* infections in the United States and Finland in 1995. This outbreak was investigated through case-control studies in Arizona, Michigan, and Finland; by isolate subtyping and by tracing and culturing of the implicated food. Alfalfa sprout consumption was the only exposure associated with *S. stanley* infections in Arizona and Finland. Alfalfa sprouts eaten by patients in six (6) US States and Finland were traced to seeds shipped by a Dutch agent. Thus, it was concluded that alfalfa sprouts grown from contaminated seeds caused an international outbreak of *S. stanley* infections in the United States and Finland. This outbreak illustrated a new mechanism through which contamination of fresh produce can cause large, widely dispersed outbreaks.

From March to October 1993, 15 cases of haemolytic-uraemic syndrome (HUS) in children were detected in a large area of Northern Italy, where only 8 cases had occurred in the previous 5 years (Tozzi *et al.*, 1994). Analysis of stool and serum specimen obtained from 14 cases showed evidence of Verotoxin-producing *E. coli* (VTEC) infection in 13. A case control study showed an association between HUS and contact with chicken coops. It was therefore concluded that risk factors for VTEC infection related to living in rural settlements include exposure to live poultry and this should be considered in outbreak investigation.

Traveller's diarrhea may be caused by food or water intake. Black (1990) stated that enterotoxigenic *E.coli* was the most frequently identified pathogen in traveler's diarrhea episodes. Studies made have shown it to be in the median of 42% in Latin America, 36% in Africa, and 16% in Asia. Other pathogens that cause diarrhea in smaller fractions of ill travelers included *Shigella, Salmonella* species, *Campylobacter jejuni, Vibrio, Aeromonas hydrophila, Entamoeba histolytica, Giardia lamblia,* rotavirus, *Plesiomonas shigelloides* and enteroadherrent *E. coli.*

Kirk *et al.* (1997) reported a prolonged outbreak of *Campylobacter* occurring over a three-month period at a training facility. An investigation of one sporting group showed that the illness was associated with consumption of

cucumber served as a self-serve salad bar. Transmission of *Campylobacter* ceased after changes were instituted to food preparation and storage in the facility kitchen.

Campylobacter, Salmonella, Escherichia coli and *Shigella* appears to be of prime importance with respect to food borne diseases and for the benefit of this study these bacteria will be discussed further.

2.1.1 Bacterial etiological agents

Campylobacter

The genus *Campylobacter* comprises of slender, spiral, gram-negative rod-shaped bacteria, which have a characteristic "corkscrew-like" motility produced by a single polar flagellum at one or both ends of the cell (Smibert, 1984). They are microaerophillic organism with a respiratory metabolism. They are also capnophillic, meaning the organism grows best under conditions of reduced atmospheric concentrations of oxygen and under high carbon dioxide concentration. The optimum atmospheric composition for growth is 5% oxygen, 10% carbon dioxide, and 85% nitrogen (Bolton and Coates, 1983). Oxygen is required for growth at concentrations of 3 to 15%. In some cases, growth occurs in aerobic conditions of 20% oxygen. Campylobacters grow at between 25^{o}C and 43^{o}C and do not ferment or oxidize carbohydrates.

Salmonella / Shigella

The genus *Salmonella* consist of Gram-negative, oxidase-negative, straight-sided, rod-shaped bacteria which are catalase-positive. They have both a respiratory and fermentative metabolism of carbohydrates. Most are motile using peritrichous flagella.

Shigella is a Gram-negative, catalase-positive and rod-shaped bacterium. They do not usually produce gas during fermentation of carbohydrates and are non-motile. Currently, the genus Shigella consists of four species: *Sh. dysenteriae* (subgroup A), *Sh. flexneri* (subgroup B), *Sh. boydii* (subgroup C) and *Sh. sonnei* (subgroup D). Separate species status has been proposed for *Sh. boydii* 13 (Varnam and Evans, 1991).

Escherichia coli

Escherichia is a member of the gram-negative *Enterobacteriaceae* and are able to ferment lactose with production of acid and gas. It is the most common aerobic organism in the gastrointestinal tract of man and many other animals and was until the late 1950s recognized as non-pathogenic normal cohabitant. In this connection, the organism has a traditional, and often misunderstood, role in food and water microbiology as an index of fecal pollution. However certain strains may induce disease, and should therefore be regarded as a potential pathogenic organism.

Escherichia coli is the predominant species among the facultative anaerobe normal flora of the intestine. Five types of diarrhea producing *E. coli* are known:

i. Enteropathogenic (EPEC), Class I and II

ii. Enterotoxigenic (ETEC)

iii. Enteroinvasive (EIEC; *Shigella*-like *E.coli*)

iv. Enterohaemorrhagic (EHEC; Verocytoxin-producing *E.coli* (VTEC)

v. Enteroadherent-aggregative (EA-AggEc).

The pathogenic strains can cause syndromes such as diarrhea, meningitis, septicemia, arteriosclerosis wound infections, hemolytic uremic syndrome and immunological diseases such as reactive and rheumatoid arthritis.

The most prominent virulence factors of *E. coli* are heat-stable and heat labile enterotoxins (ETEC), Shiga-like (vero) toxin (EHEC), adherence to epithelial cells (EPEC) and invasiveness (EIEC) (Levine, 1987). **Table 2** gives a summary of diarrheagenic strains of *E. coli.*

Fehlaber and Heseler (1989) reported that any assessment of *E.coli* in various types of foodstuffs together with an appraisal of its relevance to food hygiene would be of inadequate reliability unless the virulence properties of the *E. coli* are investigated as well.

Table 2: Summary of diarrheagenic strains of *E. coli* (Varnam and Evans, 1991)

Category	Serogroups	Virulence factors
Enteropathogenic		
Class I	055, 086, 0111, 0119, 0125, 0126, 0127, 0128ab, 0142	Shiga toxin production. Fimbrial or outer membrane protein adhesion in some strains.
Class II	018, 044, 0112, 0114	
Enterotoxigenic	06, 08, 015, 020, 025, 027, 063, 078, 080, 085, 0115, 0128ac, 0139, 0148, 0153, 0159, 0167.	Heat-labile and heat-stable toxins. Adhesive factors.
Enteroinvasive	028ac, 029, 0124, 0136, 0143, 0144, 0152, 0164, 0167	Epithelial invasion
Enterohaemorrhagic	026, 0157, 0111	High levels of Shiga toxin. Fimbrial adhesion
Enteroadherent-aggregative	Not defined	Epithelial adherence. Toxin Production

Several studies have implicated the toxin-producing strains as causes of food borne diseases. For example, heat stable enterotoxin (ST) produced by *E. coli* has been known to be a common cause of mild diarrhoeal illness in children in Italy (Guarino *et al.* 1989) whilst Fehlaber and Heseler (1989) also indicated that enterotoxin - producing *Escherichia coli* may be a cause of food borne diseases. Smith *et al.* (1991b) reported that Vero cytotoxin – producing *E. coli* (VTEC) are now recognized as important etiological agents in human disease.

The application of nucleic acid analyses to investigations of infectious disease outbreaks has resulted in useful molecular strain markers that distinguish the epidemic clone of a particular pathogen and help identify specific vehicles of infection. Plasmid profile analysis, restriction endonuclease digestion of plasmid and whole-cell DNAs, and nucleic acid hybridization have been sucessfully used to investigate outbreaks of food borne diarrheal diseases as reported by Wachsmuth *et al.* (1991). They indicated that specific DNA probes derived from cloned virulence genes of *E. coli* have been invaluable in epidemic investigation of surveys and that there is a continuing and expanding role of molecular approaches in epidemiological investigation. Smith *et al.* (1991b) reported that the use of DNA probes allowed for several hundred colonies from a sample to be examined.

DNA probes have been used to detect virulence factors in *E. coli* in several studies. Samadpour *et al.* (1990) evaluated the use of DNA probe for shiga-like toxin (SLT- I and SLT- II) for detecting SLT producing *E. coli* (SLTEC) in foods and calf fecal. Two methods were used; the colony hybridization and the dot blot technique. Of the 44 food samples and 28 fecal samples from dairy calves tested, a total of 4 food samples and 9 calf fecal samples were positive with the SLT probes. It was shown that the dot blot technique yielded results within 48 hours and could be used as a fast and sensitive method of detecting for SLTEC in foods and calf fecal samples. The colony hybridization technique however took 3-4 days but permitted recovery of the positive colonies when desired.

Samples from chickens and pork sausages were examined for the presence of Vero cytotoxin producing *E. coli* by using DNA probes for Vero cytotoxin genes (Smith *et al.*, 1991a). Hybridization was detected in 25% of the 184 sausage samples, but none of the chickens was positive.

Suthienkal *et al.* (1990), used DNA probes to identify Shiga-like toxin (SLT-I and SLT-II) producing *E. coli* in vegetables, meats, cattle and farm animals in Thailand. SLT – producing E. coli were isolated from 9% of market beef samples, from 8-28% of fresh beef samples obtained from slaughter houses and 11-84% of fecal specimen from cattle. Of the 119 SLT – producing *E. coli* isolates, 24% hybridized with SLT – I probe, 31% hybridized with the

SLT- II probe and 44% hybridized with both SLT probes. They concluded that the high prevalence of SLT – producing *E. coli* in beef producing animals suggested that exposure to animals and eating beef may pose a health risk for acquiring enterohemorrhagic *E. coli* infection in Thailand.

Notermans *et al.* (1991) used DNA-hybridization and latex – agglutination tests for screening a group of *E. coli* isolates for heat-labile enterotoxin (LT) and Shiga-like toxin (SLT- I) producing strains respectively. Strains tested originated from 162 meat samples (poultry, pigs and beef) chosen at random. The DNA hybridization technique allowed screening of large numbers of strains, whereas large scale testing of strains by latex agglutination was laborious. Of the 800 *E. coli* strains tested by DNA hybridization, none carried the gene encoding LT and only 10 carried the gene encoding SLT – I. The latex agglutination method used did not also detect any LT strain. The results obtained indicated that routine screening of *E.coli* isolated randomly from food for toxin production is not useful and should be limited to food borne disease outbreaks with an etiology resembling an *E. coli* infection.

2.1.2 Mechanism of pathogenicity

In recent years, there has been a considerable advancement in the understanding of the mechanisms of microbial pathogenicity, although in most cases understanding is far from complete. It has also become

appreciated that the pathogenicity, or non-pathogencity of an organism, cannot be viewed in isolation (Archer and Young, 1988), and a holistic approach must be taken to gain a full appreciation of the host/parasite relationship.

Ganguly and Kaur (1996) stated that besides cholera toxin (CT) other bacteria toxins like *E. coli* LT, *Salmonella* toxin, *Shigella* toxin and *Campylobacter* toxin possess an A-B structure. These toxins are composed of 1A and 5B subunits. Their action involves the role of various biochemical pathways. They act by activating the adenylate cyclase-cyclic adenosine monophosphate (cAMP) system located at the basolateral membrane of intestinal epithelial cells. The increase in cAMP levels is mainly responsible for the altered transport of Na+ and Cl-. They are also known to act through the release of prostaglandin and involvement of intramural nerves. Most of the bacterial toxins exert their effect through the involvement of ADP-ribosylating proteins whereas other toxins involve the guanylate cyclase system, calcium and protein kinases for their ultimate action.

Motility and adherence have also been reported as possible virulence factors, for example in *Campylobacter* (Lee *et al.*, 1986)

2.2 FACTORS AFFECTING THE SURVIVAL OF MICRO-ORGANISMS

Many publications have attempted to define the parameters, which permit growth and, where appropriate, toxin production by pathogenic microorganisms in foods. Parameters which have been investigated include temperature, pH value, water activity (a_W) level, redox potential, as well as the presence of various inhibitors/curing agents such as Sodium Chloride (NaCl) and nitrite.

Temperature

A study by Skirrow and Benjamin (1982) showed that temperatures used in pasteurization and cooking readily destroy *Campylobacter.* The study showed that *D* values ranged from 7.3 minutes at 50^{o}C to 1.1 minutes at 55^{o}C and *z* values from 2.8 - 5.3^{o}C with a mean of 3.7^{o}C.

Campylobacter has been shown to survive poorly during storage at room temperature (Varnam and Evans, 1991). However many factors may interact to determine the actual period of survival. Lowering the storage temperature enhances survival and in most cases this is greatest at 3^{o}C. Survival of the bacterium in frozen foods varies considerably and the physiological condition of the organism at the time of freezing may be an important determinant. Freezing cannot be relied upon to eliminate the organism even during prolonged storage.

As a generalization, salmonellas grow over the range 5°C to 47°C with an optimum of 37°C (Simonsen *et al.*, 1987). Ability to grow at temperatures below 7°C is serovar and strain dependent. For example the lowest growth temperature for strains of *S. bredeney, S. typhimurium* and *S. virchow* are at 5 to 6°C and *S. agona. S. senftenberg* and *S. hadar* are able to grow above 6°C (Alcock, 1985). Few strains can actually grow at temperatures below 10°C, but the ability to grow at relatively low temperatures may be significant where refrigeration is poor and shelf life is prolonged.

All species of *Shigella* have an optimum for growth at 37°C, but there is species variation in maximum and minimum growth temperatures. *Sh. sonnei* will grow over the range 7 to 46°C, and *Sh. flexneri* over the range 10 to 44°C (Fehlhaber, 1981). Heating readily destroys shigellas with most strains being killed by heating at 63°C for 5 minutes.

The heat resistance of *E. coli* is of the same order as for most serovars of *Salmonella*, and D values are typically 5 minutes at 55°C and 0.1 minute at 60°C. Resistance varies according to the nature of the heating medium, and there is strain variation. There is no evidence whatsoever that any strain of *E. coli* is sufficiently heat resistant to survive correctly applied pasteurization or cooking. *E. coli* is capable of prolonged survival in frozen foods. For

example, serovar O157: H7 survives for up to 9 months at -20^{o}C in ground beef (Doyle and Schoeni, 1987).

pH value

Survival of strains of bacterial enteric pathogens was investigated in two traditional fermented foods (mahewu and sour porridge) and in unfermented porridge (Simango and Rukure, 1992). The foods were inoculated with cell suspensions of *Salmonella, Shigella, Campylobacter, Aeromonas* species and pathogenic *E. coli* at a final concentration of 10^6 -10^7 cfu/ml of food. The results suggested that traditional fermented foods have bacteriostatic and bactericidal properties and may be unlikely to play a major role in the transmission of bacterial enteric pathogens.

Campylobacter is generally recognized, as being sensitive to low pH values. Optimal survival is known to be within the range 6.5 to 7.5. The organism is particularly sensitive to lactic acid as well as other organic acids, including acetic acid and ascorbic and iso-ascorbic acids. Sensitivity is attributed to oxidation products rather than to the acids *per se* (Juven *et al.*, 1988).

The behavior of *Salmonella enteritidis* and *S. typhimurium* in control gruel and fermenting siljo was evaluated (Dessie *et al.*, 1996). Acid production by fermenting lactic acid bacteria reduced the pH to less than 5.0 and this

resulted in complete inhibition of the organisms.

The use of "generally recognized as safe" (GRAS) chemicals to kill salmonellas in poultry carcasses has been investigated with particular attention being paid to succinic acid, which has been found to be highly effective against this organism (Mountney and O'Malley, 1965). Thompson *et al* (1976) were able to eliminate salmonellas from chicken carcasses using a hot dip in a solution containing 1% succinic acid and 50 mg/l chlorine. Application of this was however limited by skin discoloration. Formic acid treatment of poultry feed has been suggested as an alternative to heat treatment for elimination of salmonellas (Humphrey and Lanning, 1988).

Shigellas have been demonstrated to grow over the pH range 5.5 to 7.0 (Hentges, 1967). In the presence of formic or acetic acids growth is prevented below pH 6.0. Survival of shigellas in low pH value environments is poor, both *Sh. flexneri* and *Sh. sonnei* being inactivated within 30 minutes at pH 3.5 and within 4 hours at pH 4.0 and 4.5.

The bactericidal and bacteriostatic effect of yogurt on three strains of *E. coli* was investigated (Kotz *et al.*, 1990). It was concluded from the study that both live yogurt bacteria and a pH near 4.5 were necessary for the bactericidal activity of yogurt. There is the possibility that ingestion of yogurt could have a protective effect against infection by enteric pathogens.

The addition of a starter culture to ground pork made into nham (Thai-style fermented pork sausage) was able to decrease the number of inoculated *Staphylococcus aureus* and *E. coli* (Petchsing and Woodburn, 1990). This study recommended the use of starter culture in the preparation of nham.

E. coli is capable of growth over the pH range 4.4 to 9.0 (ICMSF, 1980). However interaction with other factors like temperature, water activity and the nature of the acidulant may affect the pH.

Curing Agents

There are variations among different species of *Shigella* with respect to nitrite sensitivity. *Sh. sonnei* was reported to be inhibited by 700 mg/L and *Sh. flexneri* by 450 mg/L at pH 5.5 (Nakamura *et al.*, 1964).

