CAMPYLOBACTER AND RELATED ORGANISMS ISOLATED FROM BROILER CARCASSES IN GROCERIES IN KHARTOUM NORTH, SUDAN

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DEDICATION

To The Soule of my Father
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Abstract

This study was carried out during the period June to August 2008. Hundred samples were collected randomly from carcass cuts (Breast, thighs and filleting) of broilers chicken from different retail market places in Khartoum state.

The study aimed to isolate *Campylobacter* spp from broiler meat using two types of selective media, Skirrow’s selective medium and *Campylobacter* agar base medium. The optimal incubation condition used for culturing was 42°C for 48°C hour.

Optimal micro aerobic growth required for *Campylobacter jejuni* was achieved by using anaerobic jar with microaerophilic gas generating kits, containing 5%O2, 10% CO2, and 85%N2.

All of samples were negative to *Campylobacter* spp, however different species of Enterobacteriaceae were identified in the condition of *Campylobacter*. The isolated Enterobacteriaceae were as follow 55% *Klebsiella* spp, 17% *Enterobacter* spp, 16% *Pasteurella. Haemolytica* spp and 12% *Listria* spp.

This finding indicated that poultry meat may introduce several species of bacteria pathogens during processing that can grow in *Campylobacter* medium.

The findings were considered to be of value in health education programs on food hygiene and safety in Sudan.
العمليات

2008 س 6. نعه. 

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\text{دواء (وعاء)} \rightarrow \text{جلد} \rightarrow \text{تولد} \rightarrow \text{كلب} \rightarrow \text{ذك} \rightarrow \text{مع} \rightarrow \text{كلب} \rightarrow \text{جلد} \rightarrow \text{وعاء} \rightarrow \text{دواء}
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\]


CHAPTER ONE
Introduction

The bacteriological safety of poultry meat and products has assumed paramount importance for industry, consumers, and public health officials. The first consumer’s right is to have products of good quality and not constituting any health hazard. Poultry meat is highly desirable, palatable, digestible and nutritious for all age's. In addition, it is low in price in comparison to beef and mutton.

Quality products are those meet which are safe to of consumers. Meat and poultry products are frequently associated with food borne outbreaks.

*Campylobacter spp,* are common contaminants of live broilers and their environment (Genigeorgis and Collins, 1986). During the last years the number of food borne infection due to *Campylobacter* was higher than those related to *Salmonella spp,* as shown by (Stern *et al.*, 2000) and Solvent and Colin (1999). Commercial broiler processing operations such as scalding, defeathering, evisceration, and chilling may affect the level of carcass contamination by food borne pathogens and spoilage microorganism. Most processing steps increase microbial contamination of broilers. The potential operation to spread contamination carcass to processing equipments, water, other carcasses and personnel has been reported (Stern *et al.*, 2001). The zoonoses which occur most frequently in the industrialized world to day are food borne infections caused by species of Salmonella and Campylobacter. The number of laboratory confirmed human cases of *Campylobacter* enteritis in England and Wales increased by 65% from 1991 to 2000 and there were approximately 53,800 cases reported in 2000 Public Health Laboratory Service (PHLS, 2001).

Worldwide, Salmonella and *Campylobacter* are the most important pathogen associated with poultry products (Bryan and Dolye, 1995).
It is well known that chickens are frequently colonized by *Campylobacter jejuni* subsp. *jejuni* therefore chicken meat is considered to be the source of most human infection with this species (Hopkins and Scott 1983; Deming *et al.*, 1987; Stern and Kazmi, 1989; Doyle, 1990; Altekruse *et al.*, 1998).

Members of genus *Campylobacter* are now recognized as the most common cause of acute bacterial enteritis, with *Campylobacter jejuni* being the dominant species in human infections.

Minimizing the risks of food poisoning due to *Salmonella, Campylobacter* spp. and similar pathogens in poultry products was discussed by many authors through the prevention and control of contamination of chicken carcasses through optimal rearing, transport and slaughter conditions (Humphrey, 1991; Mulderr, 1995; Aulik and Mourer, 1999). The use of carcass decontamination techniques should only be considered as supplementary to measures taken in the production chain.

**The objective of this study:**

1. To detect the occurrence of *Campylobacter* spp in raw poultry meat available to consumers in the retail market in Khartoum north.
2. To examine the market chicken meat for its safety for human consumption, with special reference to *Campylobacter* spp.
CHAPTER TWO

Literature Review

*Campylobacter* is a bacterial that causes gastrointestinal disease when lodges in the walls of a human intestine. There are two species of *Campylobacter* that cause human illness these are *Campylobacter jejuni* and *Campylobacter coli*. *Campylobacter jejuni*. Adding to the human and economic costs is chronic squeals associated with *C. jejuni* infection Guillain-Barré syndrome and reactive arthritis. In addition, an increasing proportion of human infections caused by *C. jejuni* are resistant to antimicrobial therapy. Mishandling of raw poultry and consumption of undercooked poultry are the major risk factors for human *Campylobacteriosis*.

*Campylobacter spp* was first described in 1880 by Theodore Escherichia sited by Friedman et al 2000. The name *Campylobacter* is derived from the Greek word "kampylos" which means curved. In 1886, Escherich observed organisms in stool samples of children with diarrhea. In 1913, Mc Faydean and Stockman identified *Campylobacter’s* in fetal tissues of aborted sheep and in 1957, Dr E. King isolated *Vibrio* from blood samples of children with diarrhea, and in 1972 clinical microbiologists in isolated Campylobacter for the first time from stool samples of patients with diarrhea (Kist, 1985). The development of selective growth media in the 1970 permitted more laboratories to test stool specimens for *Campylobacter*. Soon *Campylobacter spp.* are known to be a common human pathogen. *Campylobacter jejuni* infections are now the main cause of bacterial gastroenteritis reported in the United States (Tauxe, 1992).

The First isolation and identification of *C. jejuni* in Sudan is reported by (Ibrahim, 2006).


2.2. Bacteriology and Ecology of *Campylobacter*:

Characteristics of *Campylobacter*:-

According to Krieg and Holt (1984) *Campylobacter* spp are slender, spiral gram-negative rods which are motile by means of polar unsheathed flagella at one or both ends.

*Campylobacter* spp is microaerophilic requiring low oxygen, do not ferment sugars and have a lower guanine-cytosine (G+C) ratio (29-38 mol %). *Campylobacter* spp is oxidase and catalase positive. *C. jejuni* hydrolyzes hippurate- indoxyl and acetate and reduces nitrate, but is unable to oxidize or ferment carbohydrates. (Koenraad *et al.*, 1995).

*Campylobacter* cells dimensions of 0.2 mm too 0.8 mm Wide and 0.5 mm long. Extremely rapid, darting can be seen with a phase contrast microscope, with comma-shaped, S, or gull wing–shaped cell. As campylobacter cells begin to age, they become coccoid in shape (Moran and Upton, 1987).