Gibson and Roberts (1986) studied the effect of combinations of curing ingredients on a cocktail of "enteropathogenic" strains of *E. coli*. The upper NaCl concentration for growth was 6%, but growth in this concentration occurred only at pH values between 5.6 and 6.8 and at temperatures between 15 and 35^{o}C. At pH 5.6, growth at 10 to 15^{o}C was inhibited by 200 mg/L $NaNO_2$ and at 20^{o}C by 400 mg/L $NaNO_2$. Growth occurred in any combination of 0 to 4% NaCl and 0 to 400 mg/L $NaNO_2$ at pH values between 6.2 and 6.8 and at temperatures between 15 and 35^{o}C. The study

showed how pH and temperature may affect the effectiveness of NaCl as a curing agent.

Irradiation

Irradiation has been proposed as a means of controlling salmonellas in poultry, shellfish and frogs' legs. In Canada a dose of 3 Kgy is recommended for poultry at "on ice" temperature (Ouwerkerk, 1981) and a similar dose is used in the Netherlands for treatment of shellfish (Ley, 1983).

Serrano *et al.* (1997) determined the irradiation sensitivity of five *Salmonella enteritidis* isolates inoculated on the surface and inside of whole shell eggs. Irradiation dose of 1.5 kGy was enough to reduce *Salmonella* counts by 4 $\log_{10}$. The color and thermal characteristics remained unaffected. *Campylobacter* is less resistant to ionizing radiation than *Salmonella*, and is eliminated by a dose of 1 Kgy (Varnam and Evans, 1991). Irradiation has been proposed as a means of controlling *Shigella* in foods imported from areas where the organism is endemic (Kayser and Mossel, 1984).

Undercooked and raw meat has been linked to outbreaks of hemorrhagic diarrhea due to the presence of *E. coli* 0157:H7 and treatment with ionization radiation was investigated as a potential method in its elimination (Thayer and Boyd, 1993). It was shown from the study that irradiation was an

effective method in controlling this food-borne pathogen. The study also showed that combining irradiation with heat treatment (thermoradiation) could increase the efficiency of irradiation. The sensitivity of *E. coli* to irradiation is similar to that of *Salmonella*, and a dose of 3 Kgy proposed for control of that organism would be effective against *E. coli* (Varnam and Evans, 1991).

Water Activity (a_W)

Adequate drying as well as washing is of considerable importance in preventing the transfer of *Campylobacter* on the hands of food handlers. Drying is also important following the cleaning of equipment and utensils. The lower limit of a_W for growth is 0.93. Higher limiting values will apply when other inhibitory factors are present. The minimum a_W level for growth where other conditions are optimal is 0.95 (Coates *et al.*, 1987).

Salmonellas are destroyed in high a_W foods by heating at a minimum temperature of 74^{o}C (Simonsen *et al.*, 1987). This usually permits a margin of safety; D_{60} values for most serovars are in the range of 0.2 to 6.5 minutes, while *z* values are commonly 4 to 5 minutes. *S. senftenberg* is considerably more resistant than other serovars, but this is probably of limited consequence since the organism appears to be of low pathogenicity to man (Roberts, 1982).

Redox Potential

The survival of *Campylobacter* is greater in vacuum, or controlled atmosphere packs than in air (Blankenship and Craven, 1982, Hanninen *et al.*, 1984). A contributory factor may be the maintenance of moist conditions on the surface of the food in vacuum and controlled atmosphere packs.

The influence of modified-atmosphere packaging, storage temperature, and time of survival and growth of *E. coli* 0157:H7 inoculated onto shredded lettuce, sliced cucumber, and shredded carrot were determined by Abdul-Raouf *et al.* (1993). Packaging under an atmosphere containing 3% oxygen and 97% nitrogen had no apparent effect on *E. coli* populations, psychotrophs or mesophiles. The study indicated the ability of the organism to grow on raw salad vegetables, which are subjected to processing and storage conditions similar to those routinely used in commercial practice.

Competition with other micro-organisms

Survival of *Campylobacter* is reduced by the presence of other microorganisms. In some cases, this may be attributed to a reduction of the pH value and the production of organic acids (Varnam and Evans, 1991). The competitive ability of *Salmonella* varies according to strain. Many compete poorly with the lactic acid bacteria. This is of importance in food fermentation and may be due to a specific inhibitory mechanism as well as to acidification (Ashenafi and Busse, 1989).

E. coli is a more effective competitor with spoilage microorganisms than *Salmonella*. In food fermentation it may reach significant numbers before competition from lactic acid bacteria becomes significant. However, at low temperatures *E. coli* is likely to be overgrown in psychrotrophic spoilage organisms such as *Pseudomonas*, even if capable of growing below 5°C in the absence of competition (Varnam and Evans, 1991).

2.3 SOURCES OF TRANSMISSION AND LOCATION OF OUTBREAKS

Pets, water and contaminated foods are the main sources of transmission of enteropathogenic bacterial in humans and no single animal food source can be excluded as a potential vehicle for infection of humans. Most reports have also implicated food handlers as sources of infection.

Food

A number of studies on the microbial quality of foods in Ghana have been conducted. Raw and fried fish from the Korle Lagoon yielded high fecal coliforms, *Shigella* and *Salmonella* sp. (Nketia-Tabiri, 1976). Amoah *et al.* (1990) isolated *E. coli* and *Pleisiomonas shigelloides* from fresh water prawns. Consumption of such foods without effective heating could result in transmission of these pathogens. Hayford (1977) studied chicken pieces and isolated *E. coli* and *Shigella flexneri*. Studies carried out by Sackey in 1990 showed high numbers of fecal coliforms in "waakye" and "fufu".

Mensah *et al.* (1995) evaluated the factors associated with the transmission of diarrhea pathogens by conducting a study on the microbial quality of weaning foods consumed by pre-school children in a peri-urban community near Accra, Ghana. Food samples were collected from 65 of the children receiving supplements for microbiological analysis. *Bacillus cereus* were detected from 49 (75%) while 43 (66%) of the samples had *Staphylococcus aureus*. Fecal coliforms were detected in 48 (73%) of these samples. Enteropathogenic and enterotoxigenic *E. coli* were also detected. Poor personal and food hygiene in an environment, which lacks organized sewage disposal, appear to contribute to high levels of contamination.

Franco *et al.* (1991) isolated *E. coli* strains from 96 food samples (32 milks, 4 dairy products, 36 raw meats, 7 meat products, 7 sandwiches and 10 ready-to-eat meals). Six colonies isolated from four food samples hybridized with the probes for LT-II (3 colonies isolated from milk sample), SLT-I and SLT-II (1 colony isolated from raw bovine meat) or EAF (2 colonies from the raw chicken meat samples).

A total of 40 New Zealand white rabbits, 20 freshly slaughtered rabbits from an experimental farm and 20 processed rabbit carcasses from grocery stores in Beni-Suef city, were examined bacteriologically (Khalfalla, 1993). The freshly slaughtered rabbits showed a low bacterial load compared with processed rabbit carcasses from grocery stores. This could be attributed to

excessive and poor handling practices. Organisms isolated include *Escherichia coli, Listeria monocyto*genes, *Salmonella typhimurium, Pseudomonas aeruginosa* and *Staphylococcus aureus.* The study showed the role rabbit carcasses could play in the transmission of microorganisms and measures were suggested on how to reduce the microbial load.

Offal, such as liver, kidney and heart, is more commonly contaminated with *Campylobacter.* There is clearly wide variation with such a variable product, but surveys have shown that 30 - 47% of such offal samples may be contaminated (Bolton *et al.*, 1985; Fricker and Park, 1989).

Poultry, especially chicken, is an important cause of *Campylobacter* infections. *Campylobacter jejuni* is the usual species isolated from poultry but a high incidence of *C. coli* has been reported from the Zagreb region of Yugoslavia (Popovic-Uroic, 1989) and from France (Marinescu *et al.*, 1987). In cases where a direct food link is known, the poultry have usually been under-cooked. Cross-contamination from raw poultry to foods which are not cooked before eating, however, probably plays a large, if not a major role in sporadic cases of campylobacteriosis (Dawkins *et al.*, 1984; Coates *et al.*, 1987).

In an investigation by Pearson *et al.*, (1985), the route of transmission from chickens to salads and cooked meats via the hands of inexperienced chefs, which were contaminated during evisceration, was clearly demonstrated. Chickens from a single producer were also shown to be responsible for a large number of sporadic cases throughout Southern England.

Thermophilic campylobacters and *Arcobacter butzleri* were looked for in 57 manually shelled egg samples and 130 raw meat samples, (Zanetti *et al.*,1996). A total of 21 strains were identified, one belonging to *Arcobacter butzleri* and 20 to *Campylobacter*. The most common species was *C. jejuni* (16/20) followed by *C. coli* (4/20) and in both species, biotype I was the most frequently found.

Most egg-associated infections with *Salmonella* have involved either consumption of raw eggs or of products made with raw egg, such as home-made mayonnaise, ice cream or other desserts (Humphrey *et al.*, 1989).

Information concerning the incidence of enterotoxigenic *E. coli* (ETEC) in foods is inconclusive. ETEC was isolated from up to 10% of meat products obtained from a supermarket in Sao Paulo, Brazil, but none of the isolates was of a serogroup associated with human diarrhea in the city (Reis , 1980). A survey of milk products in West Germany detected four ETEC strains among 77 isolates from Camembert cheese, and one ETEC strain among

four isolates from yogurt (Franke *et al.*, 1984). Eight percent of *E. coli* strains isolated from a variety of foods in the USA were found to be EPEC, but the most common serogroup was O149, which is associated with illness in piglets rather than in humans (Doyle and Padyhe, 1989).

There have been few publications on the incidence of EPEC in market foods. Abbar (1988) reported that four EPEC strains were found among 60 *E. coli* isolates from cheese in Iraq and that EPEC was also isolated from Egyptian butter.

The rate of contamination with coliforms and incidence of *Escherichia coli* in raw milk supplied by farmers to dairy co-operative societies for marketing was investigated (Ombui *et al.*, 1994). The results indicated that a good number of farmers draw milk under satisfactory conditions. Again the study showed that raw milk could get contaminated with enteropathogenic strains of *E. coli* that can pose a potential risk to humans, thus calling for extra care when preparing milk and milk products that are to be consumed by human beings.

Harris *et al.* (1997) conducted a study to test feed and feed ingredients on swine farms for *Salmonella* organisms. The *Salmonella* serotypes found in the feed and feed ingredients have the potential to cause disease in pigs that consume the feed or, ultimately, in people that consume pork.

Implementing sanitary control measures continues to be a prudent recommendation.

Pets and farm animals

The isolation rate of thermotolerant campylobacters in pet monkeys and wild monkeys was reported by Tresierra-Ayala and Fernadez (1997). In this study they showed that *Campylobacter* sp. were isolated in 31.9% of the first (pet) group, being less frequent (20.9%) in the second (wild). In both groups, only biovars I and II of *C. jejuni sp. jejuni* and both biovars of *C. coli* were isolated. The results showed that domestic and wild monkeys can harbor *Campylobacter* in their intestinal tract and could be considered as reservoirs of these bacteria.

The rectal swabs of diarrheic and apparently healthy non-diarrheic dogs presented to an Amall Animal Clinic, in Trinidad, were examined for *Escherichia coli, Salmonella* and Campylobacter (Adesiyun *et al.,* 1997). The study showed that dogs harbor pathogenic microorganisms and could be responsible for its subsequent transmission.

People whose occupation brings them into close contact with animals or animal products, such as farmers, veterinarians, slaughterhouse workers, poultry processors and butchers, are at increased risk of infection. It appears that many of them become immune through regular exposure (Blaser *et al.*,

1983). Members of the general public may become infected by contact with infected pets usually in the form of a puppy or kitten with *Campylobacter* diarrhea. Although these routes of transmission are well documented, they account for only a minority of the infections as indirect transmission is more important.

The isolation rates of thermotolerant *Campylobacter* species in free-ranging domestic chickens and confined chickens from Iquitos City, Peru were determined (Tresierra-Ayalla *et al.*, 1995). *Campylobacter* sp. was isolated in 54.0% of the former group of chickens, being less frequent (35.0%) in the latter. Of the classical thermotolerant species, *C. jejuni* and *C. coli* were the most frequent. The presence of these bacteria suggests that chickens might be an important reservoir of this bacterium.

A total of 134 samples of rectal and cloacal swabs taken from apparently healthy domestic animals that were in 43 of 76 homes located in rural Ghana were examined for *C. jejuni* (Agbodadze *et al.*, 1990). *C. jejuni* was isolated from 44 (32.8%) of the animals. The highest isolation rate of 43.6% was from local domestic fowls, followed by goats (33.3%) and sheep (23%). The organism was not detected in the pigs, cats and ducks. *C. jejuni* biotype 2 was the prevailing strain in the animals in the study area.

Grados *et al* (1988) studied the route of transmission of *Campylobacter jejuni* in Lima, Peru. The risk factors identified during the study suggested that direct contact with the feces of *C. jejuni* infected chickens in the household environment were largely responsible for transmission of the organism to susceptible infants.

Food handlers

Handling of foods rather than consumption may be a risk factor in community-wide outbreaks.

To evaluate the public health hazard caused by *Salmonella* carriers as food handlers, a study was carried out to gather more quantitative data about fecal contamination of hands after stools (de Wit and Romboutt, 1992). Fecal *E. coli* was used as a model bacterium for *Salmonella*. It appeared that hands might be contaminated with *E. coli*, regardless of toilet use. Thus the presence of Enterobacteriaceae on hands is not a good indicator for toilet hygiene. Hand washing after stools reduced the numbers of *Enterobacteriaceae* and *E. coli* on the hands. If normal hygiene such as washing hands is practiced after stools, the number of contaminated hands will be very low. Furthermore the number of fecal microorganisms will be very small and transmission would be reduced.

It should be appreciated that any food, which is not heated directly before consumption, handled by a *Shigella* infected person with poor personal hygiene, is a potential vehicle of food-borne shigellosis (Smith, 1987).

Water

Investigations conducted by Abrahams *et al.* (1985), on the portability of drinking water available to some rural and urban communities illustrated the role of water in the transmission of diarrhea pathogens in Ghana. The study showed that poor handling of pre-treated water supplies could result in the contamination of this water. They further stated that chlorinated pipe-borne water could also become contaminated if regular checks on pipes are not carried out thus allowing sewage to enter the distribution system through cracks in the system.

EIEC serogroup O124 has also been implicated as the cause of outbreaks of gastroenteritis involving soft cheese, with more than 380 people becoming affected. The source of contamination was improperly treated river water used for cleaning at the producing plant. Serogroup O124 has also been associated with a water-borne outbreak in Hungary (Doyle and Padyhe, 1989).

Oo *et al.* (1991) conducted a 3-month study in a suburban community in Yangon, Myanmar to isolate the major bacterial pathogens from drinking

water. Of 113 water samples collected, *E. coli* and *V. cholerae* non-01 were isolated from 29 and 5 each respectively. Of the 29 *E. coli* isolates, 3 produced ST. All water samples were negative for *Salmonella.* The study underscored the importance of bacterial contamination of drinking water and stressed the need to improve environmental sanitation.

Water has been responsible for both large outbreaks and sporadic cases of *Campylobacter* enteritis. Contamination of stored drinking water by wild birds was responsible for two outbreaks, the first involving contamination of a community supply in Florida, USA, which affected 871 people (CDC, 1983), the second involving the tank supplying an English boys' school (Palmer *et al.*, 1983).

Community outbreaks in the US have also been attributed to untreated spring water, which formed the supply to remote settlements (CDC, 1978, Metzing, 1981).

Sporadic outbreaks have usually involved water not intended for human consumption and many of those affected have been walkers who drank from mountain springs and streams (Taylor *et al.*, 1983; Pacha *et al.*, 1987).

2.4 PERSONS AT PARTICULR RISK FROM MICROBIAL INFECTION

Several factors may be responsible for humans acquiring any microbial infection. These factors may include susceptibility of the individual and may depend on the age and/or underlying disease. The infective dose to the organism may also put the individual at risk. Sometimes the organism may multiply during certain particular seasons and the risk of becoming infected may increase.

Susceptibility

A study to describe clinical and laboratory data for, and to propose pathogenesis and management of, children from poor communities with *Campylobacter* bacteremia was carried out by Reed *et al.* (1996). It was concluded from the study that malnourished children might be more likely to have gastrointestinal *C. jejuni* infection. Immunodeficiency and intestinal mucosal compromise secondary to malnutrition may render such children at increased risk of potentially life-threatening diarrhea.

The effect of chronic iron intake on diarrheal disease was evaluated in children in a community of low socio-economic stratum in Santiago, Chile (Brunser *et al.*, 1993). The study showed that those areas with inadequate environmental sanitation, chronic iron supplementation might have negative effects on diarrhea morbidity, despite improving iron nutritional status.

Djuretic *et al.* (1996) analyzed routinely available data on infectious intestinal diseases occurring in people aged 65 years and over in England and Wales from 1990 to 1994. Data available indicated that infectious intestinal disease, which is largely preventable, could cause substantial morbidity and mortality in elderly people. It was suggested that appropriate food hygiene and infection control measures, particularly in institutions, would help to reduce the incidence of infectious intestinal disease in elderly people.

Systemic *Campylobacter* infections are almost always found in patients who have an underlying predisposing condition. Such conditions include malignant disease such as leukemia, immunosuppression, alcoholism, liver cirrhosis, diabetes, chronic renal failure and gastrectomony (Skirrow, 1984). Pregnancy is also a risk factor, probably due to the suppression of cell-mediated immunity (Weinberg, 1984). With the obvious exception of pregnancy, systemic *Campylobacter* infections usually involve males aged more than 45 years.

AIDS could also be considered to be of particular importance as a predisposing factor for campylobacteriosis. Infections may be overlooked due to the unusual symptoms; chronic or recurrent infections of both septicemia and a digestive nature; and it is likely that AIDS is of greater importance than previously thought (Bernard *et al.*, 1989). *Salmonella* should

also be considered among the pathogens associated with human acquired immunodeficiency virus (AIDS) (Celum *et al.*, 1987), and a recurrent *Salmonella* bacteraemia may be the first indication of the syndrome (Sperber and Schleupner, 1987). Food counseling has been advised for AIDS sufferers to enable them to avoid foods, which are of high risk for *Salmonella* (Griffin and Tauxe, 1988).

Within the community, susceptibility to *Salmonella* infection varies. Individual susceptibility varies according to a number of factors and is partly genetically controlled. High susceptibility is common in the very young and old, for whom symptoms are often more severe, and those with underlying illnesses including malignancies. *Salmonella* infections may be associated with Reiter's syndrome, which has a high predilection for young males (Varnam and Evans, 1991).

Greater than normal susceptibility to *Shigella* infections is found among the old, the young and those with an underlying disease. A gradual increase in the average age of males infected with *Sh. flexneri* in the USA (5 to 24 years between 1968 and 1984) suggested increased male homosexual transmission (Wachsmuth and Morris, 1989).

Susceptibility to *E. coli* infections follows the usual pattern of the old, the young and those with underlying infections being at greatest risk. Infections

due to both EPEC and ETEC strains are particularly common in infants, and classical EPEC serovars are usually associated only with infections in children aged less than 18 months. Within this age group, there is evidence that males are more susceptible than females (Antai and Anozie, 1987).

Exposure

Exposure to *Campylobacter* is greatest in developing countries where standards of hygiene are poor. *Campylobacter* is associated with travelers' diarrhea and, in Sweden, between 50 and 75% of infections with the organism are associated with overseas travel. Multiple infections with two, or more, serovars is common in developing nations such as Mexico, but does not occur in industrialized nations (Sjogren *et al.*, 1989).

Occupational exposure is also an important risk factor in the acquisition of *Campylobacter* infection (Mancinelli *et al.*, 1987). Farm workers, veterinary surgeons, slaughterhouse and poultry plant workers, and butchers are all at enhanced risk of infection. Contact with domestic animals, primarily puppies, but also cats and kittens, is also a risk factor in *Campylobacter* infections. *C. jejuni* infection from a coyote has also been reported (Fox *et al.*, 1989).

Dietary habits may increase the risk of exposure to campylobacters with, as ever, the conscious consumption of raw milk and meat being significant factors (Varnam and Evans, 1991).

People may be at greater risk from *Salmonella* because of greater than average exposure rather than individual susceptibility. People working with animals, handlers of unprocessed meat and poultry, and some other workers such as sewer workers, face a higher risk of exposure particularly if personal hygiene is poor or preventive measures inadequate (Varnam and Evans, 1991).

Seasonal trends

Campylobacter enteritis is more common in summer than in winter (Blaser *et al.*, 1983). CDC reports for England and Wales show a striking seasonal consistency (Skirrow, 1987); there is a sharp rise in May, with a peak in July and thereafter a gradual fall to winter levels in December. The reason for the seasonal variations was unknown. Willis and Murray (1997) investigated the possible seasonal trends in the *Campylobacter jejuni* carrier state of market broilers. This study also showed that the highest recovery was during the warmer months of the year, i.e. May, and the lowest during December and January. It is known that during the summer periods, most people gather outdoors and engage in barbecues and other outdoor cooking. If infected meats are undercooked, it could lead to a rise in infections during the period.

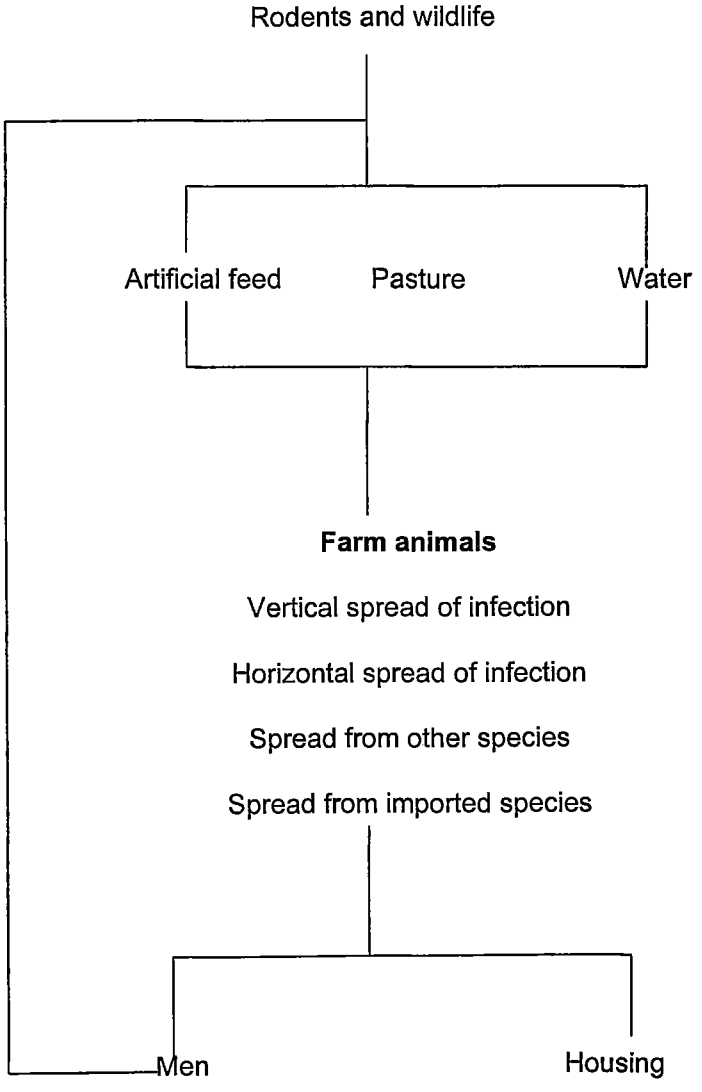
2.5 CONTROL OF FOOD-BORNE PATHOGENIC ORGANISMS

Animals and birds utilized for meat are subject to infection by a wide range of microorganisms, which are important pathogens of man. Recent publicity has focused on the role of modern intensive agricultural practices in the spread of food borne infection, and there is no doubt that many current practices are undesirable. Farm animals may be infected via a number of routes **(Figure 1)**.

The relative importance of these different routes varies from species to species. Within a species, there is also considerable variation related to differences in agricultural practice. From the viewpoint of food safety, every stage in the rearing of an animal, from birth to arrival at the abattoir, comprises a critical point at which spread of infection should be controlled through attention to good practice.

It should be stressed that there is no conflict between requirements of food safety and good husbandry and that both producer and consumer benefit from observation of basic precautions.

Figure 1: Pathways of infection of farm animals (Varnam & Evans, 1991).



During slaughtering of animals and subsequent meat processing, the water becomes polluted with organic matter of animal origin (i.e. protein and fat). Fransen *et al.* (1996) investigated the possibility of the use of this organic sludge as a product suitable for animal feeding by determining the microbiological contamination level. The prevalence of *Campylobacter jejuni/coli* was higher in flocculated poultry sludge than in both flocculated pig sludge and aerobically activated pig sludge.

It was obvious that decontamination of the sludge should be mandatory if it is to be applied as a feed constituent. This is to prevent the creation of bacterial cycles in livestock, as well as the spread of human pathogenic bacteria such as *Campylobacter, Salmonella* and *Yersinia.* It will also minimize the loss of protein quality by the microbial breakdown of amino acids and the formation of possible toxic metabolites in sludge during storage.

The efficacy of a new pelleting process in eliminating naturally occurring *Escherichia coli* and *Salmonella* from poultry mash was assessed by comparing the microbial load in raw and processed mash (McCapes *et al.*, 1989). Within limitations of the sampling and analytical tests utilized, the new pelleting process eliminated *Salmonella* from all mash in which the organism was known to be present but eliminated *E. coli* in only three trials. The process appeared to be 100% effective against both organisms when mash

entering the pellet mill from the conditioner had a temperature of 85.7°C and moisture content of 14.5% and had been retained and treated with steam and hot gases for 4.1 minutes.

From September 1991 until August 1993 an epidemiological study involving 20 Dutch broiler farms was conducted to identify risk factors and risk reducing measures for *Campylobacter* infections in broiler flocks (van de Giessen *et al.*, 1996). The results obtained indicated that application of specific hygiene measures during the rearing period were important. Hygiene measures such as washing hands before tending the broiler flocks, the use of separate boots for each broiler house and the use of footbath disinfecting when entering a broiler house, may significantly reduce the risk of *Campylobacter* infections in broiler flocks.

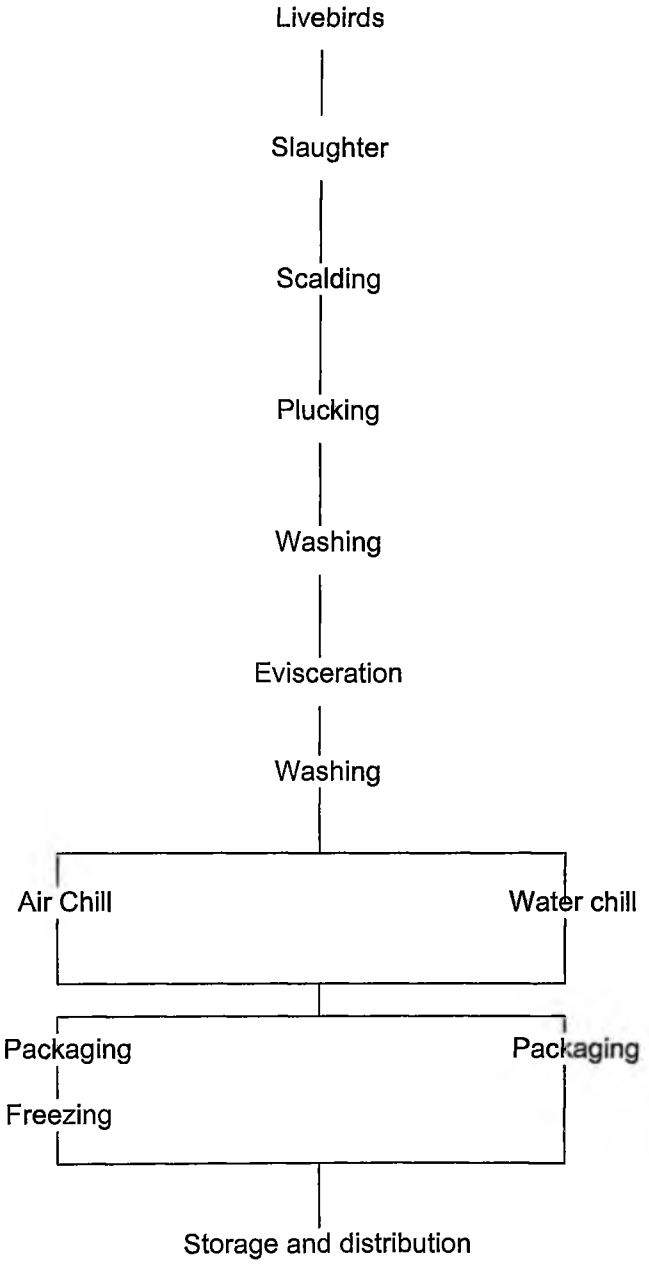
To get a better understanding of the epidemiology of Campylobacter, a chicken farm was studied for 16 weeks by Berndtson *et al.* (1996). Samples from each flock were taken weekly from input until the flock became colonized with *Campylobacter* and were slaughtered. Samples were also taken from fresh droppings and from drinkers during the rearing period, as well as from the environment in empty houses. The spread of *Campylobacter* during the slaughter process was also surveyed. No *Campylobacter* was found in samples from newly hatched or one-week-old chickens or their drinkers. All flocks, with the exception of one, were

colonized at two to five weeks of age. All *Campylobacter* isolates belonged to the same sero- and biotype; *C. jejuni Penner* 2. The spread of Campylobacter in the flock was rapid and all samples were positive once colonization had been proven. *C. jejuni* was isolated from flies in anterooms as well as from air in chicken units in houses with positive chicken flocks. Samples were taken at slaughter when some of the *Campylobacter* positive flocks from the farm were slaughtered. *Campylobacter* were isolated from all sampled equipment along the processing line, from the chicken transport crates to the chillers, as well as from the air.

The slaughter and preliminary butchery of animals and birds inevitably involves contamination of the carcass. Contamination may occur at virtually all stages of handling, but major sources are the skins and feathers, especially if heavily contaminated with feces, and the gastrointestinal tracts. The scale and nature of operations in packing plants leads to particular problems of cross - contamination. The process is summarized in **Figure 2.**

In June and September 1988, the USDA Food Safety and Inspection Service sampled raw chicken carcasses at a federally inspected slaughter establishment in Puerto Rico to determine the effects of changing the scalding equipment on bacterial contents of raw poultry products (James *et al.,* 1992).

Figure 2: Flow diagram of poultry packing (Varmam & Evans., 1991).



Flow diagram is based on process for chickens.

The findings indicated that although simple scalder changes contribute substantially to the improvement of the bacterial quality of chicken carcasses, additional interventions in the chilling process (such as chlorinating of chill water) are important to control cross-contamination and to preserve the positive effects obtained by the scalder changes.

An investigation was also carried out to assess the occurrence and distribution of *Salmonella* infection on all stages of turkey production (Hafez *et al.*, 1997). Samples from turkey parent flocks, hatchery, meat turkey flock, turkeys feed and finally from the surveillanced flocks at different steps in processing plant were examined. The study showed that cross contamination was very common during processing and even in several flocks contamination appeared to have started already during transport.

In most cases, the control of slaughter and butchery of meat animals and birds is limited to minimizing contamination and controlling temperature. Various attempts have been made to positively reduce the numbers of micro-organisms on the animal carcasses including washing either with hot water or with various antimicrobials including chlorine sprays (Kelly *et al.*, 1974), acetic acid and lactic acid (Snijders *et al.*, 1985).

Trisodium phosphate (TSP) has been approved by the United States Department of Agriculture as a post-chill antimicrobial treatment for raw

poultry. Somers (1994) studied the effectiveness of TSP against planktonic (suspended) and biofilm (attached) cells of various organisms.

They included *Campylobacter jejuni, Escherichia coli* 0157:*H7, Listeria monocytogenes* and *Salmonella typhimurium* at room temperature (RT) and 10^{o}C. Trisodium phosphate appeared to be an effective treatment for reducing populations of *C. jejuni, E. coli* 0157:H7 and S. typhimurium. The study showed the potential TSP has in the reduction of bacterial counts on other food products besides raw poultry or on food and non-food contact surfaces.

In March 1989, the USDA Food Safety and Inspection Service sampled raw chicken carcasses and giblets at a federally inspected slaughter establishment in Puerto Rico to determine the effects of adding chlorine to carcass and giblet chill water on bacterial contents of raw poultry products (James *et al.*, 1992). The study showed that placing raw chicken carcasses in chlorinated chill water reduced aerobe, *Enterobacteriaceae*, and *E. coli* plate counts. The prevalence of carcasses with salmonellae however remained nearly the same.

Particular concern over levels of *Salmonella* and *Campylobacter* in poultry has led to the development of a large number of decontamination methods.

Lactic and succinic acids have been used in various dip or spray treatments, and are sometimes combined with freeze thaw process (Stern *et al.*, 1985) while hydrogen peroxide has also been used in combination with lactic acid.

Various types of ready-to-serve food items were evaluated for their bacteriological profile and holding temperatures by Ashenafi (1995). These included roasted offal, fish soup, cooked and sauced macaroni and spaghetti, and shiro sauce. Several bacterial genera were isolated but Micrococcus and Bacillus sp. dominated the aerobic microflora. The unhygienic conditions of the food service environment, possibilities of cross contamination from utensils and keeping food items at ambient temperatures for several hours were considered to be critical points for the high load of microorganisms. Thus proper food handling and the use of right temperature holding equipment were suggested as practices that could minimize contamination.

Various factors are known to cause a decrease in bacterial enteropathogens in food during fermentation. Fermentation could therefore control food borne pathogenic organisms and various studies have been undertaken in this direction. Mensah *et al.* (1991) simulated unhygienic conditions of a typical rural community in a developing country in the laboratory by inoculating fermented maize dough porridge with *Shigella flexneri* and enteropathogenic *E. coli* (ETEC). The study assessed the antimicrobial effects of the different

processes involved in the preparation of maize dough porridge. The study showed that the antimicrobial effect of fermented maize dough was not due to the pH per se and that fermentation of maize dough appeared to be a useful strategy for reducing contamination of weaning food by *Shigella flexneri* and ETEC.