*Campylobacter* is a fastidious organism that is capable to survive in wide range of environment. It has been isolated from rivers, estuarine, and coastal waters, at populations ranging from 10 to 230 colonies–forming units (cfu) (Bolton *et al.*, 1982, 1987). *Campylobacter jejuni* and *Campylobacter coli* are being able to grow at 37°C to 42°C with an optimum growing temperature of 42°C but will not capable to growth below 30°C (Friedman *et al.*, 2000). However, study by (Decease *et al.*, 2002) found that *C. jejuni* survived in excess of 27°C and 60% to 62% relative humidity on some common clean or soiled food contact surfaces. It has a d-value of less than one min at 60°C and is easily inactivated by heat. Freeze-thawing also reduces the population of
Campylobacter (Stern and Kazmi., 1989). The best degree of temperature to isolate Campylobacter species from broilers is 37°C – 42°C for 48 hours (Kreg and Holt., 1984). Campylobacter is inactivated by frozen storage at 5°C in 3 days (Stern and Kotula., 1982) however freezing does not eliminate the pathogen from contamination foods (Lee et al., 1998) and Hazeleger et al. (1995) discovered that aging C. jejuni cells survived the longest at 4°C. Campylobacter will not survive below PH 4.9. It’s capable of growing in the range of 4.9 to 9.0 PH, and optimally at PH 6.5 to 7.5. Campylobacter jejuni is unusually sensitive to oxygen and dehydration. Enzymes present in C. jejuni such as SuperOxide Dismutase (SOD), catalase, peroxidase, glutathione synthetase, and glutathione reductase are believed to play a vital role in providing protection against oxygen toxicity (Pesci et al., 1994, Purdy and Park, 1994.) Campylobacter requires a special atmosphere, which usually consists of 5% oxygen, 10% carbon dioxide, and 83% nitrogen for growth in or on laboratory media (Stern and Kazmi, 1989). Doyle and Roman (1982) examined the sensitivity of C. jejuni to drying. They demonstrated that several factors influenced the rate of activation of Campylobacter dried on a glass surface including bacterial strain, temperature, humidity, and the suspension medium.

The result of Doyle and Roman (1982) suggest that C. jejuni is quite sensitive to appropriate humidity, large numbers may survive drying and remain viable for several weeks.

2.3. Classification:

The classification of bacteria within the genus Campylobacter has changed frequently. Campylobacter spp. Show morphological and some biochemical similarity to Vibrio spp (Park, 1961). There are currently 18 spp, previously
classified as *Campylobacter spp* had been reclassified in the genus *Helicobacter* e.g. *Helicobacter pylori*, formerly *Campylobacter pylori* (Elmer, 1994). The genus *Campylobacter* is now classified along with the genus *Arcobacter* in a separate family *Campylobacteraceae*.

### 2.3.1. Species considered being of clinical importance:

(a) *C. jejuni*

It is found in the intestinal tract of poultry, dogs, cats, sheep and cattle. Enteric infection in animals has been reported (Krieg and Holt, 1984). It causes abortion in sheep and fever and enteritis in man, it infected people of all ages, more frequently diagnosed in children than adults (Krieg and Holt, 1984). Penner (1988) showed that *C. jejuni* is usually present in high number in diarrhea stools of individual.

(b) *C. coli*

*Campylobacter coli* are found in the intestinal tract of poultry and causes enteric infection in man (Krieg and Holt, 1984).

(c) *C. fetus*

This spices of *Campylobacter* infects human and animals it is associated with sporadic abortion in cattle and sheep. In man it causes fever and other symptoms it is usually isolated from blood culture from patients with some other debilitating condition (Krieg and Holt, and the other species:-

*C. lari*, *C. doylei*, *C. fetus ssp fetus*, *C. canaedie*, *C. cryarophila*, *C. fennelliae*, *C. hyointestinalis*, *C. mucosais*, *C. sputorum ssp bubulus*, *C. upsuliansis*. All these species except *C. fetus subsp fetus* are known as thermophilic *Campylobacter*
because they will grow at 42-43°C and 37°C but not at 25°C (Penner, 1988) and Elmer (1994).

2.4. Epidemiology:-

Farm animal reservoirs:

Poultry has been shown to be highly contaminated with *C. jejuni* and high isolation rates have been reported, ranging from 30% (Stern *et al.*, 1985) to 100% (Ibrahim, 2006). Consequently poultry, especially chicken has been considered a major reservoir of *C. jejuni*. The Communicable Disease Centre in the USA showed a strong correlation implicating chicken as a major vector to human enteric disease and indicated that chicken accounted for an a etiological factor of 70% of the risks contributing to the disease (Deming *et al.*, 1987). In the UK, (Pearson *et al.*, 1987) concluded that there was a definite correlation between human infection and consumption of chicken.

In another studies, sporadic cases were traced back to a chicken producer whose birds had the same serotype (*C. jejuni* Lior 1, Penner 4), as seen in clinical cases. These workers showed that when this strain was removed the human disease frequency diminished by 35%.

Park *et al.* (1991) suggested that the rearing of Campylobacter-free poultry was not economically feasible and that reduction in incidence should be attempted at the processing stage, through the addition of a solute or by drying operations, in order to reduce the amount of available water, thus creating a hurdle for the growth of these organisms.

*Campylobacter* is commensally organism routinely found in cattle, sheep, swine, and avian species. And this is the most common host for *Campylobacter*, probably because of their higher body temperature (Skirrow, 1977).
Campylobacter are also found in raw milk (Hutchison et al., 1985), in river water (Knill et al., 1982) and sewage (Arimi et al., 1988) which is all possible sources of infection. Campylobacter jejuni is the predominant species associated with food borne infection derived from poultry. Campylobacter coli and C.lari are occasionally recovered from the intestinal tract of poultry and have also been implicated in food borne infection (Cynthia et al., 2005). However, cattle frequently carry large numbers of Campylobacter’s in their intestinal contents, and sometimes suffer from mastitis due to Campylobacter, so that the source of infection for humans could be contaminated milk, or surface water contaminated from farm manure. Red meat offal also tend to be more highly contaminated than meat (Fricker and Park 1989; Bolton et al., 1999) Campylobacter enteritis is a world wide zoonotic disease and it is regarded as a food born disease rather than food poisoning (Robinson, 1981).

Campylobacter jejuni and Campylobacter coli account for the majority of human infection (Friedman et al., 2000). According to (Simmons and Gibbs, 1979) C.jejuni and C.coli are common in the intestine of many animals and as a consequence, are recovered from the surface of raw meat particularly chicken and offal. (Beutling, 1998) showed that the most cases of Campylobacteriosis are associated with handling raw poultry are eating raw or under cooked poultry meat, very small number of Campylobacter organism (fewer than 500 ) can cause illness from raw chicken meat can infected a person.

Outbreaks of infection have followed the drinking of untreated water from river, streams and lakes, and raw or improperly pasteurized milk from cows that have contaminated from their faces or infected udders (Robinson and Jones, 1981). Outbreaks have also occurred from row or improperly pasteurized cow’s milk and from sewage-polluted water (Finch and Blake 1985; Tauxe, 1992).
Campylobacter also survive well on meat, pork or poultry derived from infected animals, which may cause infection if eaten lightly cooked salted or smoked. Persons handling or preparing the raw flesh may infect themselves (Skirrow, 1982).

Contaminated water may introduce infection into poultry flocks, and unchlorinated water derived from Adam River or shallow well should be regarded as possible source (Cynthia and Lee, 2005). Rat’s wild bird, and house flies can infect flocks, equipment and foot wear contaminated with feces from an infected source may also serve as a vehicle of transmission (Cynthia and Lee, 2005). Once C. jejuni has been introduced into the environment, rapid transmission within the flock occurs, with subsequent or laying strain poultry. It is unclear whether C. jejuni can be transmitted vertically, either on the surface of eggs or by Tran’s ovarian transmission. (Cynthia and Lee, 2005).

Drinking water has sometimes been found to be the source of infection (Pearson et al. 1993) or a very significant risk factor (Kapperud et al., 1993). Similar observations have been made concerning detection of Campylobacter in the air and in flies and other sources, such as feed (Rosef and Kapperud 1983; Kazwala et al., 1990; Berndtson et al., 1996; Gregory et al., 1997).

Symptom less human carrier is mane common in developing countries but their role in the transmission of the disease is unknown (Riztiniemi, 1998). However, Buswell et al. (1998) suggested that C. jejuni can survive, although not multiply, in the biofilm which forms inside the water supply system, this would justify and chlorination of all water, and regular thorough dismantling and cleaning of the water supply system.
Betty and Diane (1993) showed that the incidence of *C. jejuni* and *C. coli* in a number of European countries was discussed their non significant source of outbreaks of human food poisoning. *C. jejuni* and *C. coli* are the predominant species associated with *Campylobacter* gastroenteritis in Sudan (Ibrahim, 2000).

Analysis of contamination showed by Solvate and Colin, (1999) that across contamination during processing is the main risk factors for the presence of *Campylobacter* spp in poultry carcasses. On study even suggested that handling raw chicken was protective (Adak *et al.*, 1995), although the high prevalence of *Campylobacter* infection among young male college students has been attributed to their preparing raw chicken in the kitchen (Hopkins and Scott 1983; Pearson *et al.*, 1987).

Fedrigh and Coppelier, (1995) found that one way of broilers to become infected is to cut the poultry meat on a cutting board and use the unwashed that cutting board or to prepare vegetables or other tightly cooked food.

**2.5. Prevalence of *Campylobacter*:-**

In the United States, estimated 2.1 to 2.4 in the million cases of human *Campylobacteriosis* (illnesses ranging from loose stools to dysentery) occur each year (Tauxe, 1992). Although recent reports by The Centers for Disease Control and Prevention (CDC), proclaim that the incidence of bacterial food borne pathogens is declining, *Campylobacter* is still the second most common bacterial pathogen after *Salmonella* in humans, with an incidence of 12.6 cases every 100,000 people for the year 2003. Many more cases go un diagnosed and therefore un reported (Omer, 2005). The pathogenesis of Guillian-Barré syndrome (GBS) and Reiter syndrome is not completely understood. It has been estimated that 500 persons die each year due to complications with *Campylobacter* infections. Some
strains of *C. jejuni* have also been incriminated in the production of Guillian-Barré syndrome, an acute neuromuscular paralysis, and Reiter syndrome, a reactive arthropathy (Omer, 2005).

Commonly reported symptoms of patients with laboratory-confirmed infections (a small subset of all cases) include diarrhea, fever, and abdominal cramping. In one study, approximately half of the patients with laboratory-confirmed *Campylobacteriosis* reported a history of bloody diarrhea (Blaser *et al.*, 1983).

The incidence of *Campylobacteriosis* in HIV-infected patients is higher than in the general population. For example, in Los Angeles County between 1983 and 1987, the reported incidence of Campylobacteriosis in patients with AIDS was 519 cases per 100,000 populations, 39 times higher than the rate in the general population. (Sorvillo *et al.*, 1991). Common complications of Campylobacteriosis in HIV-infected patients are recurrent infection and infection with antimicrobial-resistant strains (Peterson, 1994). Reports from both developing nations including Mexico (Marquez-Davila *et al.*, 1989), Brazil (Borges *et al.*, 1989), Bangladesh (Blaser *et al.*, 1980), Vietnam (Megraud *et al.*, 1989, 1991) and Thailand (Varavithya *et al.*, 1990) as well as from western countries including Scotland (Brunton and Heggie, 1977), Italy (Crotti *et al.*, 1989), the Netherlands (Severin, 1978) and the USA (Nachamkin *et al.*, 1992) have shown *Campylobacter* isolations to be equal to, and sometimes higher than those of any other enteric pathogen. *Campylobacter*’s belonging to the thermophilic group including *C. jejuni, C. coli, C. lari* and *C. upsaliensis* are now the most frequently isolated pathogens in many part of the world. In addition this upward trend in isolations both on the British mainland and in Northern Ireland is showing no sign of lessening. Unlike the salmonella and other enteric pathogens, the majority (case
99%) of clinical reports concerning *Campylobacter* is sporadic and *Campylobacter enteritis* outbreaks are rare (Cowden, 1992). *C.jejuni* and *C.coli* are the predominant species associated with *Campylobacter* gastroenteritis in Sudan (Elamin, 2000).

**2.6. Pathogenesis and Clinical Features:-**

The pathogenesis of *C. jejuni* infection involves both host- and pathogen-specific factors. The health and age of the host (Tauxe, 1992). In a volunteer study, *C. jejuni* infection occurred after ingestion of as few as 800 organisms (Black *et al.*, 1988).

Rates of illness appeared to increase when inoculate were ingested in a suspension buffered to reduce gastric acidity (Black *et al.*, 1988). The number of bacteria needed for infection to occur differs, for many types of bacteria this is in excess of 100,000 bacterial cells, but for *Campylobacter* the infecting dose may be as low as six cells. (Brooks, 2006).

Many pathogen-specific virulence determinants may contribute to the pathogenesis of *C. jejuni* infection, but none has a proven role (Ketley, 1997). Suspected determinants of pathogenicity include chemotaxis, motility, and flagella, which are required for attachment and colonization of the gut epithelium (Ketley, 1997). Once colonization occurs, other possible virulence determinants are iron acquisition, host cell invasion, toxin production, inflammation and active secretion, and epithelial disruption with leakage of serosal fluid (Ketly *et al.*, 1997). The all important factors in the virulence of *Campylobacter* enabling it to penetrate the viscous mucus which cover the epithelial surface of the gut. Studies with *C.jejuni* have demonstrated a chemotactic response to ward the sugar L-fucose, a number of
amino acids, and intestinal mucus from the mice and pigs (Adams and Moss, 2000).

Although *Campylobacter* does not normally process fimbriae it probably possesses other adhesions that enable it to adhere to epithelial cells once the mucosal barrier has been penetrated. It has been shown to be invasive in cell cultures and a number of toxins with cholera–like or cytotoxic activity has been described (Adams and Moss, 2000).

The incubation period is from 1 to 11 days, most commonly 3-5 days with malaise fever, severe abdominal pain and diarrhea as the main symptoms. The diarrhea is self–limiting and persists for up to a week, produces stool containing $10^6$-$10^9$ cells, which are often foul–smelling and can vary from being profuse and watery to bloody and dysenteric. Less frequently, *C. jejuni* infections produce bacteremia, septic arthritis, and other extra intestinal symptoms (Pesterson et al., 1999).

Gastrointestinal symptoms are sometimes preceded by a prodromal stage of fever headache and malaise which lasts about a day. Excretion of the organism continues for up to 2-3 weeks. Vomiting is a less common feature.

Complications are rare although reactive arthritis can develop and *Campylobacter* has been shown to cause the serious neurological disease, Guillain-Barré syndrome (GBS) (Adams and Moss, 2000).

(GBS) is a demyelating disorder resulting in acute neuromuscular paralysis, is a serious sequel of *Campylobacter* infection (Allos, 1997). An estimated one case of GBS occurs for every 1,000 cases of Campylobacteriosis (Allos, 1997). Up to 40% of patients with the syndrome have evidence of recent *Campylobacter*
infection (Allos, 1997). Approximately 20% of patients with GBS are left with some disability, and approximately 5% die despite advances in respiratory care. Campylobacteriosis is also associated with Reiter syndrome, a reactive arthropathy. In approximately 1% of patients with Campylobacteriosis, the sterile post infection (Peterson et al., 1994). Multiple joints can be affected, process occurs 7 to 10 days after onset of diarrhea particularly the knee joint. Pain and incapacitation can last for months or become chronic. Both GBS and Reiter syndrome are thought to be autoimmune responses stimulated by infection. Many patients with Reiter syndrome carry the HLA B27 antigenic marker (Peterson et al., 1994). The pathogenesis of GBS (Shoenfeldy et al., 1996) and Reiter syndrome is not completely understood. Deaths from C. jejuni infection are rare and occur primarily in infants, the elderly, and patients with underlying illnesses (Tauxe, 1992).