As part of a program to formulate foods to aid the control of diarrhea diseases, Olukoya *et al.* (1994) developed an improved ogi (the commonest weaning food in Nigeria) named DogiK using Lactobacillus starter cultures. DogiK, produced by this culture, possessed antimicrobial activity against diarrhoeagenic bacteria and also possessed amylolytic activity. The survival of diarrhoeagenic bacteria was investigated in locally fermented ogi and in DogiK and it was shown that DogiK may have the potential for use in the prevention and treatment of diarrhea.

Mensah (1990) studied the antimicrobial effects of fermented maize dough and showed that fermented maize porridge had fewer levels of bacteria than unfermented product. The study showed that fermentation of maize dough could be an effective method for the control of diarrhea pathogens.

The survival of strains of *Campylobacter jejuni* and enteropathogenic and enterotoxigenic (LT) *E. coli* were investigated in mahewu, a traditional fermented cereal gruel by Simango and Rukure (1992). Results obtained

suggested that mahewu has some antimicrobial qualities and is unlikely to play a major role in the transmission of these enteric pathogens.

2.6 SUSCEPTIBILITY OF ENTERIC PATHOGENS TO ANTIMICROBIALS

Campylobacter

Campylobacter infections are often self-limiting, and no treatment is required. In severe cases, dehydration may occur and a glucose electrolyte mix may be prescribed. Chemotherapy is indicated in severe cases or where complications are present. Erythromycin is generally accepted as the drug of choice (Anders *et al.*, 1982; Pai *et al.*, 1983). However, erythromycin resistance may be a problem (Bernard *et al.*, 1989). Tetracycline, particularly minocycline, is also effective but the high level of resistant strains means that laboratory control is essential. Nalidixic acid is effective against *C. coli* and *C. jejuni*, but the emergence of resistant strains is causing increasing problems (Altwegg *et al.*, 1987). Murphy *et al.* (1996) reported that Ciprofloxacin and azithromycin resistant *Campylobacter* caused traveler's diarrhea in U.S. troops deployed to Thailand in 1994.

Susceptibility to antibiotics of 39 *Campylobacter jejuni/coli* strains was studied using the modified disc method (sowing by loop) (Sicinschi, 1996). All the strains were resistant to rifampicin, polymyxin M sulphate, ketokonazole, cephalotin, cephazolin; most of the strains were resistant to

carbenicillin, one third to ampicillin and almost one fifth to polymyxin E. The findings enabled the recommendation of these antibiotics (except penicillin) as ingredients for the selective media for *Campylobacter* isolation. All the strains were sensitive to ciprofloxacine and chloramphenicol; most of the strains were sensitive to doxycicline, tetracycline and nalidixic acid. Three strains were appreciated as intermediate sensitive to cephotaxime, two strains to erythromycin and one strain to clindamycin. These antimicrobial agents were therefore recommended in the treatment of the campylobacterioses according to the susceptibility testing results of each isolated strain. All strains were also sensitive to gentamycin, kanamycin, streptomycin and tobramycin.

More than 200 strains of *Campylobacter jejuni/coli* isolated in 1985 and 1987/88 from human fecal specimens were tested for their susceptibility to erythromycin and ciprofloxacin (Hirschl *et al.*, 1990). All strains were regarded as susceptible to ciprofloxacin. Only 3.6% of the *C. coli* strains were resistant to erythromycin. All 209 strains of *C. jejuni* proved to be susceptible to erythromycin.

Agbodadze *et al.* (1990) studied the antibiogram of 24 strains of *C. jejuni* isolated from domestic animals in rural Ghana. All strains showed resistance to cephalotin, cephalexin and sulfamethoxazole/trimethroprim. The most effective antibiotics were ampicillin, minocyclin, chloramphenicol, fosfomycin,

gentamycin, amoxicillin, erythromycin and nalidixic acid.

To get better knowledge about clinical and bacteriological features in *Campylobacter* sp. bacteremia a study was conducted over a period of 8 years (1987-1994) (Font *et al.*, 1997). An increasing ciproflaxin resistance in *C. jejuni* strains was detected during this period, which reached up to 75% in the last years. Antimicrobial susceptibility to erythromycin and aminoglucosides was kept in all the strains. It was concluded that *Campylobacter* spp. bacteremia had a remarkable mortality rate and that this was probably related to immunosuppressive underlying diseases in affected patients. An increasing fluoroquinolone resistance was detected over the years while susceptibility to erythromycin and aminoglycosides was maintained.

Salmonella / Shigella

Enteric fever, and other severe systemic infections due to human-adapted serovars of *Salmonella*, is usually treated by oral antibiotic therapy. Typical regimes for treatment of *S. typhi* infections include amoxycillin, cotrimoxazole and chloramphenicol. More recently, ceftriaxone has been suggested as a superior alternative to chloramphenicol (Islam *et al.*, 1988). Treatment with amoxycillin and cotrimoxazole is usually continued for 14 to 21 days, with chloramphenicol for 10 days and with ceftriaxone for 7 days. Continued excretion of *S. typhi* usually requires the treatment to be repeated. Rowe *et*

al, 1987 indicated that chloramphenicol should be a drug of choice for commencing treatment whilst awaiting antibiotic sensitivity results.

Treatment with antibiotics is not desirable in enteritis since it is ineffective. It increases the likelihood of the development of a carrier state, and may promulgate the further spread of antibiotic resistance. The situation is aptly summarised by Duguid et al. (1978): '**Thus the pratitioner, although he may have a feeling of futility in not being able to offer a specific treatment, should refrain from exhibiting what are in cases of *salmonella* food poisoning useless and potentially harmful drugs**'.

However, antibiotic therapy is advocated in *Salmonella* enteritis where there is associated septicaemia. Clinical determination of septicaemia is difficult in the early stages of the illness when antibiotic treatment is most successful. In elderly patients, delay may lead to severe dehydration and often, fatal illnesses. Early use of antibiotic therapy may, therefore, be advisable in elderly patients with severe diarrhoea (Mandal and Brennand, 1988). Chloramphenicol, ampicillin and cotrimoxazole are most commonly used. In all types of *Salmonella* infection, rehydration and restoration of the electrolyte balance may be required if diarrhea is particularly severe.

Arakawa *et al* (1996) studied the drug susceptibility and serovars for a total of 1,110 strains of *Salmonella* isolated from humans during a period from

1987-1993 in Aichi prefecture in Japan. In drug susceptibility tests using 8 drugs, the frequency of drug resistance and number of patterns of resistant isolates were 46 and 33% patterns, respectively. There were different resistant rates and characteristic resistance patterns in each serovar of the isolates. Some of the resistance patterns of isolates derived from food poisoning were rare during the year of occurrence. Thus, it seems that surveillance of *Salmonella* may provide valuable fundamental epidemiological data.

Salmonella isolates from 295 layer and 294 broiler flocks in Canada were examined to determine resistance to antimicrobial agents, plasmid profiles, biochemical properties, and susceptibility to polyvalent bacteriophages (Poppe *et al*, 1996). A high number of strains were found to be resistant to spectinomycin (97.8%). Less than 2% of the strains were resistant to cephalothin, chloramphenicol, cotrimoxazole, gentamicin, kanamycin, neomycin, nitrofurantoin, and/or polymyxin B. About 3% of the strains were resistant to ampicillin, carbenicillin and/or tetracycline, whereas 8% of the strains were resistant to sulfisoxazole. *Salmonella anatum* var. 015+ and *S. typhimurium var. Copenhagen* strains were resistant to multiple antimicrobial agents.

To establish the relationship between the presence of plasmid and their antimicrobial resistance of *Salmonella* strains, Luque *et al.* (1994) tested 171

strains of *Salmonella* isolated from different sources: natural waters (73 strains), food (23 strains), and from clinical samples (75 strains). Antimicrobial resistance of the strains significantly varied according to their primary isolation sources. Strains isolated from the water environment exhibited a full susceptibility to cephalothin and colistin, whilst all the strains isolated from food were sensitive to ampicillin, gentamicin, kanamycin, neomycin, tobramycin and trimethoprim-sulphamethoxazole. On the other hand, resistances to colistin, gentamicin and trimethoprim-sulphamethoxazole were not found in clinical isolates. From the 171 *Salmonella* strains tested, only 12.2% were sensitive to all the antimicrobials. *Salmonella* was, however, commonly resistant to streptomycin (49.3%), tetracycline (33.1%), nalidixic acid (30.7%) and 0.6% to gentamicin.

In most cases, shigellosis is a self-limiting disease, and no treatment is required except in severe cases where fluid replacement is seen as the basis for successful therapy (Berkow, 1982).

Antibiotic therapy may be used if symptoms are particularly severe or, more commonly, to decrease morbidity and limit the secondary spread of infection (Weissman *et al.*, 1974). Ampicillin and cotrimoxazole are recommended, but the situation has been complicated by the increasing incidence of multiple drug resistance among shigellas. Since multiple, transferable drug resistance was first noted in Japan by Ochiai *et al.* (1959), there has been a dramatic

increase in drug resistance in the USA, England and Wales (Thomas and Tillett, 1973; Gross and Rowe, 1981) and elsewhere. For this reason, the decision to use antimicrobials in mild cases should be balanced against the risk of producing resistant strains (Anon, 1987).

Inappropriate administration of antimicrobials is a risk factor for the development of haemolytic uraemic syndrome. This complication has arisen where resistant strains of *Shigella* have been treated with ampicillin or cotrimoxazole (Parsonnet and Greene, 1989).

Infections caused by multiple resistant strains of *Shigella* that require antimicrobial therapy may be treated with nalidixic acid or norfloxacin but care must be taken with children because of possible side effects (Anon, 1988).

Wharton *et al.* (1990) reported of a food outbreak caused by *Shigella sonnei.* This organism was resistant to ampicillin, tetracycline, and trimethoprim-sulfamethoxazole. Hoge *et al.* (1995) also reported of a nalidixic acid resistant *S. dysenteriae* 1 that emerged in a community in Thailand.

Escherichia coli

In most cases infections with diarrhoeigenic *E. coli* are self limiting, and treatment is usually restricted to general care, although in severe cases

rehydration and restoration of the fluid balance is necessary. Antibiotic therapy is normally neither effective nor desirable, but is indicated when infection with adherent EPEC strains leads to persistent and life-threatening diarrhoea, and severe small intestine enteropathy (Hill *et al.*, 1988). Intravenous administration of antibiotics includes gentamicin and trimoxazole (Thoren *et al.*,1980).

Grigor'eva *et al.* (1989) studied the antibiogram of 1303 strains of pathogenic *Escherichia coli.* The following antibiotic range was established: streptomycin - chloramphenicol (chloromycetin) - neomycin - sulfate - tetracyline - kanamycin - polymyxin - gentamycin, thus the least number of strains was characterized by gentamycin resistance.

Eighty percent (80%) of *Enterobacteriaceae* isolates from battery poultry exhibited drug resistance in a survey among university and commercial poultry flocks (Ojeniyi, 1989). These birds, being a source of human food, may serve as an important reservoir for human pathogenic drug-resistant enteric organisms. Feeds used on University and Commercial poultry farms were found to be inhibitory to strains of *Staphylococcus aureus* and *Escherichia coli* K 12 J5 $NA^{+}Lac^{-}$. Feed additives, which purportedly were mineral and vitamin supplements, were highly laden with antibacterials. A quantitative estimation showed that one of them, termed A & D Crumbles

contained as much as 3000 ug of antibiotic / gm, while another additive known as ADVIT contained 130 ug of antibiotic /gm of the feed supplement. These are routinely added to poultry feeds, a practice that may easily lead to development of drug resistant strains that may reach humans and complicate therapy of human bacterial infections.

3.0 MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Sampling

3.1.1.1 *Sample location*

Locally bred and imported chickens were obtained from three (3) poultry farms, two (2) open markets and nine (9) supermarkets.

Poultry farms

The poultry farms were selected on the basis of the level of experience of the supervising personnel and the demographic distribution of its products. Three farms were selected and these are coded Farms A, B and C.

Farm A (teaching/commercial) has competent managerial personnel and a wide demographic distribution of its products. It has a great variety of farm animals with free movement by some. Farm B (commercial farm) is also managed by competent managerial personnel but has a small demographic distribution of its products. Here, only chickens are reared and the birds are strictly caged. Farm C (commercial) has managerial personnel with little training in agricultural management. Other farm animals are available and are caged. Its products have a wide demographic distribution.

In all the farms only apparently healthy birds were selected for analysis. **PLATES 1 & 2** show the external and internal view respectively of Farm A.

PLATE 1: Outside surroundings of Farm A (Observe tanks that are sometimes left opened which enable flying birds to drink from them)

PLATE 2: Inside view of Farm A showing birds, water and feeding troughs (the water and feed are easily contaminated by fecal and extraneous matter).

Supermarkets

The supermarkets were selected on the basis of their size, availability of advanced vending and storage equipment and the level of patronage. Those visited were well-known supermarkets using very advanced technology in their day to day activities and had the reputation of being patronized by high-income earners.

Nine supermarkets were visited in all with many of them being patronized by expatriates. **PLATE 3** shows how chicken is displayed in one of the supermarkets.

Open markets/Coldstore

The open markets were also selected on the basis of their level of patronage. Two open markets were selected and are coded Market 1 and 2. These are popular and patronized by majority of residents in Accra. **PLATE 4** shows how chicken is displayed in the open market.

Samples were collected from a very popular coldstore where imported products are stored before sale to the general public. **PLATE 5** shows how imported chicken is packed and the scale used in weighing during vending.

PLATE 3: Display of chicken meat in a supermarket (Observe meat neatly packed and arranged in a refrigerator)

PLATE 4: Display of chicken meat on an open market (Meat and vending scale are both exposed to the atmosphere)

3.1.1.2 Sample size

A total of one hundred and eighty four (184) samples were analyzed. Of these, ninety-seven (97) were live poultry birds and eighty-seven (87) were carcasses.

Out of the 97 live birds, 40, 27 and 30 birds were examined from Farms A, B, and C respectively. Out of the 87 carcasses, 20, 10, and 10 carcasses were also sampled from Farms A, B and C respectively.

From the Supermarket, Market 1, Market 2 and the Coldstore 14, 14, 6, and 13 carcasses, ranging in size from 0.5 to 1.0 Kg, were examined from each respectively.

3.2 METHODS

3.2.1 Field methods

3.2.1.1 Specimen collection

Chicken Carcasses

Chicken carcasses were purchased from the various sample locations and placed in sterile plastic bags (Stomacher bags) to avoid post-contamination before being transported to the laboratory.

Live birds (Swab sample method)

The Swab procedure was used for sampling live chicken. In this, sterile swabs (prepared in the laboratory) were used to swab the cloaca of the bird. The swab was transferred into Cary-Blair (Oxoid CM 519) transport medium. **PLATE 6** shows the swab sample method being performed on a farm.

3.2.1.2 *Transport*

The chicken carcasses in sterile plastic bags (Stomacher bags) and the swab samples in the Cary-Blair transport medium were transported to the laboratory in ice chests with ice packs. They were then stored in a coldroom and analyzed within three (3) hours of collection.

3.2.2 Laboratory methods

3.2.2.1 Glassware and media preparation

Glassware was sterilized using a Hot-Air Oven (Yamamoto Fine Oven DH 62).

Media were prepared according to the manufacturer's specifications. Media used were sterilized as required, for example using an autoclave (Sanyo Autoclave MLS-2420). The media were then incubated at 37^{o} C after dispensing using an incubator (Yamamoto IC-62 or a Sakura IF-4) to check for sterility.

PLATE 5: Display of imported meat retailed outside the Coldstore (The meat is retailed as-is from the cartons)

PLATE 6: Swab method being performed on a Farm.

3.2.2.2 Surface rinse method (for carcasses)

The surface rinse procedure was used for sampling chicken carcasses. 500 ml of Phosphate Buffered Saline (Oxoid "Dulbecco A" BR 14a) solution was added to the samples in the sterile plastic bags. The carcass was rinsed by shaking and massaging and allowed to drip before removal.

3.2.2.3 Sample analysis

The rinse from the sample was centrifuged at 11,500 x g for 30 min at 4°C using an automatic high speed refrigerated centrifuge (Hitachi 20 PR-520). The supernatant fluid was then discarded and the pellet examined for pathogenic bacteria. Loopfuls of the Cary-Blair transport medium with the swab innoculum were also examined.

3.2.2.3.1 *Campylobacter*

Isolation

100 µl of the pellet or 2 - 3 loops of the transport medium, prepared in **Section 3.2.2.3,** was streaked onto plates of Skirrow's agar (Oxoid Blood Agar Base No.2 CM 271 + 5% Sheep Blood + Oxoid *Campylobacter*-Selective Supplement (Skirrow) SR 069E). This was then incubated at 37°C for 48 hours under microaerophillic conditions. Microaerophilic conditions (5% O_2, 10% CO_2, 85% N_2) were achieved using an atmosphere gas generating systems (BBL Campy pak Microaerophillic System Envelopes).