2.7. Sensitivity and Resistance to antimicrobial agents:-

*Campylobacter’s* are usually sensitive to erythromycin, nalidixic acid, ciprofloxacin, norfloxacin, tetracycline, and chloramphenicol (Elmer, 1994, and Rozhalostina et al., 1994, and DU Pont, 1991). They are highly resistant to trimethoprim and colistin, moderately resistant to penicillin and other beta-lactam antibiotic (David, 1995; and Pennerj, 1988). Fifty percent of strains are now resistant to tetracyclines, but 90% are still sensitive to <8µg/ml of chloramphenenicole (Tenover et al., 1992), all isolated strain of *Campylobacter. Jejuni* shoed almos the same pattern of antimicrobial sensitive to erythromycin, norfloxacin, ciprofloxacin, gentamicin and tetracycline. (Elamin, 2000).

Most strains are resistant to penicillin tetracycline and kanamycin resistance incretion C.jejuni strains (Cynthiam et al., 2005). *Campylobacter jejuni* they were
resistant to cotrimoxazole (Elamin, 2000). All isolated species of Campylobacter they were resistant to cotrimoxazole. (Fatima, 2003). Antimicrobial resistance can prolong illness and compromise treatment of patients with bacteremia. The rate of antimicrobial-resistant enteric infections is highest in the developing world, where the use of antimicrobial drugs in humans and animals is relatively unrestricted. In 1994 study found that most clinical isolates of C. jejuni from U.S. troops in Thailand were resistant to ciprofloxacin. Additionally, nearly one resistant to azithromycin (Murphy et al., 1996). In the third of isolates from U.S. troops located in Hat Yai were industrialized world; the emergence of fluoroquinolone-resistant strains of C. jejuni illustrates the need for prudent antimicrobial use in food-animal production (Piddock, 1995). Experimental evidence demonstrates that fluoroquinolone-susceptible C. jejuni readily become drug-resistant in chickens when these drugs are administered (Jacobs et al., 1996). After fluoroquinolone use in poultry was approved in Europe, resistant C. jejuni strains emerged rapidly in humans during the early 1990s (Piddock, 1995). Similarly, within 2 years of the 1995 approval of fluoroquinolone use for poultry in the United States, the number of domestically acquired human cases of ciprofloxacin-resistant Campylobacteriosis doubled in Minnesota (Smith and Muldoon, 1998). In a 1997 study conducted in Minnesota, 12 (20%) of 60 C. jejuni isolates obtained from chicken purchased in grocery stores were ciprofloxacin-resistant (Smith et al., 1998). Results of susceptibility testing of the nine strains of Campylobacter compared with result given by (Alawia Nadia and Hassan) indicated that the resistance of campylobacter to Quinolons and Macrolides has not developed yet in Sudan (Elamin, 2000).
2.8. Control and prevention:-

Prevention of *Campylobacter* infection in commercial species is based on strict bio security, decontamination of housing between successive flocks, exclusion of rodents and wild birds, and insect eradication. Chlorination of drinking water to 2ppm and operation of farms on a strict “all in, all out” basis occasionally reduces the prevalence of infection (Cynthia and Lee, 2005).

Schoeni (1986) suggested that one source of *C. jejuni* contamination of retail fresh mushrooms was handling of the product by staff with poor personal hygiene. However washing and thorough drying of contaminated hands has been shown to be effective in the elimination of this organism. Carcass chillers: poultry carcasses are required to be cooled rapidly to prevent bacterial growth (USDA, 2003). *Campylobacter* document it potential for cross-contamination in water chillers (Sanchez et al., 2002; Whyte et al., 2002). In the chill tank, chlorination with up to 50mg/l at ph 6.0 is required to control cross-contamination of poultry carcasses due to the increased organic load (Mcvicker et al., 1958; Mallman et al., 1959).

Study by Sanchez *et al.* (2002) found that *Campylobacter* levels and chilled carcasses were significantly higher in immersion chilling than air chilling. Most hazards to consumers could be eliminated if all carcasses were decontaminated immediately after slaughter, and dressing. Ionizing radiation would be most effective because it could be applied to warm, chilled or frozen carcasses and would affect appearance and organoliptic properties least (Carry and Atabay, 2001). To reduce the rate of transmutation water should be properly treated and milk should be properly heated and control of infection in broiler chicken (David, 1995). The need for enforcement of meat, to prevent cross contamination was emphasized. Proper hygienic handling of meat as prescribed in various meat
hygiene regulations. Apart from hygienic, the decontamination of end products might be considered way controlled campylobacter infection, treatment with lactic acid, or irradiation of meat offer the most promise (Oosterom et al., 1983).

2.8.1. On the Farm:

Drinking of chlorinated water had lower intestinal colonization rates than poultry that drank unchlorinated water (Kapperud et al., 1993; and Pearson et al., 1996). Experimentally treatment of chicks with commensal bacteria (Stern and Kazmi, 1988) and immunization of older birds (Widders, 1996) reduced C. jejuni colonization. Because intestinal colonization with Campylobacter's readily occurs in poultry flocks, even strict measures may not eliminate intestinal carriage by food-producing animals (Jacobs-Retsma et al., 1995).

2.8.2. At Processing:

Slaughter and processing provide opportunities for reducing C. jejuni counts on food-animal carcasses. Bacterial counts on carcasses can increase during slaughter and processing steps. In one study, up to a 1,000-fold increase in bacterial counts on carcasses was reported during transportation to slaughter (Stern et al., 1995). In studies of chickens (Tzat et al., 1988) and turkeys (Acuff et al., 1986) at slaughter, bacterial counts increased by approximately 10- to 100-fold during defeathering and reached the highest level after evisceration. However, bacterial counts on carcasses decline during other slaughter and processing steps. In one study, forced-air chilling of swine carcasses caused a 100-fold reduction in carcass contamination (Oosteron et al., 1983). In Texas turkey plants, scalding reduced carcass bacterial counts to near or below detectable levels (Acuff et al., 1986). Adding sodium chloride or trisodium phosphate to the chiller water in the
presence of an electrical current reduced \textit{C. jejuni} contamination of chiller water by 2 log\textsubscript{10} units (Liyb \textit{et al.}, 1995).

\textbf{2.8.3. Treatment:}

Antibiotics such as erythromycin can be administered in drinking water. In the context of commercial production in the USA where earth floored housing is used and litter is recycled, pre harvest control of \textit{C. jejuni} is impractical, innovative methods of prevention in the near future (Cynthia \textit{et al.}, 2005). The increasing rate of human infections caused by antimicrobial-resistant strains of \textit{C. jejuni} makes clinical management of cases of Campylobacteriosis more difficult (Murphy \textit{et al.}, 1996; and Pidock, 1995). Erythromycin, the drug of choice for \textit{Campylobacter} diarrhea in humans, also effective in other animals Gentamicin, farazolidone, and Doxycycline and Ampicillin are relatively inactive used. Ampicillin is relatively inactive against most strains of \textit{Campylobacter} (Cynthia \textit{et al.}, 2005).many of the routine drugs (e.g. Cotrimoxazole) that are used for treating bacterial gastroenteritis in Sudan are not suitable for the treatment of \textit{Campylobacter} gastroenteritis which is usually treated by Macrolides and Quinolones (Elamin, 2000). Macrolides, including Erythromycin and Roxytomycin and Quinolones including Nalidixic acid, Norfloxacin, and Ciprofloxacin are the drugs of choice for the treatment of \textit{Campylobacter} infection Laurece \textit{et al.}, (1997) and Betram, (1998).
2. Other bacterial pathogen in broiler carcasses offered for sale to consumers:-

2.1. *Klebsiella* spp:-

2.1.1. Bacteriology and Ecology of *Klebsiella*:

*Klebsiella* is a genus of Gram-negative, rod shaped bacteria, non-motile Oxidase-negative, with a prominent polysaccharide capsule. This capsule encases the entire cell surface, accounts for the large appearance of the organism on gram stain, and provides resistance against many host defense mechanisms. (Ryan *et al.*, 2004). While originally considered to be without clinical significance and restricted to aquatic, botanical, and soil environments, Frequent human pathogens, *Klebsiella* organisms can lead to a wide range of disease states, notably pneumonia, urinary tract infections, septicemia, Ankylosing spondylitis, and soft tissue infections (Podschun and Ullmann, 1998).

2.1.2. Classification:

The taxonomy of *Klebsiella* is characterized by a nomenclature reflecting its colorful taxonomic history. Originally, the medical importance of the genus *Klebsiella* (family *Enterobacteriaceae*) led to its being subdivided into three species corresponding to the diseases they caused: *K. pneumoniae*, *K. ozaenae*, and *K. rhinoscleromatis*. In the early 1980s, *Klebsiella* isolates from the environment, which had previously been classified as “*Klebsiella*-like organisms” (groups J, K, L, and M), were increasingly being classified into provisional taxa (Gavini, 1977) these groups gave rise to four new species:
K. terrigena (Izard et al., 1981), K. ornithinolytica (Sakazaki et al., 1989), K. planticola (Bagley et al., 1981) and K. trevisanii (Ferragut et al., 1983). In 1986, the last two species were combined into one species, K. planticola, because of their extensive DNA sequence homology (Gavini et al., 1986). K. terrigena and K. planticola have recently been reported as occurring in human clinical specimens (Mori et al., 1989; Podschun et al., 1994). Thus, at present it seems possible that in addition to K. pneumoniae and K. oxytoca, a third Klebsiella species exists that is able to cause human infections.

2.1.3. Epidemiology:-

Klebsiella spp is ubiquitous in nature. Klebsiellae probably have two common habitats, one being the environment, where they are found in surface water, sewage, and soil and on plants, and the other being the mucosal surfaces of mammals such as humans, horses, or swine, which they colonize. (Matsen et al., 1974; Bagley et al., 1978; Edberg et al., 1986).

In humans, K. pneumoniae is present as a saprophyte in the nasopharynx and in the intestinal tract. The detection rate in stool samples ranges from 5 to 38%, while rates in the nasopharynx range from 1 to 6% (Rose and Schreier, 1968; Davis and Matsen, 1974). It is a common hospital-acquired pathogen, causing urinary tract infections, nosocomial pneumonia, and intra abdominal infections. K. pneumoniae is also a potential community-acquired pathogen. Klebsiella pneumoniae is also well known in the environment and can be cultured from soil, water and vegetables. In fact, it is likely that we have K. pneumoniae in our intestine from eating raw foods such as salads. Two research papers on surveys of bacteria in sprouts found K. pneumoniae to be a predominant part of the microflora. (Soriano et al., 2000) Many hospital-acquired infections occur because of the invasive treatments that are often needed in hospitalized patients. For
example, intravenous catheters used for fluid administration, catheters placed in the bladder for urine drainage and breathing tubes for people on a breathing machine can all increase the susceptibility to infection.

Reported carrier rates in hospitalized patients are 77% in the stool, 19% in the pharynx, and 42% on the hands of patients (Cooke et al., 1979; Johanson et al., 1969; Smith et al., 1973; Pollack et al., 1974).

The principal reservoirs for transmission of Klebsiella in the hospital setting are the gastrointestinal tract of patients and the hands of hospital personnel (Montgomerie, 1979). The ability of this organism to spread rapidly (Kühn et al., 1993) often leads to nosocomial outbreaks, especially in neonatal units (Hart, 1993). Of the 145 epidemic nosocomial infections reported in the literature published in English between 1983 and 1991, 13 were caused by Klebsiella (Doebbeling, 1993).

According to the statistics of the Centers for Disease Control and Prevention, Klebsiella spp. account for 8% of endemic hospital infections and 3% of epidemic outbreaks (Stamm, 1981).

Especially feared are epidemic hospital infections caused by multi resistant strains. In the 1970s, these strains were chiefly aminoglycoside-resistant Klebsiella strains (Curie et al., 1978; Mangan and Snyder, 1976). Recently published reports from the United States, Israel, and Europe support our observations. Neither Vergis et al. (2000). From the United States nor Lieberman et al. (1996). From Israel found a single case of K. pneumoniae pneumonia in large multicenter studies of community-acquired pneumonia in the 1990s. Nine European studies published since 1990 show that only 14 (2.3%) of 621 patients admitted with severe community-acquired pneumonia requiring intensive-care unit admission had K.

### 2.1.4. Pathogenesis and Clinical Features:

*Klebsiella* infections most commonly involve the urinary and respiratory tracts. Since these two body sites differ considerably with respect to the host defense mechanisms, it should be expected that the pattern of virulence factors found in UTI-causing strains of *Klebsiella* will differ from that observed in strains isolated from pulmonary sources of patients with pneumonia.

*Klebsiella* usually develop prominent capsules composed of complex acidic polysaccharides. The capsular repeating subunits, consisting of four to six sugars and, very often, uronic acids (as negatively charged components), can be classified into 77 serological types (Kabha *et al.*, 1995). Capsules are essential to the virulence of *Klebsiella* (Cryz, 1985, Highsmith *et al.*, 1985).

*Klebsiella* pneumonia tends to affect people with underlying diseases, such as alcoholism, diabetes and chronic lung disease. Classically, *Klebsiella pneumonia* causes a severe, rapid-onset illness that often causes areas of destruction in the lung. (Patterson and Woodburn, 1980).

Infected persons generally get high fever, chills, flu-like symptoms and a cough productive of a lot of mucous. The mucous (or sputum) that is coughed up is often thick and blood tinged and has been referred to as "currant jelly" sputum due to its appearance. (Patterson and Woodburn, 1980).

There may also be pus surrounding the lung (known as empyema), which can be very irritating to the delicate lung tissue and can cause scar tissue to form.
At times, surgery may be needed to "rescue" a lung that is trapped in irregular pockets of pus and scar tissue.

*Klebsiella* can also cause less serious respiratory infections, such as bronchitis, which is usually a hospital-acquired infection. Other common hospital-acquired infections caused by *Klebsiella* are urinary tract infections, surgical wound infections and infection of the blood. All of these infections can progress to shock and death if not treated early in an aggressive fashion.

Mortality in *Klebsiella pneumonia* is around 50% due to the underlying disease that tends to be present in affected persons. The mortality rate for untreated cases is around 90%.

2.1.5. Sensitivity and Resistance to antimicrobial agents:-

However, the percentage of ceftazidime-resistant strains may be much higher, because the conventional disc diffusion criteria used in the routine laboratory underestimate the incidence of these isolates (Jacoby and Han, 1997). Since ESBL production frequently is accompanied by multiresistance to antibiotics, therapeutic options become limited. So far, however, ESBL-producing *Klebsiella* strains have been susceptible to carbapenems such as imipenem or meropenem. Both antibiotics are the drugs of choice in the treatment of infections due to ESBL-producing organisms. In this respect, a recent observation is very disturbing. For the first time, ESBL-producing *K. pneumoniae* strains which showed an additional resistance to imipenem have been isolated (Bradford, 1997).

Enhancement of the zone of inhibition around the cephalosporin disc towards the clavulanate-containing disc indicates the presence of an ESBL-producing strain. A commercially available product is the ESBL screening E test
strip (AB Biodisk, Solna, Sweden). This method is based on the evaluation of the difference between the antimicrobial activity of ceftazidime alone compared to that of ceftazidime plus clavulanic acid (Katsanis, 1994). Risk factors for acquisition of these strains seem to be the length of stay in hospital and the performance of invasive procedures (Lucet et al., 1996).