In addition, 500 µl of the pellet or 2 - 3 loops of the transport medium were inoculated into about 10 ml of Brucella Broth (Difco 0495-17-3) with Preston *Campylobacter* Selective Supplement (SR 117E) in test-tubes. This was incubated at 37°C for 24 hours for enrichment of the organisms under microaerophillic conditions. Two or three loops of this were then streaked onto Skirrow's agar.

Growths from these two agar plates were identified as follows.

Identification

Detection of *Campylobacter* was made using the colonial morphology on the selective agar. Colonies seen to be smooth, convex, and glistening with a distinct edge or flat, translucent, shiny, and spreading with an irregular edge; colorless to grayish or light cream; usually 1 to 2 mm in diameter but ranging from pinpoint to 4 to 5 mm in diameter **(PLATE 7)**, were screened for their motility, morphology and their oxidase test response.

Cultures that were found to be positive from the initial screening test **(Figure 3)** were inoculated on a blood agar at 37°C for 48 hours under microaerophillic conditions to obtain pure cultures. These were identified using an API CAMPY (Bio-merieux 20800) **(PLATE 8)**. This Kit uses 22 different Biochemical reactions (**Appendix 1**) to differentiate between

Campylobacter serovars.

Antibiotic sensitivity

Tryptone Soy Broth (TSB) (Oxoid CM 129) was inoculated with a pure culture till a turbidity of 0.5 McFarland standard (as supplied in kit) was attained. Sterile cotton swab was then dipped into the suspension and streaked on the entire surface of a blood agar (in a 150-mm plate). The antimicrobial disks were then placed on the plate and incubated at 37^{o}C for 48 hours under microaerophillic conditions and the zone diameters measured in mm using a straight-line ruler. The zone diameter was interpreted using an interpretive chart for zone sizes (**Appendix 2**). **TABLE 3** gives the list of the antimicrobial agents used in the Study.

TABLE 3: List of antimicrobial agents

Antimicrobial agent	Quantity
Fosfomycin	200 ug
Minocyclin	200 ug
Nalidixic Acid	30 mcg
Cefadroxil	30 ug
Chloramphenicol	30 ug
Ampicillin	10 ug
Ceftrioxone	30 ug
Penicillin	10 IU
Amoxycillin	30 ug
Kanamycin	30 ug
Cefotiam	30 ug
Tetracycline	30 ug
Erythromycin	15 ug

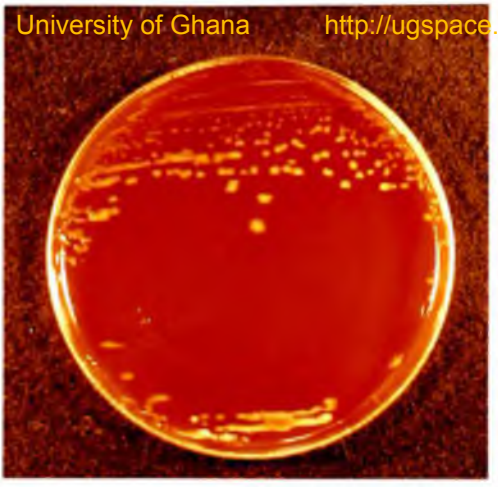


PLATE 7: Colonial appearance of *Campylobacter* sp (flat, translucent, shiny, and spreading with an irregular edge; light cream; ranging from pinpoint to 5 mm in diameter)

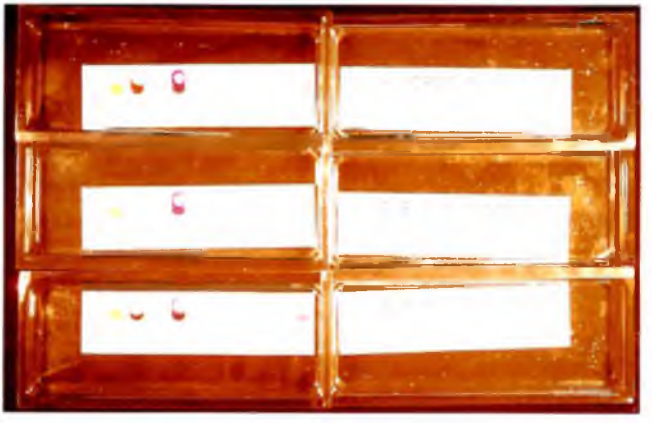
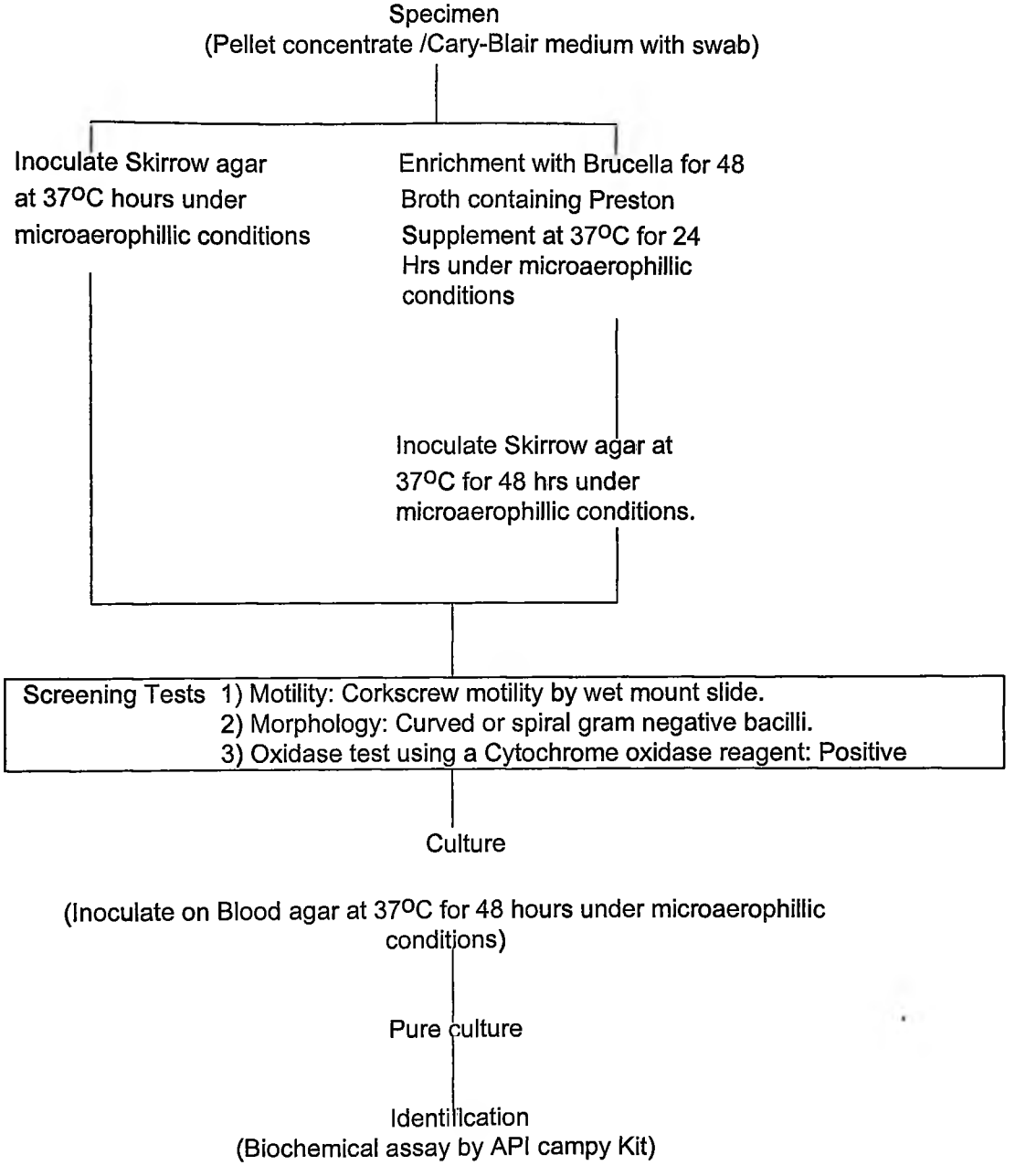


PLATE 8: API Campy Kit showing positive reaction for *Campylobacter*

Figure 3: Flow diagram for the isolation and identification of *Campylobacter* species.



3.2.2.3.2 *Salmonella / Shigella*

Isolation

100 µl of the pellet or 2-3 loops of the transport medium, prepared in **Section 3.2.2.3,** were streaked onto plates of *Salmonella-Shigella* (SS) agar (BBL *Salmonella-Shigella* Agar 11597) and incubated at 37° C for 24 hours under aerobic conditions.

In addition, 500 µl of the pellet or 2-3 loops of the transport medium were inoculated into about 10 ml of Selenite F broth (Eiken 42001) in test tubes and incubated at 37°C for 24 hours under aerobic conditions. Two to three loops of this were then streaked onto a *Salmonella-Shigella* agar as in direct plating. The colonies were identified as follows:

Identification

Colonies suspected to be *Salmonella* (Black colonies or transparent colonies with black centers) or *Shigella* (Pale color, indicating that lactose is not utilized fermentatively or colorless colonies) were selected and identified using standard methods (ICMSF, 1986). The following biochemical assays were performed:

i. Lactose and Sucrose fermentation, gas and H_2S production test were performed using a Triple Sugar Iron (TSI) agar (Oxoid CM 277).

ii. Urease test was performed using Urea Agar base (Oxoid CM53) with

40% Urea solution (Oxoid SR20).

iii. Indole and Motility tests were performed using Lysine Indole Motility semi solid agar ("Eiken" 19001) or Sulphide Indole Motility medium ("Nissui" 05106).

iv. Oxidase test was performed using a Cytochrome Oxidase reagent strip ("Nissui" 05180).

Slide Agglutination Test

The cultures that were identified as positive with the API were subjected to slide agglutination tests to identify the species. *Salmonella* Antisera (O grouping and Vi-sera) ("Seiken" No 21041) and *Shigella* Antisera (III) ("Seiken" No 1453) were used.

Cultures that gave the required results of the biochemical assay (**Figure 4**) were confirmed using an API 20E (2010) kit. The kit uses 27 different biochemical reactions to confirm and differentiate between serovars (**Appendix 3**). **PLATES 9 & 10** show colonial appearance and biochemical characteristics of *Salmonella* and *Shigella* respectively.

Antibiotic sensitivity

Antibiotic sensitivity was performed as in **Section 3.2.2.3.1** but in this a Mueller-Hinton (Oxoid CM 337) Agar medium was used and incubation was

at 37°C under aerobic conditions.

3.2.2.3.3 *Escherichia coli*

Isolation

100 µl of the pellet or 2 - 3 loops of the transport medium, prepared in **Section 3.2.2.3**, were streaked onto plates of Eosin Methylene Blue (EMB) agar (Oxoid "Levine" CM 69) and incubated under aerobic conditions at 37°C for 48 hours. Colonies suspected to be *E. coli* (Mettalic sheen colonies) were selected and identified using standard methods (ICMSF, 1986). Biochemical assay was performed as in **Section 3.2.2.3.2.** Cultures that gave the required positive biochemical results **(Figure 5)** were confirmed using an API '20E 2010' kit. This was performed as in **Section 3.2.2.3.2.**

Slide Agglutination test

Cultures that were identified as positive using the API were subjected to slide agglutination test to identify the serovar. *Escherichia coli* Antisera (1) O sera (Seiken 24506) was used. **PLATE 11** shows morphological and biochemical characteristics of *E. coli*.

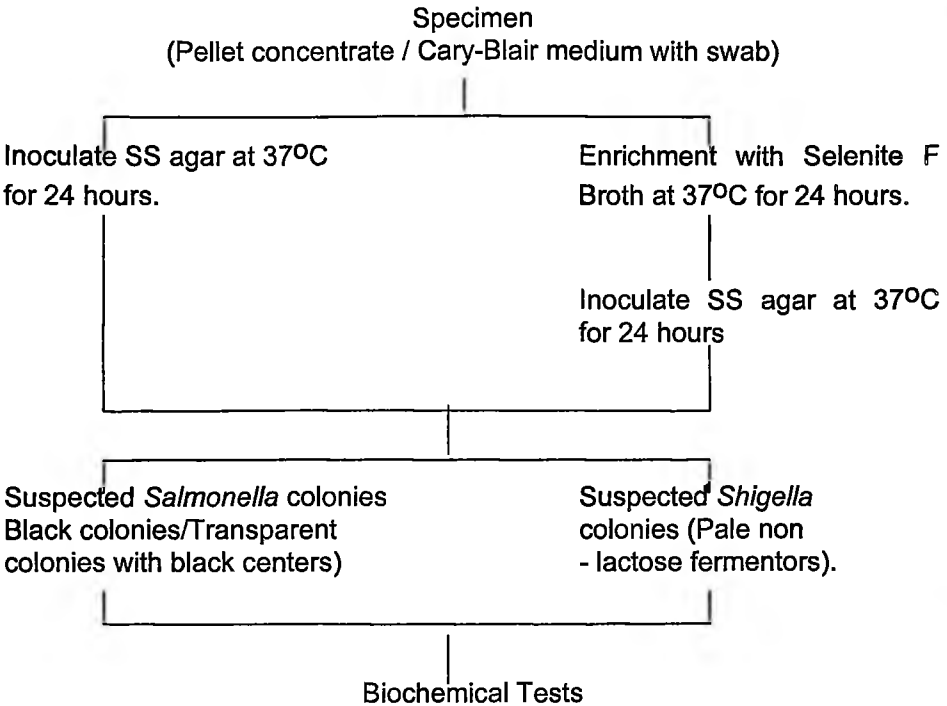
Antibiotic Sensitivity

Antibiotic sensitivity test was performed as in **Section 3.2.2.3.2 (PLATE 12).**

PLATE 9: Colonial appearance and biochemical characteristics of *Salmonella* (Observe black color due to H_2S production)

PLATE 10: Colonial appearance and biochemical characteristics of *Shigella* (Colorless colonies and positive indole (pink color) production)

Figure 4: Flow diagram for the isolation and identification of *Salmonella* / *Shigella* species.



	TSI				SIM / LIM			
	Slant	Butt	H_2S	Gas	Indole	Mot	Oxi	Urease
Salmonella	K	A	+	+	-	+	-	-
Shigella	A	A	-	+ -	+ -	-	+	-

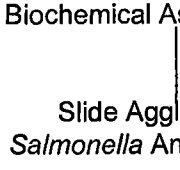
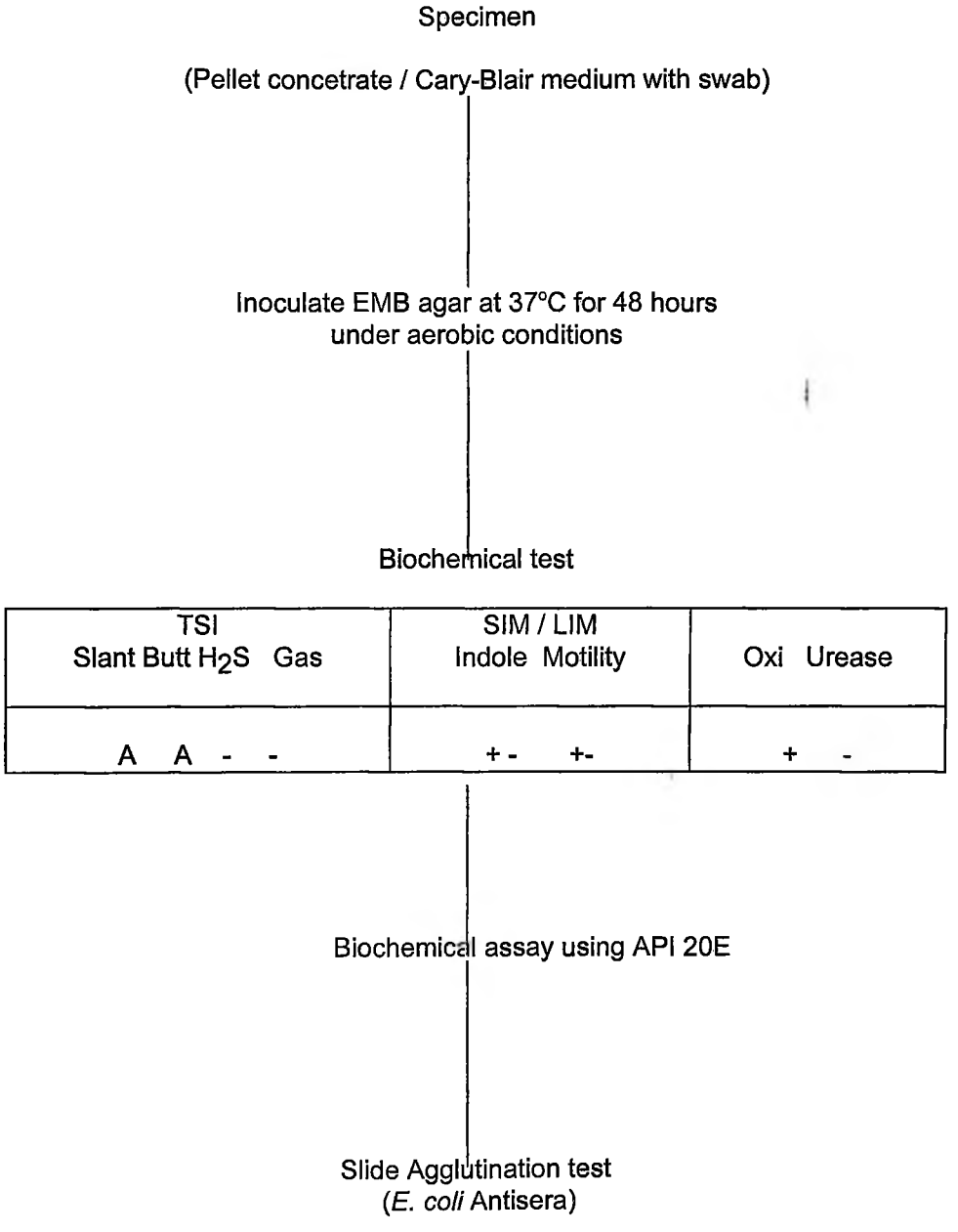


Figure 5: Flow diagram for the isolation and identification of *Escherichia coli* species.