*Klebsiella* strains have been susceptible to carbapenems such as imipenem or meropenem. Both antibiotics are the drugs of choice in the treatment of infections due to ESBL-producing organisms. In this respect, a recent observation is very disturbing. For the first time, ESBL-producing *K. pneumoniae* strains which showed an additional resistance to imipenem have been isolated (Bradford et al., 1997).

2.1.6. Control and prevention:-

To control *Klebsiella* infections is the regulation of antibiotic use in the hospital to prevent misuse and overuse of antibiotics. Furthermore, nosocomial infection surveillance is necessary to collect data that are used in the prevention and control of nosocomial *Klebsiella* infection (Nassif and Sansonetti, 1986).

2.2. *Enterobacter* spp:

2.2.1. Bacteriology and Ecology of *Enterobacter* spp:

Gram negative bacteria, rods shap, peritrichous flagella, some encapsulated, motile and non spore forming. facultatively anaerobic, *E. cloacae* has also been shown to be capable of growth in 5% dextrose solution.
2.2.2 Classification:

The genus *Enterobacter* belongs to the family *Enterobacteriaceae* and can be readily distinguished from the genus *Klebsiella* in that the former is motile, usually ornithine decarboxylase positive, and urease negative (Farmer, 1995).

There are 14 species or biogroups of *Enterobacter* listed in the most recent edition of *Manual of Clinical Microbiology* (Farmer, 1995). Not all of these have been implicated as causes of diseases in humans. Among those that have, the most commonly encountered species include *Enterobacter aerogenes*, *Enterobacter cloacae*, *Enterobacter agglomerans*, and *Enterobacter sakazakii* (Farmer, 1995; Smith and Brenner, 1985; Gallagher, 1990; Gaston, 1988; Haddy *et al.*, 1991). *Enterobacter taylorae*, *Enterobacter gergoviae*, *Enterobacter asburiae*, and *Enterobacter amnigenus* are only rarely isolated from clinical specimens (Burchard *et al.*, 1986; Chow *et al.*, 1991; Haddy *et al.*, 1991).

2.2.3. Species considered being of clinical importance:

*E. aerogenes* and *E. cloacae* are by far the most frequently encountered human pathogens among the genus *Enterobacter* (Andresen *et al.*, 1994; Burchard *et al.*, 1986; Haddy *et al.*, 1991).

**Other Enterobacter spp.**  *E. aerogenes*, *E. cloacae*, *E. gergoviae*, *E. amnigenus*, *E. asburiae*, *E. intermedius*, *E. hormaechei*, *E. cancerogenus*, *E. sakazkii*, *E. agglomeran* (new genus *Pantoea agglomerans*).

2.2.4. Epidemiology of Enterobacter:-

*Enterobacter* infections can be acquired from either endogenous or exogenous sources. This is not surprising, given the ubiquitous nature of the organism. Various species can be found in the feces of humans and animals and in water,
plants and plant materials, insects, and dairy products (John et al., 1982, Loessner et al., 1993). Single-source outbreaks have been traced to contaminated intravenous solutions, blood products, distilled water, endoscopes, hands of personnel, hydrotherapy water, stethoscopes, cotton swabs, cryopreserved pancreatic islet infusions, lipoidal solutions, and devices used for monitoring intraarterial pressure (Bennett et al., 1995). However, most nosocomial infections cannot be traced to a single common exogenous source or to any of a number of modes of nosocomial transmission (John et al., 1982, Falkiner, 1992). Most nosocomial *Enterobacter* infections appear to arise endogenously from a previously colonized site in the involved patient (Falkiner, 1992, Kühn et al., 1993). Which explains the early outbreaks reported with this organism (Gaston, 1988, Matsaniotis et al., 1994).

### 2.2.5. Pathogenesis and Clinical Features:

Species of *Enterobacter* are clearly opportunistic pathogens and rarely cause disease in the otherwise healthy individual. As opportunistic pathogens that have only recently become important causes of nosocomial infections. As gram-negative pathogens, they possess endotoxin and thus have all of the pathogenetic properties imparted to an organism by this virulence factor (Bone, 1993).

The incubation period of *Enterobacter* bacteremia has been estimated from common-source outbreaks in which the organisms were infused directly into the bloodstream (Matsaniotis, 1984).

The time for appearance of signs and symptoms has varied from as short as 2 h to as long as 20 days, with most occurring in a few hours to 2 days. In a pediatric outbreak, the incubation period was a mean of 6 days (Matsaniotis, 1984). Signs and symptoms are generally similar to those noted during bacteremia with other
Enteric bacilli in both adults and children. This includes leukopenia, thrombocytopenia (Bouza et al., 1985), hemorrhage (Bodey et al., 1991), and jaundice (Fung et al., 1988) have each been noted in a few series. The syndrome of disseminated intravascular coagulopathy has been recognized in 0 to 6% of bacteremic episodes (Bodey, et al., 1991. Fung et al, 1988). Most of the usual cutaneous manifestations associated with bacteremia have been noted occasionally. These include purpura fulminans (Gu¨rses and Oskan 1988), hemorrhagic bullae (Loessner et al., 1993), and eczema gangrenosum (Redjeb et al., 1989). Cyanosis and mottling has been encountered in two-thirds of bacteremic children (Matsaniotis, 1984). Most of the species of Enterobacter have been implicated in a wide spectrum of lower respiratory infections, including asymptomatic colonization of respiratory secretions, purulent bronchitis, lung abscess, pneumonia, and empyema (Karnad et al., 1987, Lerner, 1980s; Parej et al., 1992).

2.2.6. Sensitivity and Resistance to Antimicrobial Agents:

Enterobacter spp. are sensitive to aminoglycosides, chloramphenicol, tetracyclines, TMP-SMX, nalidixic acid, nitrofurantoin. (Toala et al., 1970).

Most strains of E. cloacae and E. aerogenes are resistant to cephalothin; resistance to ampicillin. (Toala et al., 1970)

Susceptibility to Disinfectant: Susceptible to many disinfectants - 1% sodium hypochlorite, 70% ethanol, 2% glutaraldehyde, iodines, phenolics, formaldehyde. (Toala et al., 1970).
2.3. Pasteurella SPP:

2.3.1. Bacteriology and Ecology of Pasteurella:

Pasteurella is gram-negative bacteria, cocobacilli, nonmotile, nonsporforming, not acid fast, fermentative (Cynthia, 2005), Growth between 22-44°C, Optimum 37°C Facultative anaerobe, with fermentative metabolism (Noel and John, 2005). It produces acid but not gas through using dextrose, glycerol, inositol, lactose, maltose, and mannose. It is oxidase- and catalase - positive and is able to reduce nitrate to nitrite (Astrid, 2006).

Pasteurella bacteria are part of the natural oral, respiratory, genital, and gastrointestinal floras of various wild and domestic animals; for example, *P. multocida ssp. gallicida* is most frequently isolated from birds and occasionally from pigs, and *P. multocida subsp. septica* is isolated from cats more than from dogs and infrequently from humans. Some of these bacteria are also considered pathogenic to animals and even humans. Interactions among Pasteurella and members of the flora of the different mucosal and its host are not known (Prokaryotes) (Astrid, 2006).

2.3.2. Classification:

Pasteurella A genus of gram-negative bacteria in the tribe Pasteurella, family Pasteurellaceae.

Species:

*Pasteurella aerogenes*; Pasteurella haemolytica (*Mannheimia* haemolytica); *P. avium*; *P. bettyae*; *P. caballi*; *P. canis*; *P. dagmatis*; *P. gallinarum*; *P.
2.3.3. Epidemiology:-

They are distributed worldwide, and diseases caused by them are common in sheep and goats of all ages, also birds and human. *Pasteurella haemolytica* and *pasteurella trehalosi* are the species most often associated with disease. These bacteria are the primary agents involved in respiratory disease, septicemia, arthritis, meningitis, and mastitis and many also be important secondary invaders in respiratory diseases of ruminants. There are 12 serotypes of *P. haemolytica* is most commonly associated with pneumonic *pasteurellosis*. The virulence of *P. haemolytica* is mediated by the action of several factors (including endotoxin, leukotoxin, and capsular polysaccharide) that afford the bacteria advantages over host immunity and are important in the pathogenesis of disease. *P. haemolytica, P. multocida* and *P. trehalosi* is common commensal organisms of the tonsils and nasopharynx of healthy sheep and goats. Transition form infection to disease appears to be facilitated by various stressors, including concurrent infection; changes in climate, pasture, or feed; and other management factors. (Cynthia, 2005),

2.3.4. Pathogenesis and Clinical Features:-

*Pasteurella pneumotropica* is an opportunistic pathogen that is not often associated with clinical diseases. However, when infecting a host, it can generally be recovered from the respiratory tract, the urogenital tract, or conjunctiva from the host: common hosts include mice, rats, hamsters, guinea pigs, rabbits, cats, and other laboratory animals.
Mannheimia (Pasteurella) species are common commensals on mucous membranes of most domestic animals worldwide (Biberstein, 1979). Most animals are asymptomatic carriers of *M. haemolytica* and *P. trehalosi* and also carry strains of *P. multocida*. *M. haemolytica* is associated with disease in cattle causing pneumonic pasteurellosis, hemorrhagic septicemia and abortion (Timoney, 1988; Ward, 1990) pneumonia, mastitis and septicemia in domestic sheep and isolates in domestic goats (Midwinter, 1985; Viera *et al.* 1993).

In case of humans, many strains from *Pasteurella multocida subsp. multocida*, *Pasteurella multocida subsp. septica*, *Pasteurella canis*, *Pasteurella stomatis*, and *Pasteurella dogmatis* have been isolated from infected humans. Toxins were produced only by one strain of *P. multocida subsp. multocida* and *P. canis*; in addition, other than one severe case of necrotizing cellulitis caused by *P. dagmatis*, *P. multocida subsp. multocida* or *P. multocida subsp. septica* was involved in the more serious cases of infection. Symptoms of a *Pasteurella* infection vary on which body organ is involved and how long the disease is present. One of the most common symptoms is during respiratory infection and manifests as a nasal discharge. Others include sneezing, congestion, conjunctivitis, and clogged tear ducts. *Pasteurella* infections also can cause abscesses under the skin that can be chronic, requiring surgery to correct. Some abscesses can go as far as causing central nervous symptoms like oscillations of the eyes], circling to one side, and severe tilting of the head [wry neck or torticollis] (Christensen *et al.*, 2006).

### 2.3.5. Control and prevention:-

The risk of *Pasteurella* infection and illness can be reduced, although not eliminated. After contact with other rabbits and cats, thoroughly wash hands with
soap and warm water and towel dry; you can also add an alcohol-based sanitizing gel. Change your clothes and shoes if you've been visiting the rabbits at the shelter before you enter your own bunnies' area. Feeding her fresh vegetables, and having an emotionally healthy rabbit that gets lots of loving attention. Studies show that human blood pressure drops when petting an animal- cuddling is therapeutic for everyone. Antibiotics can do a lot to help the body fight off bacteria. However, some rabbits do develop a chronic low-grade infection that can be kept under control with life-long antibiotics,( Christensen et al., 2006).

2.4. **Listeria spp:**

2.4.1. **Bacteriology of Listeria spp**

*Listeria* spp is gram -positive bacteria, coccoid to bacillus-shaped, nonspore forming that tends to form long filaments, particularly in order cultures (Cynthia, 2005). *Listeria* spp able to grow in temperatures ranging from 4°C the temperature of a refrigerator, to 37°C the body's internal temperature (Southwick, 2007).

2.4.2. **Classification:**

*Listeria* is a bacterial genus containing six species. Named after the English surgeon, Joseph Lister, *Listeria* species are Gram-positive bacilli and are typified by *L. monocytogenes*, the causative agent of listeriosis. *Listeria ivanovii* is a pathogen of ruminants, and can infect mice in the laboratory, although it is only rarely the cause of human disease.

**Other Species of Listeria:** *L.grayi, L.innocua, L.ivanovii, L.welshimeri L.monocytogenes, L.seeligeri.* (Cowan, 1985).
2.4.3. Epidemiology of *Listeria spp*:

*Listeria spp* is a bacterium commonly found in soil, stream water, sewage, plants, and food (Southwick, 2007). It is widely distributed among avian species, it has been isolated from apparently normal birds and from birds dying of causes other than uncompleted *Listeriosis*. (Cynthia, 2005). *Listeria* has been found in uncooked meats, uncooked vegetables, unpasteurized milk; foods made from unpasteurized milk, and processed foods. There is a chance that contamination may occur in ready-to-eat foods such as hot dogs and deli meats because contamination may occur after cooking and before packaging, and animals can also be carriers, (Cynthia, 2005).

These bacteria can be spread by contact with infected hands or counter tops during food preparation. Although the risk of *listeriosis* associated with foods from deli counters is low, persons at high risk may choose to avoid these foods or thoroughly reheat cold cuts before eating. Furthermore, *listeriosis's* deadliness can be particularly attributed to the infection's ability to spread to the nervous system and cause meningitis (Southwick, 2007).

The Center for Science in the Public Interest has published a list of foods that have sometimes caused outbreaks of *Listeria*: hot dogs, deli meats, raw milk, cheeses raw and cooked poultry, raw meats, ice cream, raw vegetables, raw and smoked fish and the green lip mussel, (Galbraith *et al.*, 2007). You get *listeriosis* by eating food contaminated with *Listeria* bacteria. Babies can be born with *listeriosis* if their mothers eat contaminated food during their pregnancy.
Finally, *Listeria* has a particularly high occurrence rate in newborns because of its ability to infect the fetus by penetrating the endothelial layer of the placenta, (Kenneth, 2003).

2.4.4. Pathogenesis and Clinical Features:-

Listeriosis is a serious infection caused by eating food contaminated with the bacteria *Listeria monocytogenes*. The majority of *Listeria* bacteria are targeted by the immune system before they are able to cause infection. Those that escape the immune system's initial response, however, spread though intracellular mechanisms and are therefore guarded against circulating immune factors (Kenneth, 2003).

To invade, *Listeria* induces macrophage phagocytic uptake by displaying D-galactose in their teichoic acids that are then bound by the macrophage's polysaccharide receptors. Once phagocytosed, the bacteria is encapsulated by the host cell's acidic phagolysosome organelle, (Southwick, 2007). *Listeria*, however, escapes the phagolysosome by lysing the vacuole's entire membrane with secreted hemolysin (Tinlev, 1989). Now characterized as the exotoxin listeriolysin O. (Southwick, 2007). The bacteria then replicate inside the host cell's cytoplasm, (Kenneth, 2003). *Listeria* must then navigate to the cell's periphery to spread the infection to other cells, (Kenneth, 2003).

As the bacterium infects the host, the temperature of the host melts the structure and allows translation initiation for the virulent genes. Symptoms have been noted to occur within as few as 3 to as many as 70 days after consumption of a contaminated food, and most commonly within 3 weeks. Persons with listeriosis usually have a fever, severe headache, constipation, muscle aches, and sometimes
nausea or diarrhea. If the infection spreads to the nervous system, symptoms such as headache, stiff neck, confusion, loss of balance, or convulsions may occur. Serious illness it can sometimes lead to death.