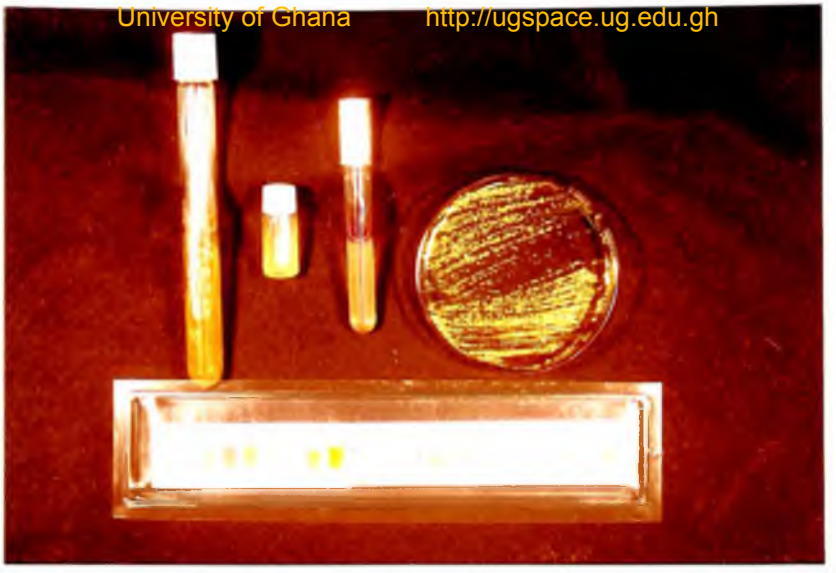


PLATE 11: Colonial appearance and biochemical characteristics of *E. coli* (Observe metallic sheen appearance on EMB Agar)

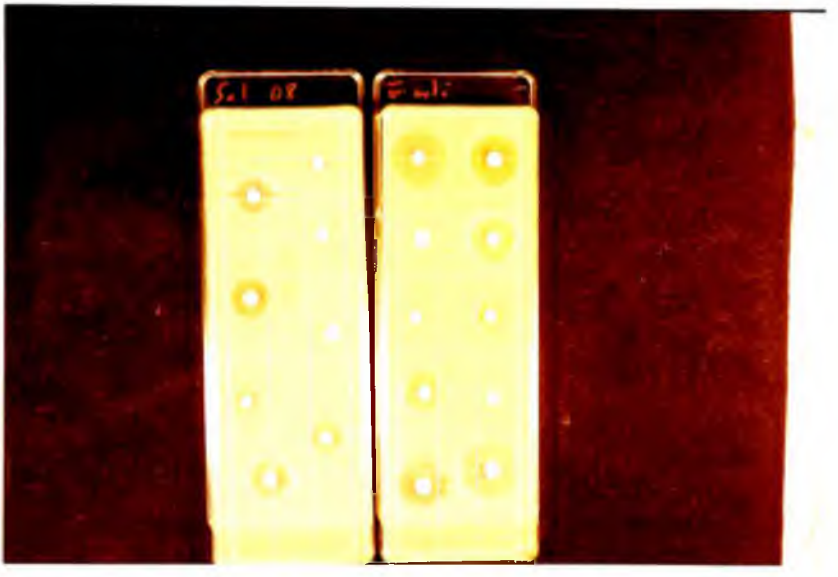


PLATE 12: Antibiotic sensitivity test of *Salmonella* isolate (08) and *E. coli.*

Detection of heat labile and heat stable enterotoxins of E. coli

The *E. coli* isolate were tested for heat labile toxin (LT), heat stable enterotoxin (ST). DNA-DNA hybridization assays were performed using enzyme-linked oligonucleatide probes. The alkali phosphatase-phosphatase oligonucleotide probes were synthesized by Toyobo Co Ltd Biochemical Operations Div. Japan (Tamatsukuri *et al.*, 1991). Both human (ST-h) and porcin (ST-p) ST probes were used. The sequence for ST-h was 5'TGAAGACTCTACTGGTTAGCATC (PRB-003) and ST-p 5'GAAGAGTCAAGTGATTCAGTTGAC (PRB-002). The sequence of the LT-3 probe was 5'AGATATGAGATTCTTAATGTGTCC (PRB-001).

Stored *E.coli* were cultured on Tryptic Soy Agar (TSA) at 37^{o}C for 18-24 hours. The organisms were transfered onto Gene Screen Plus (Biotechnology System, NEN Research Products) with pencil marks using sterile toothpicks. The membrane was then placed on Whatman 3mm filter paper and wetted with 0.5 N NaOH + 1% sodium lauryl sarcosine (SLS) and kept at 50^{o}C for 10 mins to lyse the cells.

The membrane was transferred onto Whatmann 3mm filters wetted with 1M Tris HCl (pH 7.4) at room temperature to neutralise the pH. Cell-debris was gently rubbed using a sponge in 5 x standard saline citrate (SSC) and 1% sodium dodecyl sulphate (SDS). The membrane was then dried at room

temperature for 30-60 min. The membrane with DNA was soaked in 5 x SSC for 5 minutes and pre-hybridized using hybridization buffer (5 x SSC, 0.5% bovine serum albumen, 0.5% polyvinyl pyrrolidine and 1% SDS) for 15 mins in plastic hybridization bags (Betheseda Research Laboratories). ST-h and ST-p were pre-hybridized at 55^{o}C and LT at 50^{o}C.

Hybridization with enzyme labeled probes were performed in heat sealable bags with 5µl enzyme labeled probe and 1.0 ml of hybridization buffer as above. The filters were washed twice in washing buffer (2 x SSc, 1% SDS) with shaking at 55 °C for 10 minutes, followed by another wash in 1 x SSC with 1% Triton X-100 at room temperature.

The washed membrane was then immersed in 5ml substrate buffer (0.1M Tris-HCL, 0.1M NaCl, 0.1M MgCl2, 0.1M ZnCl2, 0.005% NaN3, pH 8.5) with 22ul nitro-blue-tetrazolium (NBT), 17µl (75mg/ml in 70% dimethylfomamide and 22µl of 5-bromo-4-chloro-3-indonyl phosphate (BCIP)(50mg/ml in dimethyl-formamide) and sealed. After sealing, the bag was kept at 37°C in an incubator for 30-60 mins and were observed for positive (blue) and negative (colorless) results.

E.coli 309775 LT and *E.coli* J196 ST were used as control for LT and ST respectively. Refer to **Appendix 4** for preparation of various reagents.

3.2.3 Statistical Analysis

Computerised Business Statistics (CBS) software was used to analyse the data. A "no significant difference" hypothesis was set and Chi-square analysis used to accept or reject it.

4.0 RESULTS AND DISCUSSION

Chicken is now a popular source of animal protein to the Ghanaian as indicated by many stands where chicken parts are roasted for sale. Although chicken is usually produced locally, the trade liberalization program has resulted in the importation of frozen chicken from many developed countries. The imported chicken is however relatively cheaper and enjoys much patronage from the general public.

There appears not to be adequate, if any, microbiological monitoring of these products due to the limited number of laboratories presently available in the country. The objective of this study was therefore to assess the enteropathogenic bacterial quality of chicken from some selected farms, wholesale and retail outlets in Ghana using the Accra Metropolitan Area as a unit case. It evaluated the prevalence of *Campylobacter, Salmonella, Shigella* species and *Escherichia coli*. A qualitative analysis of these bacteria was made on cloacal swabs of live birds and the surface wash of carcasses. The antimicrobial sensitivity patterns of all isolates were also assessed.

Cloacal swabs of a total of 97 live birds were collected from three different farms in Accra and 87 carcasses obtained from the 3 farms, 9 supermarkets, 2 open markets and one wholesale outlet (Coldstore).

It was observed during the Study, that the poultry birds from the selected farms were slaughtered on a large scale. Scalding was done in a bucket and the feathers plucked on the floor. The hot water was used continuously until it cooled. The slaughtered birds were then eviscerated and washed before being dressed for sale **(See PLATES 13, 14 & 15).**

Fourteen (14), representing 14.4% of the 97 live birds examined for intestinal contaminants were positive for *Campylobacter* **(Table 4)**. These constitute Farm A (1), Farm B (7) and Farm C (6) representing 1.0%, 7.2% and 6.2% respectively. None of the chicken carcasses was positive for *Campylobacter.*

Table 4: Incidence of *Campylobacter* in live chicken birds from poultry farms.

Location	No of positive Samples / visit			Total/ location	% +ve	(%)[a]
	1	2	3			
Farm A	0/10	0/10	1/20	1/40	2.5	1.0
Farm B	0/10	7/17		7/27	39.6	7.2
Farm C	0/10	6/20		6/30	20.0	6.2
TOTAL	0/30	13/47	1/20	14/97		14.4

[a] = Percent Positive of total live bird samples

PLATE 13: Plucking of feathers from farm birds (Observe the birds being plucked on bare floor)

PLATE 14: Washing of plucked chicken on a Farm (Same water is used to wash several birds)

PLATE 15: Dressing of Farm birds (Observe the birds being dressed on a single large bench)

Animals have been known to be reservoirs of enteropathogenic bacteria, for example, *Campylobacter, Salmonella* etc. Birds are often the principal reservoirs of *Campylobacter* (Blaser *et al.*, 1983). Poultry especially chicken have been found to be an important source of *Campylobacter* infection (Marinescu *et al.*, 1997).

The presence of these enteropathogenic bacteria in the intestinal content of the birds from the poultry farms is a cause for concern as transmission to man through handling and consumption could occur. Transmission could occur as a result of cross contamination during the slaughter of the birds. Cross contamination has been known to be greatest when mass slaughter of birds occurs especially on large farms. Infected birds could contaminate the water, the floors and benches on which evisceration and dressing is carried out. This could also result in cross contamination to other uninfected birds. It is known that in situations where conveyer belts are used, transporting from one end to another during processing could also result in cross-contamination to uninfected birds, which could subsequently be transferred to humans. In general, however, conveyer belts are not utilized during processing in Ghana.

Farm workers, slaughterhouse and poultry plant workers and butchers are all at an enhanced risk of infection from these enteropathogenic bacteria. This group of people could be a source of infection to healthy individuals with

whom they come into contact if adequate personal hygiene practices are not adhered to.

Statistical analysis (**Appendix 6**) showed that there were no significant differences in the level of *Campylobacter* contamination of the cloacal swabs from the three (3) different farms.

Campylobacter, however, was not detected in the dressed birds and carcasses. Storage time and exposure to air have been noted to affect the detection of *Campylobacter* (Willis and Murray, 1997). *Campylobacter* is known to be very much associated with chicken livers, freshly dressed birds and offal (Bolten *et al.*, 1985). None of these were examined since the study analyzed for only poultry meat available for retail. Commercial chicken livers were not available on the markets that were surveyed and therefore not examined.

Despite the above, a high isolation rate in other studies from dressed birds have been made. It has to be noted that the incidence of contamination on chilled birds could exceed 50% at retail level as shown by Fricker and Park (1989).

Four *Campylobacter* serovars, *C. jejuni I, C. jejuni* 2, *C. jejuni var doylei* and *C. upsaliensis* were isolated (**Table 5**) from the live birds. *C. jejuni I* occured

most frequently, forming 42.8% (6) of the total isolates, followed by *C. upsaliensis,* 28.5% (4), *C. jejuni var doylei*, 21.5% (3) and *Campylobacter jejuni 2,* (7.2%) (1).

Zanetti *et al.*, 1996 noted that the most common *Campylobacter* species associated with chicken was C. *jejuni* followed by *C. coli.* Tresierra-Ayalla *et al.* (1995) indicated similar results when he also showed that *C. jejuni* followed by *C. coli* were the most frequent thermotolerant species in chickens.

Table 5: *Campylobacter* serovars isolated from live chicken birds.

SEROVAR	LOCATION			TOTAL(%)[a]
	FARM A	FARM B	FARM C	
C. jejuni 1	-	5	1	6 (42.8)
C.upsaliensis	-	1	3	4 (28.5)
C. jejuni var Doylei	1	-	2	3 (21.5)
C. jejuni 2	-	1	-	1 (7.2)
Total	1	7	6	14

[a] = Percent positive of total isolates

The study therefore confirmed the findings of these previous studies as *C. jejuni* biotype I was the most frequent isolate in the study (**Table 5**). This

should be of great concern since *Campylobacter jejuni* Biotype I and II are known to be leading bacterial causes of gastroenteritis in man (Black, 1990).

Seven live birds (7.2 %) from the poultry farms were positive for *Salmonella*. This constitute Farm A, 6 (6.2%) and Farm B, 1 (1.0%). None of the live birds from Farm C was positive for *Salmonella* (**Table 6**).

Table 6: Incidence of *Salmonella* species in live chicken birds from poultry farms.

Location	Number of positive Samples / visit			Total / Location	%+ve	(%)[a]
	1	2	3			
Farm A	0/10	0/10	6/20	6/40	15.0	6.2
Farm B	0/10	1/17		1/27	3.7	1.0
Farm C	0/10	0/20		0/30	0.0	0.0
TOTAL	0/30	1/47	6/20	7/97		7.2

[a] = Percent Positive of total live bird samples

Animal feeds have been known to be contaminated with enteropathogenic bacteria, for example, *Salmonella* (Harris *et. al.*, 1997). Birds feeding on such contaminated feeds could become infected. Feed analysis was not undertaken during the study. It is however important that these feeds are

examined for their microbial load as they could be a source of infection.

Studies have implicated water as a source of infection for enteropathogenic bacteria. Oo *et al.* (1991) and Berndston *et al.* (1996) implicated water as a source of infection of *Salmonella* and *Campylobacter* respectively.

Water was implicated as a possible source of infection of *Salmonella* on Farm A. This information was given by the Management during a follow up investigation at Farm A after the third visit, where 6 out of the 20 birds sampled were positive for *Salmonella.* The Management reported a high death rate among the birds and suspected that the source of infection could be from the supply of drinking water. This water was taken from big tanks (illustrated in **PLATE 1** on page 60) which could easily be contaminated by flying birds that were known to also drink from them when left opened. A contaminated bird could introduce the bacterium into the drinking water by defecating into the tank. This showed the possibility of animal to animal transmission. This drinking water was not analyzed because at the time these findings were made the water had been exhausted.

There is therefore the need to use potable water directly from the taps for watering these birds. The unreliability of this source of water in Ghana however, makes it necessary to store potable water. It is necessary that this stored supply of water is provided with an outlet so that it is covered all the

time. This would prevent other animals from drinking from it directly so that contamination from both within and without could be avoided. It is advisable to sterilize the water, by boiling, before using it for drinking purposes.

As was observed during the study, the feeding troughs and drinking water were contaminated with a lot of fecal matter. This was as a result of the birds defecating into them. The feed and water could therefore be easily contaminated if the birds are carriers of these enteropathogens. Other birds consuming this contaminated feed and water could also become infected. New improved feeding and drinking containers whereby only the heads of the birds get assess to the food and water should be used. These containers apart from preventing fecal contamination in the food and water also saves cost by reducing wastage.

The mass production of birds on the farms do not only encourage the spread of organisms but also a build up of potential infection in the environment. It is necessary therefore to constantly clean and disinfect the environment of these birds. The feeding troughs and drinking containers should be constantly cleaned. The floor covering material (sawdust in this case) should be changed on regular basis.

Statistical analysis (**Appendix 7**) showed that there were no significant differences in the level of *Salmonella* contamination in the cloacal swabs

from the three (3) different farms.

Eggs have been associated with bacteria infection, especially *Salmonella* food-borne diseases (Humphrey *et al.*, 1989) and infection has been known to be at the trans-ovarian stage. Birds could therefore become infected even before they are hatched. The consumption of raw eggs and or products made with raw eggs could therefore result in *Salmonella* infection if the birds are contaminated as was shown in this study. It is essential that eggs are properly cooked before intake. Consumption of raw eggs should be discouraged.

Most homes in the developing world, including Ghana, purchase live birds and slaughter them at home unlike the developed countries where dressed birds are mostly preferred. This situation may provide a good opportunity for transmission during slaughter and evisceration and the subsequent contamination to other food materials if handling practices are poor. Cross contamination from the slaughtered birds to food, which is not cooked, undercooked or not reheated before consumption could therefore play a major role in cases of food borne infections in our homes.

Raw meat, including poultry are known to be commonly contaminated with enteropathogenic bacteria including *Salmonella and Campylobacter.* Six chicken carcasses (6.8%) were positive for *Salmonella* and these were from

only the market samples. This was made up of Market 2 (2.3%), Coldstore (2.3%), Market 1 (1.1%) and Supermarkets (1.1%). None of the carcasses from the poultry farms was positive for *Salmonella* (**Table 7**). Fricker and Park (1989) indicated that the incidence of enteropathogenic bacteria contamination varied according to species with poultry being very high and also varies according to agricultural practices and hygiene standards during slaughter and subsequent handling. Morton (1993) implicated poultry products as food sources for *Salmonella* infection leading to gastrointestinal infection.

Table 7: Incidence of *Salmonella* on chicken carcasses from open markets and supermarkets

Location	Number of positive Samples / visit		Total/ Location	%+ve	(%)[a]
	1	2			
Supermarkets	1/10	0/4	1/14	7.1	1.1
Open market					
Market 1	1/4	0/10	1/14	7.1	1.1
Market 2	2/6		2/6	33.3	2.3
Coldstore	2/9	0/4	2/13	15.3	2.3
Total			6/47		6.8

[a] = Percent Positive of total carcass sample

Four (4) different *Salmonella* serovars were identified from the 13 isolated i.e. 7 from the live birds and 6 from the carcasses. These are *Salmonella*

serovars 08, 09, 018 and, 021. The highest number isolated was seven from Farm A (**Table 8**). Two were isolated from Market 2 and one each from Market 1, the Coldstore and Farm B. *Salmonella* serovars 08, 018, and 021 were isolated from the intestinal contaminants from the poultry birds whilst *Salmonella* serovar 09 was from the dressed carcasses. The predominant isolate from the intestinal content was *Salmonella* serovar 021 forming 38.4% of the total *Salmonella* isolated. There were no isolates from the chicken carcasses from the poultry farms.

Table 8: *Salmonella* strains isolated from live chicken birds and carcasses.

LOCATION	SEROVAR 08	09	018	021	Total
Farm A	1	-	1	5	7
Farm B	1	-	-	-	1
Farm C	-	-	-	-	0
Supermarkets	-	1	-	-	1
Openmarkets					
Market 1	-	1	-	-	1
Market 2	-	2	-	-	2
Coldstore	-	1	-	-	1
Total (%)[a]	2(15.3)	5(38.4)	1(7.6)	5(38.4)	13

[a] = Percent positive of total isolates

None of the live poultry birds was positive for *Shigella*. However six (6) carcasses representing 6.9% of the market samples were positive for

Shigella. Samples from the Coldstore gave the highest, 4.6%, with Market 2 giving 2.3% of the total samples. Also, none of the chicken carcasses from any of the Farms, Supermarkets and Market 1 was positive for *Shigella* **(Table 9).**

Table 9: Incidence of *Shigella* on chicken carcasses from open markets.

LOCATION	Number of positive Samples / visit		Total/ Location	%+ve	(%)[a]
	1	2			
Open market					
Market 1	0/4	0/10	0/14	0.0	0.0
Market 2	2/6		2/6	33.3	2.3
Coldstore	4/9	0/4	4/13	30.7	4.6
TOTAL			6/33		6.9

[a] = Percent positive of total carcass samples

The results from this study indicate that the problem of the spread of *Salmonella/Shigella* is not only confined to dirty environments as could be found in the open markets but also in supermarkets, that had their products neatly packed in refrigerators (illustrated in **PLATE 3** on pg. 62). Statistical analysis (**Appendices 8 & 9**) showed that there were no significant differences in the level of *Salmonella/Shigella* contamination of the chicken carcasses from the Open markets, Supermarkets and the Cold store.

Therefore regardless of where the chicken is purchased, one could still be at risk of infection by these bacteria. In some cases the chicken is repackaged at the supermarket level and this could result in the microbial contamination from a single bird being transferred to others through hands and equipment. Samples from Market 2 and the Coldstore showed 33.3% and 30.7% infection rate with *Shigella.* This is a high contamination rate. The contaminated birds were imported and it could be likely that contamination could have taken place prior to shipment into the country. Products on the markets are taken directly from the opened cartons and sold immediately (illustrated in **PLATE 5** on pg. 65). There is no refrigeration and the frozen meat is kept in the cartons till they are sold out. They are placed back in the coldstore if not sold out. Inadequate or intermittent refrigeration could be an important factor in the transmission of *Shigella* as these could allow the organisms to multiply. The presence of *Shigella* only on the imported market samples could result in cross contamination from the food samples to the handlers or vice versa. This is a serious finding and one wonders what is happening at the ports of entry.

Weighing scales used during retail were not cleaned between intervals of weighing and vending. Contaminated meat previously weighed could easily contaminate others through these scales. Low wages and lack of job security place pressure on these food handlers to work while unwell. Contamination could occur from young and inexperienced food handlers through the failure

to provide adequate hygiene training. These were very evident at the Coldstore and also could be the case in the countries of origin.

Two different *Shigella* serovars were identified from the open market and the Coldstore. These are *Shigella boydii* (57.1%) and *S. flexnerri* (42.9%) **(Table 10)**. One sample was positive for both serovars.

Table 10: *Shigella* serovars isolated from chicken carcasses obtained from open markets.

LOCATION	SEROVAR		
	S. boydii	*S. flexneri*	Total
Market 1	-	-	0
Market 2	1	2	3
Coldstore	3	1	4
Total (%)[a]	4 (57.1)	3 (42.9)	7

[a] = Percent positive of total isolates

Hayford (1977) isolated *Shigella flexneri* from fried chicken pieces. *Shigella boydii, Sh. flexneri, Sh. dysenteriae* and *Sh. sonnei* are all known to be causes of shiggellosis. The presence of *Shigella boydii* and *Shigella flexneri* in the samples (**Table 10,** above) therefore indicates the danger these products pose to humans when consumed without adequate washing and heating during preparation.

Chicken is known to be readily colonized by small populations of *E. coli* and they can become long-term excreters. In the Study, sixteen live birds (16.5%) were positive for *E.coli* in their intestinal content. All three farms showed positive results with Farm A having 8 (8.2%), Farm B, 6 (6.2%) and Farm C, 2 (2.1%) of the total birds sampled **(Table 11).** Statistical analysis **(Appendix 10)** showed that there were no significant differences in the level of *E. coli* contamination of the cloacal swabs from the three farms.

Table 11: Incidence of *Escherichia coli* in live chicken birds from poultry farms.

Location	Number of positive Samples / visit			Total/ Location	% +ve	(%)[a]
	1	2	3			
Farm A	0/10	2/10	6/20	8/40	20.0	8.2
Farm B	1/10	5/17		6/27	22.0	6.2
Farm C	0/10	2/20		2/30	6.6	2.1
TOTAL	1/10	9/47	6/20	16/97		16.5

[a] = Percent positve of total live bird samples

Five (5) of the chicken carcasses (5.7%) were positive for *E. coli*. These were from Farm A (2.3%), Farm B (2.3%) and Farm C (1.1%) **(Table 12).** Only the dressed birds from the poultry farms were positive for *E.coli*. .

Statistical analysis (**Appendix 11**) showed that there were no significant differences in the level of *E. coli* contamination of the chicken carcasses from the three (3) farms

Table 12: Incidence of *Escherichia coli* on chicken carcasses from poultry farms.

LOCATION	Number of positive Samples / visit		Total/ Location	%+ve	(%)[a]
	1	2			
Farm A	0/10	2/10	2/20	10.0	2.3
Farm B	2/10		2/10	5.0	2.3
Farm C	1/10		1/10	10.0	1.1
TOTAL			5/40		5.7

[a] = Percent positive of total carcass sample

Twelve different *E. coli* serovars were identified from the total of 21 positive samples (**Table 13**). These in order of magnitude isolated are *E. coli* 0158 (14.3%), 0125 (14.3%), 025 (9.5%), 028ac (9.5%), 0159 (9.5%), 015 (9.5%), 0126 (9.5%), 063 (4.8%), 0143 (4.8%), 026 (4.8%), 078 (4.8%), 0164 (4.8%). Statistical analysis (**Appendix 12**) showed that there were no significant differences in the levels of contamination of *E. coli* isolated from the cloacal swabs and chicken carcasses from the three (3) farms.

Similar factors as discussed above could be true for the prevalence of *E. coli* in the chicken samples. As have been discussed earlier, it is possible that poultry meat being retailed could be contaminated at the farms or through transport before being brought to the retail outlets. For example, *E. coli* 025 was isolated from the live and dressed birds simultaneously from the same farm (**Table 13**). This showed possible cross contamination during slaughter and handling. It is therefore necessary, no matter how tedious, that the dressed birds should be re-washed with potable water at the market level before retail to eliminate earlier contamination.

Fehlaber and Heseler (1989) reported that any assessment of *E.coli* in various foods together with an appraisal of its relevance to food hygiene will be of inadequate reliability unless the virulence properties of the *E. coli* are investigated as well. In this study therefore the *E. coli* strains isolated were tested for toxin production, specifically heat stable enterotoxin (ST-h and ST-p) and heat labile enterotoxin (LT) using DNA-DNA hybridization. This method has been proven to be highly sensitive in detecting virulence factors in *E. coli* (Samadpour *et al.,* 1990; Suthienkal *et al.*, 1990).

Table 13: *Escherichia coli* serovars isolated from live chicken birds and carcasses.

Serovar	LIVE BIRDS Farm			CARCASSES					
							Open Mkt	Super Mkt	Total(%)[a]
	A	B	C	A	B	C			
025	-	1	-	-	1	-	-	-	2 (9.5)
028ac	-	-	-	-	1	1	-	-	2 (9.5)
063	1	-	-	-	-	-	-	-	1 (4.8)
0143	1	-	-	-	-	-	-	-	1 (4.8)
0159	-	-	-	2	-	-	-	-	2 (9.5)
026	-	-	1	-	-	-	-	-	1 (4.8)
0158	2	1	-	-	-	-	-	-	3 (14.3)
0125	2	1	-	-	-	-	-	-	3 (14.3)
078	-	-	1	-	-	-	-	-	1 (4.8)
0164	1	-	-	-	-	-	-	-	1 (4.8)
015	1	1	-	-	-	-	-	-	2 (9.5)
0126	-	2	-	-	-	-	-	-	2 (9.5)
Total(%)[a]	8	6	2	2	2	1	-	-	21

[a] = Percent positive of total isolates

None of the *E. coli* isolates hybridized with the enzyme-linked oligonucleotide probes used indicating negative (colorless) results. The control strains used, i.e. *E. coli* 309775 LT and *E. coli* J 196 ST, showed positive (blue) results. Routine examination of *E. coli* isolates from chicken by Smith *et al.* (1991a)

using DNA probes also showed the absence of toxin producing strains. This reiterates the findings by Notermans *et al.* (1991) that the routine screening of *E. coli* isolated randomly from food for toxin production may not be useful and should be limited to investigation of outbreaks of foodborne disease with an etiology suggestive of *E. coli.*

From these findings It could be said that the presence of these enteropathogenic bacteria in live chicken and meat as found in the study should be of public health concern. Even though efforts are being made by poultry farmers and retailers to reduce contamination load, it should be noted that none of the currently available procedures could provide a pathogen-free poultry bird or meat. Thus for the foreseeable future, proper handling of poultry birds and meat is essential to prevent foodborne illness from *Campylobacter*, salmonellae and other enteric pathogens. Some suggestive critical control points to prevent foodborne illness include: preventing cross contamination from the raw products to ready–to-eat foods, adequate times and temperatures for cooking, avoiding recontamination after cooking by surfaces previously contaminated with the meat, and properly chilling and storing of the meat after cooking.

There is the need to monitor the microbial quality of chicken (live/meat products) on the Ghanaian market. The absence of microbiological monitoring could allow the importation of inferior quality chicken into the

country.

The antibiotic sensitivity of the various enteropathogen strains isolated was tested using the disk diffusion method. This method is known to provide a simple and reliable test specially applicable in routine Clinical Bacteriology. Commercially prepared dry antibiotic disks (refer to **Table 3** on pg. 69) were used for the assay and the degree of sensitivity determined using an interpretive chart for zone sizes (**Appendix 2**).

Results of disk diffusion tests vary with a number of experimental conditions that are difficult to standardize. It is therefore necessary to evaluate them by comparison with those of a control test with a standard organism of known sensitivity. It should be noted that this was not done due to the unavailability of control strains at the time of analysis.

The antibiogram study of the *Campylobacter* strains showed that fosfomycin, minocyclin, nalidixic acid, chloramphenicol, penicillin, amoxycillin, erythromycin and kanamycin were the most effective drugs against all the *Campylobacter* serovars isolated (**Table 14**). All the strains were resistant to Cefadoxil, ampicillin, ceftrioxone, cefotiam and tetracycline.

These results were similar to a study in rural Ghana (Agbodaze *et al.*, 1990). They examined the prevalence of *Campylobacter* in domestic birds free from

antibiotics. The strains isolated were sensitive to chloramphenicol, erythromycin, nalidixic acid, fosfomycin and gentamycin. In that study also, ampicillin (30ug) was effective against *Campylobacter* and this could have resulted from the higher dosage used as compared to that used in this study (10ug).

Sicinschi (1996) showed that most strains of *Campylobacter jejuni / coli* were susceptible to tetracycline, nalidixic acid, and all were susceptible to chloramphenicol, streptomycin and kanamycin. Murphy *et al.* (1996) reported that Ciprofloxacin and azithromycin resistant *Campylobacter* caused traveler's diarrhea in U.S. troops deployed to Thailand in 1994. Hirschl *et al.* (1990) showed that all strains of *C. jejuni/coli* proved to be susceptible to ciproflaxin, and most strains were susceptible to erythromycin. Altwegg *et al.* (1987) reported that nalidixic acid was effective against *C. jejuni* and *C. coli* but with time some resistant strains have been detected. Our work has confirmed the findings of most of these studies.

Pai *et al.* (1983) recommended erythromycin and nalidixic acid as effective drugs against *Campylobacter spp.* Results from this study also has shown that these antibiotics could be recommended for therapy.

Table 14: Antibiotic Susceptibility Test on *Campylobacter* isolates from live chicken birds.

Antimicrobial agent		*C. Jejuni* 1	*Jejuni* II	*Campy. Upsalienis*	*C. jejuni var doylei*
Fosfomycin	200ug	++	++	++	++
Minocyclin	200ug	++	++	++	++
Nalidixic Acid	30mcg	++	++	++	++
Cefadroxil	30ug	R	R	R	R
Chloramphenicol	30ug	++	++	++	++
Ampicillin	10ug	R	R	R	R
Ceftrioxone	30ug	R	R	R	R
Penicillin	10 IU	++	++	++	++
Amoxycillin	30ug	++	++	++	++
Kanamycin	30ug	++	++	++	++
Cefotiam	30ug	R	R	R	R
Tetracycline	30ug	R	R	R	R
Erythromycin	15ug	++	++	++	++

R = Resistant ++ = Susceptible

NP = Not Performed + = Moderately susceptible

Antibiotic susceptibility tests performed on the *Salmonella* serovars indicated varied results with the different antibiotics (**Table 15**). Fosfomycin was effective against *Salmonella* serovars 08, 09, and 018 with 021 being resistant. *Salmonella* serovars 018 and 021 were resistant to minocyclin whilst serovar 09 was susceptible and serovar 08 moderately susceptible. Nalidixic acid was moderately effective against serovars 08, 09,and 018 and very effective against serovar 021. Chloramphenicol was moderately

effective against 09, 018, 021 with serovar 08 being resistant to it. All the isolates were resistant to erythomycin, cefotiam, penicillin, ampicillin and cefadroxil. *Salmonella* serovars 08, 018, and 021 were resistant to tetracyclin. Ceftrioxone was effective against *Salmonella* 018 and 021, moderately susceptible against serovar 09 with serovar 08 being resistant to it. Serovar 08 was the only strain that showed resistance to amoxycillin.

Only fosfomycin and chloramphenicol were effective against *Shigella boydii* and *Shigella flexnerii* **(Table 15).** Minocyclin, nalidixic acid and kanamycin were only moderately effective against the 2 strains of *Shigella*. Ampicillin was not tested in this instance due to its unavailability. Erythromycin was also not tested against *S. flexnerii* for the same reason.

The study detected multiple resistant strains of *Salmonella* species. Multiple resistance has been shown in strains of *S. typhi* and this include resistance to chloramphenicol, trimethoprin, ampicillin, and sulphanamides (Luque *et al.*, 1994, Poppe *et al.*, 1996). Rowe *et al.* (1987) indicated that chloramphenicol should be a drug of choice for commencing treatment whilst awaiting antibiotic sensitivity results.

Poppe *et al.* (1996) showed a high strain suceptibility to chloramphenicol, cotrimaxazole, gentamicin, kanamycin, neomycin and nitrofurantoin. Mandal and Brennard, 1988 also recommended chloramphenicol, ampicillin and

cotrimoxazole for the treatment of *Salmonella* enteritis infection.

Table 15: Antibiotic Susceptibility test on *Salmonella / Shigella* isolates from live chicken birds and carcasses.

Antimicrobial agent		*Salmonella*				*Shigella*	
		08	09	018	021	*boydii*	*flexneri*
Fosfomycin	200 ug	++	++	++	R	++	++
Minocyclin	200 ug	+	++	R	R	+	+
Nalidixic Acid	30 mcg	+	+	+	++	+	+
Cefadroxil	30 ug	R	R	R	R	R	R
Chloramphenicol	30 ug	R	+	+	+	++	++
Ampicillin	10 ug	R	R	R	R	NP	NP
Ceftrioxone	30 ug	R	+	++	++	R	R
Penicillin	10 IU	R	R	R	R	R	R
Amoxycillin	30 ug	R	+	++	+	R	R
Kanamycin	30 ug	+	+	R	R	+	+
Cefotiam	30 ug	R	R	R	R	R	R
Tetracycline	30 ug	R	+	R	R	R	R
Erythromycin	15 ug	R	R	R	R	R	NP

R = Resistant NP = Not Performed

++ = Susceptible + = Moderately susceptible

Varied antimicrobial resistance among different strains of *Salmonella* has been shown in this study and this therefore emphasizes the need to undertake antimicrobial susceptibility testing before making recommendation for therapy. From the results of this study, chloramphenicol and nalidixic acid

are also recommended for the treatment of *Salmonella* infection.

Anon (1988) recommended the use of nalidixic acid in shigellosis. Nalidixic acid or norfloxacin has been recommended for multiple resistant strains of *Shigella* that may require antimicrobial therapy. The current study confirmed findings from previous studies where nalidixic acid was moderately effective against *S. boydii* and *S. flexneri*. Weissman *et al.* (1974) recommended ampicillin and cotrimexazole. Since these 2 antibiotics were not tested one cannot comment on them. Ampicillin was not tested against the *Shigella spp.* due to its unavailability. Erythomycin was not used against *S. flexneri* for the same reason.

Cefadroxil, ampicillin, penicillin, cefotiam, tetracyclin and erythromycin were ineffective against all strains of *E. coli* isolated **(Table 16)**. Minocyclin was effective against all strains with the exception of *E. coli* 0159, 025, 0164 and 063 which were moderately susceptible. All strains with exception of *E.coli* 0164 were susceptible to Fosfomycin. Nalidixic acid, chloramphenicol, kanamaycin, ceftrioxone and amoxycillin all showed varied effectiveness against the strains isolated.

The high proportion of strains of *E. coli,* which showed resistance to these antibiotics could be related to widespread use of antibiotics by the public. *E. coli* form part of the normal micro-flora found in the gut of man. Thus

indiscriminate use of antibiotics could result in the possible development of resistant strains.

However, as the strains showed varied resistance against the various antimicrobials recommendation of any antibiotic agent against any Enteropathogenic *E. coli* should therefore not be generalized but must be based on laboratory results. In general intravenous administration of antibiotics like gentamicin and trimoxazole have been recommended (Thoren *et al.*, 1980).

It should be said that several studies (Duguid *et al.*, 1978; Thoren *et al.*, 1980; Berkow, 1982) have indicated that infection by these enteropathogens are often self limiting. Generally treatment with antibiotics is not desirable since they are often ineffective and also could increase the likelihood of development of a carrier state which would promulgate the further spread of antibiotic resistance. In cases of severe diarrhea, rehydrative restoration of electrolyte balance may be required. In general, glucose electrolyte mix, or oral rehydrative salt (ORS) is recommended for treatment.

Table 16: Antibiotic susceptibility tests on *E. coli* isolates from live chicken birds and carcasses.

Antimicrobial agent		*Escherichia coli* Serovar LIVE BIRDS (LB)							
		0158	026	0143	0126	0125	078	063	0164
Fosfomycin	200ug	++	++	++	++	++	++	++	R
Minocyclin	200ug	++	++	++	++	++	++	+	+
Nalidixic Acid	30mcg	+	+	+	++	+	+	+	+
Cefadroxil	30ug	R	R	R	R	R	R	R	R
Chloramphenicol	30ug	R	++	++	++	++	++	++	++
Ampicillin	10ug	R	R	R	R	R	R	R	R
Ceftrioxone	30ug	++	+	R	+	+	+	+	++
Penicillin	10 IU	R	R	+	++	R	R	R	R
Kanamycin	30ug	+	+	+	+	+	+	+	+
Cefotiam	30ug	R	R	R	R	R	R	R	R
Tetracycline	30ug	R	R	R	R	R	R	R	R
Erythromycin	15ug	R	R	R	R	R	R	R	R
Amoxycillin	10 IU	NP	NP	R	R	NP	NP	NP	NP

Table 16: Continued

Antimicrobial agent		*Escherichia coli* Serovar LB CARCASSES			
		015	*0159*	*025*	*028ac*
Fosfomycin	200ug	++	++	++	++
Minocyclin	200ug	++	+	+	++
Nalidixic Acid	30mcg	R	+	R	+
Cefadroxil	30ug	R	R	R	R
Chloramphenicol	30ug	++	R	++	++
Ampicillin	10ug	R	R	R	R
Ceftrioxone	30ug	R	+	+	R
Amoxycillin	10 IU	R	NP	R	R
Penicillin	10 IU	+	R	R	+
Kanamycin	30ug	+	+	R	+
Cefotiam	30ug	R	R	R	R
Tetracycline	30ug	R	R	R	R
Erythromycin	15ug	R	R	R	R

R = Resistant NP = Not Performed

++ = Susceptible + = Moderately susceptible

Oxytetracycline is an antibiotic used in the diet of these birds **(Appendix 5)**. The presence of this antibiotic could have resulted in the resistance of

almost all the strains isolated from the birds to tetracycline. Feed additives, which are laden with antibacterials could lead to the development of drug resistance among the enteric pathogens that are exposed to them (Ojeniyi, 1989).

The ineffectiveness of tetracycline against almost all the strains in the study could have resulted from the selection of resistant strains due to constant exposure from the feed. *Salmonella* serovar 09 was not isolated from the intestinal content but rather from the market samples. This could explain why serovar 09 was moderately susceptible to tetracycline, as it may not have been previously exposed to it.

It is therefore necessary that the use of oxytetracycline as a constituent of poultry diet should be suspended for some time to reduce the prevalence of resistant strains to a minimum.

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

The objective of this study was to assess the enteropathogenic bacterial quality of chicken from some selected farms, wholesale and retail outlets in the Accra Metropolitan Area. It evaluated the prevalence of *Campylobacter, Salmonella, Shigella* and *Escherichia coli* species.

It can be concluded from the study that:

i. Chicken on sale in the Accra Metropolis could be a source of *Salmonella, Shigella, Campylobacter sp.* and enteropathogenic *E. Coli.*

ii. *Campylobacter spp.* appears to be particularly associated with cloacal contents of live birds and *Shigella spp.* with imported chicken carcasses.

iii. Enteric pathogens from locally produced chicken showed resistance to oxytetracycline, a common antibiotic in chicken feed.

iv. There are no significant differences in the prevalence of the enteric pathogens in the various retail outlets.

5.2 RECOMMENDATIONS

Based on the above it is recommended as follows:

i. There is the need for microbiological monitoring of both locally produced and imported chicken.

ii. The use of oxytetracycline as a constituent of poultry diet should be suspended to reduce the prevalence of resistant strains to a minimum.

iii. Bactericidal steps, like the use of curing and pH reducing agents, should be encouraged in the slaughtering and packaging chains.

iv. Weighing scales used during vending of poultry meat should be regularly cleaned during vending or be covered with different polythene layers between each vending process to prevent cross contamination.

v. Effective heating during cooking should be ensured, as this will prevent the survival and transmission of these enteropathogens.

vi. Hand washing during preparation of food with poultry, both for home consumption and commercial purposes, should be an integral part of personal hygiene.

vii. Further studies should be carried on the possible sources of enteric pathogens on farms and during vending.

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APPENDICES

APPENDIX 1: Biochemical tests on API Campy strip

Biochemical test	Strip Abbreviation
Urease	URE
Nitrate production	NIT
Esterase production	EST
Hippurate hydrolysis	Hip
Gamma Glutamyl transferase	GGT
Triphenyl Tetrazolium chloride	TTC
Pyrrolidonyl Arylamidase	Pyr A
L-Arginine Arylamidase	Arg A
L-Aspartate Arylamidase	Asp A
Phosphatase Alkaline Phosphatase	PAL
Hydrogen sulfide production	H_2S
Glucose Assimilation	Glu
Succinate Assimilation	SUT
Nalidixic Acid Susceptibility	NAL
Cefazoline Susceptibility	CFZ
Acetate Assimilation	ACE
Propionate Assimilation	Prop
Malate Assimilation	MLT
Citrate Assimilation	CIT
Erythromycine susceptibility	ERO
Catalase test	CAT

APPENDIX 2: Interpretative chart for zone sizes[a]

Antibiotic Disc Agent		Diameter of zone inhibition (mm) Susceptible potency		
		Resistant	**Moderately**	**Susceptible**
Amikacin	30 ug	<14	15-16	>17
Ampicillin	10 ug	<13	14-16	>17
Benzyl-penicillin	10 IU	<28	-	>28
Ceftriazone	30 ug	<13	14-20	>21
CefuroximeSodium	30 ug	<14	15-17	>18
Cefalotin	30 ug	<14	15-17	>18
Chloramphenicol	30 ug	<12	13-17	>18
Clindamycin	2 ug	<14	15-20	>21
Cotrimoxazole	25 ug	<10	11-15	>16
Erythromycin	15 ug	<13	14-22	>23
Gentamicin	10 ug	<12	13-14	>15
Nalidixic Acid	30 ug	<13	14-18	>19
Nitrofurantoin	300 ug	<14	15-16	>17
Oxacillin	1 ug	<10	11-12	>13
Piperracillin	100 ug	<17	18-20	>21
Sulfonamides	300 ug	<12	13-16	>17
Tetracyclin	30 ug	<14	15-18	>19
Trimethoprim	2 ug	<10	11-15	>16

[a] National Comittee for Clinical Laboratory Standards. 1990. Voluntary consensus standards clinical laboratory testing. Villanova,PA, NCCLS.

APPENDIX 3: Biochemical tests on API 20

Biochemical test	Strip abbreviation
Beta-Galactosidase	ONPG
Arginine Dihydolase	ADH
Lysine Decarboxylase	LDC
Ornithine Decarboxylase	ODC
Citrate deSimmons	CIT
H_2S production	H_2S
Urease	URE
Tryptophan Deaminase	TDA
Indole	IND
Voges Proskauer	VP
Gelatin Hydrolysis	GEL
Glucose	GLU
Mannitol	MAN
Inositol	INO
Sorbitol	SOR
Rhamnose	RHA
Saccharose / Sucrose	SAC
Melbiose	MEL
Amygdalin	AMY
Arabinose	ARA
Oxidase	OX
Nitrite Production	NO_2
Nitrogen Production	N_2
Motility	MOB
Growth on MacConkey	McC
Oxidation	OF/O
Fermentation	OF/F

APPENDIX 4: Preparation of reagents for the detection of virulence mechanism of E.coli using DNA-DNA hybridization.

20 X SSC. 1% SDS

175g sodium chloride, 88g sodium citrate dehydrate pH 7.0, brought up to 1.0 liter with deionized water. Filter buffer through an 0.45 um nitrocellulose filter and store at room temperature.

5 X SSC. 1% SDS

250 ml of 28 X SSC diluted to approximately 500 ml with deionized water, mix. Add 10g Sodium dodecyl sulfate (SDS), warm, mix and dilute to 1.0 liter with deionized water. Store at room temperature.

2 X SSC. 1% SDS

50 ml of 20 X SSC, dilute to 450 ml wit deionized water, mix, add 10 ml Triton-100, mix well, adjust volume to 495 ml wiyh deionized water.

0.5 NaOH. 1% SLS

Dissolve 20g sodium hydroxide pellets and 10g Sodium N-Lauryl sarcosine in 1.0 liter deionized water. Store in a plastic bottle at room temperature.

1 M Tris-HCl

Dissolve 121.14g tris aminomethane in 500 ml deionized water. Adjust pH 7.4 with HCl, fill to 1.0 liter with deionized water.

APPENDIX 5: Content of poultry diet

Ingredient	Weight (Kg)
Maize	520
Copra-cake	140
Fish meal	200
Wheat Bran	82
Shell	57
Salt	1
Vetrimycin A/D Premix	2
Vetrivite	2

Vetrimycin A/D is a premix for poultry and livestock, containing 2.5 grammes oxytetracyclin in 454 grammes vitamin A and D, valuable B-vitamins and growth factors.

Vertrivite is a vitamin feed supplement for poultry to boost vitamin levels.

APPENDIX 6: Chi –Square Results Expectation for Table 4

FARM	1	2	3	TOTAL
A	0	0.047	0.003	0.050
B	0	0.383	0.027	0.410
C	0	0.280	0.020	0.300
TOTAL	0	0.710	0.050	0.760

Number of Columns = 3

Number of Rows = 3

Alpha Error = 0.05

Degrees of Freedom = 4

Results

Critical χ^2 = 9.48773

Computed χ^2 = 0.7600

P – Value = 0.9436

Conclusion

Do not reject hypothesis

Appendix 7: Chi –Square Results Expectation for Table 6

FARM	1	2	3	TOTAL
A	0	0.043	0.257	0.300
B	0	0.007	0.043	0.050
C	0	0	0	0
TOTAL	0	0.050	0.300	0.350

Number of Columns = 3

Number of Rows = 3

Alpha Error = 0.05

Degrees of Freedom = 4

Results

Critical χ^2 = 9.48773

Computed χ^2 = 0.3500

P – Value = 0.9868

Conclusion

Do not reject hypothesis

Appendix 8: Chi –Square Results Expectation for Table 7

LOCATION	1	2	TOTAL
Super Market	0.10	0	0.10
Market 1	0.25	0	0.25
Market 2	0.33	0	0.33
Coldstore	0.22	0	0.22
TOTAL	0.90	0	0.90

Number of Columns = 2

Number of Rows = 4

Alpha Error = 0.05

Degrees of Freedom = 3

Results

Critical χ^2 = 7.81473

Computed χ^2 = 0

P – Value = 1

Conclusion

Do not reject hypothesis

Appendix 9: Chi –Square Results Expectation for Table 9

LOCATION	1	2	TOTAL
Market 1	0	0	0
Market 2	0.33	0	0.33
Coldstore	0.44	0	0.44
TOTAL	0.77	0	0.77

Number of Columns = 2

Number of Rows = 3

Alpha Error = 0.05

Degrees of Freedom = 2

Results

Critical χ^2 = 5.99147

Computed χ^2 = 0

P – Value = 1

Conclusion

Do not reject hypothesis

Appendix 10: Chi –Square Results Expectation for Table 11

FARM	1	2	3	TOTAL
A	0.051	0.298	0.152	0.500
B	0.039	0.232	0.118	0.390
C	0.010	0.060	0.030	0.100
TOTAL	0.100	0.590	0.300	0.990

Number of Columns = 3

Number of Rows = 3

Alpha Error = 0.05

Degrees of Freedom = 4

Results

Critical χ^2 = 9.48773

Computed χ^2 = 0.5217

P – Value = 0.9716

Conclusion

Do not reject hypothesis

Appendix 11: Chi –Square Results Expectation for Table 12

FARM	1	2	TOTAL
A	0.12	0.08	0.20
B	0.12	0.08	0.20
C	0.06	0.04	0.10
TOTAL	0.30	0.20	0.50

Number of Columns = 2
Number of Rows = 3
Alpha Error = 0.05
Degrees of Freedom = 2

Results

Critical χ^2 = 5.99147
Computed χ^2 = 0.5000
P – Value = 0.7735

Conclusion

Do not reject hypothesis

Appendix 12: Chi –Square Results Expectation for Table 13

E. coli	FARM A	FARM B	FARM C	TOTAL
025	0.952	0.762	0.286	2
028 ac	0.955	0.762	0.286	2
063	0.476	0.381	0.143	1
0143	0.476	0.381	0.143	1
0159	0.952	0.762	0.286	2
026	0.476	0.381	0.143	1
0158	2.381	1.905	0.714	5
0125	2.381	1.905	0.714	5
078	0.476	0.381	0.143	1
0164	0.476	0.381	0.413	1
015				
0126				
TOTAL	10	8	3	21

Number of Columns = 3

Number of Rows = 10

Alpha Error = 0.05

Degrees of Freedom = 18

Results

Critical χ^2 = 28.8693

Computed χ^2 = 25.8475

P – Value = 0.1032

Conclusion

Do not reject hypothesis