2.4.5. Control and Prevention:

Prevention of *Listeria* as a food illness involves effective sanitation of food contact surfaces. Alcohol has proven to be an effective topical sanitizer against *Listeria*. Quaternary ammonium can be used in conjunction with alcohol as a food contact safe sanitizer with increased duration of the sanitizing action. Nonflammable Alcohol Vapour in carbon dioxide NAV-CO2 systems or sodium hypochlorite is frequently used to sanitize surfaces to prevent *Listeria*. Refrigerated foods in the home should be kept below 4°C to discourage bacterial growth, *Listeria* is killed by pasteurization and cooking (Maple, 2008).

2.4.6. Treatment:

Antibiotics effective against *Listeria* species include ampicillin, vancomycin, ciprofloxacin, linezolid, azithromycin. Intralytix has created a virus spray with bacteriophages to be applied to food for the prevention of *Listeriosis* by killing six strains of *L. monocytogenes* bacterium (Greenemeier, 2008). Food Safety has created and put a similar product on the market, LISTEX P100. LISTEX P100 prevents Listerios in food by using bacteriophages for killing *Listeria*. Those with a healthy immune system with the help of these antibiotics have no worries if they are concerned about living through it (Laine *et al.*, 1998).
3.1. Study Area:

Samples were collected from Khartoum North. This area has a supermarket where poultry meat product from different places are purchased. During the period from June to August in 2008.

3.2. Collection of specimens and transportation:

Hundred specimens were collected randomly from retail markets. From each sample, 25 grams of breast, wing, and leg muscles were taken in sterile plastic bags using aseptic long handled forceps, knives, and scissors. Samples were labeled and placed in ice boxes and transported to the laboratory. They were examined immediately for any bacterial isolates.

3.3. Criteria used for the identification of the isolate:

- Microscopic examination.
- Cultural characteristics on solid media
- Biochemical tests

3.4. Culture Media:

Solid, semisolid, and liquid media were used. Media were either prepared from original ingredients or in a dehydrated form under aseptic conditions. All media were prepared according to the methods described by the manufacturers.
3.4.1. Solid Media:-

3.4.1.1. Campylobacter Agar Base (Oxoid):-

This medium consist: -10g Lab lemco powder, 10 g Peptone, 5 g Sodium chloride, and 12g of Agar.

The campylobacter agar base medium was prepared by dissolving 18.5 g in 475ml of distilled water brought to the boiling to dissolve. Then it was sterilized by autoclaving at 121°C for 15 minutes, Cooled to 50°C and 25ml of lysed horse blood and 1 vial of Antibiotic campylobacter selective supplement which was dissolved in 4ml of sterile distilled water, this mixture added was prepared completed in agar base media which was there poured into plates in 20ml volumes.

3.4.1.2. Skirrow’s Campylobacter Selective Medium (1977):-

Oxoid Blood Agar Base No.2 from which 20g was suspended in 500ml of distilled water, dissolved and sterilized by autoclaving at 121°C for 15 minutes, and cooled to 55°C. Sterile saponin-lysed horse blood (25ml) was added and mixed gently. One vial of antibiotic mixture (Vancomycin, Polymixin B and Trimethoprim) was dissolved in 4ml of sterile distilled water and added to the mixture which was there poured into plates in 20ml volumes.

3.4.1.3. Nutrient agar (Oxoid):-

This medium consist of 10g heart infusion agar, 10g Tryptone ,5 g Sodium chloride and 15g agar.

The medium was prepared by dissolving 28g of the medium in 1000ml distilled water. The PH was adjusted to 6.8 brought to the boiling to dissolve, mixed and
sterilized by autoclaving at 121°C for 15 minutes, cooled to 55°C and then distributed into sterile petri dishes in 20ml volumes.

3.4.1.4. MacConkey’s Agar medium:-

This medium was obtained in a dehydrated from Oxoid consisted of: 17g peptone, 10g lactose, 1.5 g bile salts, 5g sodium chloride, 13.5g agar and 0.03g neutral red.

25g prepared by dissolved in 1000 ml distilled water and sterilized at 121°C for 15 minute under presser and dispended in 15ml volume into sterile petri dishes.

3.4.1.5. Hugh and Leifson’s (Oxidation Fermentation) medium:-

The oxidation fermentation medium was used to test the ability of organism to break down the carbohydrates by oxidation and/or fermentation. The medium contained peptone, Sodium chloride dipotassium hydrogen phosphate, agar and 0.2% aqueous solution of Andrade’s. It was prepared according to Cowan and Steel (1985) by the dissolved the solids in distilled water. The PH was adjusted to 7.1 the medium was filtered and the indicator was added. Sterile solution of appropriate carbohydrate was then added aseptically to give a final concentration of 1%; the content was distributed aseptically in 10ml amounts into sterile test tubes and covered with sterile cotton wool.

3.4.2. Semisolid Media:-

3.4.2.1. Motility medium (Difco): -

This medium composed of tryptose, Sodium chloride and agar. It was prepared by dissolving 20g dehydrated powder in 1liter distilled water. The PH was adjusted
to 7.4, distributed in 5ml amount in tests tubes covered with cotton wool and sterilized by autoclaving at 121°C for 15 minutes.

3.4.3. Liquid Media:

3.4.3.1. Campylobacter Enrichment Broth (Oxoid):

Media consist: 1.0g Lab-Lemco powder, 2.0g yeast extract, 5.0g peptone, and 5.0g Sodium chloride PH 7.4.

This medium was prepared by dissolving 13g of the medium in 1000ml of distilled water. The PH was adjusted to 7.4, then mixed well and sterilized at 121°C for 15 minutes campylobacter antibiotic selective supplement was added then the medium was distributed into screw capped bottles in 20ml amounts.

3.4.3.2. Peptone Water (Oxoid L37):

This medium was used as a base for the carbohydrate utilization tests. It was prepared by dissolving 10g of peptone water powder and 5g of Sodium chloride in 1000ml of distilled water. The PH was adjusted to 7.2 distributed into screw capped bottles and sterilized by autoclaving at 121°C for 15 minutes.

3.4.3.3. Nitrate medium:

The medium was prepared by dissolving 1g of potassium nitrate in 1 liter of nutrient broth medium, and then it was distributed into bijou bottles in 3ml and sterilized by autoclaving at 115°C for 15minutes.

3.4.3.4. Carbohydrate fermentation media:

These media were prepared according by an amount of 900ml peptone water, PH was adjusted to 7.1,10g peptone water ,5g Sodium chloride ,distilled water 900ml,1% aqueous solution of Andrade’s were added and the medium was then
sterilized. The sugar was prepared by dissolved 10g of sugar in 90ml of distilled water and steam for 30minutes. Sugar solution was added in to the sterile peptone water, and distributed in 3ml amounts into sterile bijou bottles with inverted Durham’s tubes. All the bottles were labeled before use.

3. 4.3.5. **Campylobacter Antibiotic supplement – III (Skirrow):**

Antibiotic supplement recommended for the selective isolation of Campylobacter species was added to prevent over growth by other enteric species. The supplements consist of 5.00Vancomycin, 1,250IU Polymyxin B, 2.50mg Trimethoprin per vial, sufficient for 500ml medium. The supplements was dissolved in 2ml sterile distilled water and mix well then added with sterile precaution to the sterilized and cooled medium.

3.5. **Chemical reagent and indicators:**

3.5.1. **Reagents:**

3.5.1.1. **Staining reagents and stains:**

These were prepared according to Robert (1976) as follow:

Ammonium oxalate – Crystal violet

Solution A: 10 grams of Crystal violet and 100 ml of (95%) ethanol were mixed and dissolve.

Solution B: 1% Ammonium Oxalate aqueous solution 20ml of solution A were mixed with 80ml of solution B before use.
Strong carbol fuchsin:

Solution A:

Basic Fuchsin 10 grams
Ethanol (95%) 100ml

These ingredients were mixed and dissolved and kept at 37°C over night.

Solution B:

Basic Fuchsin 5 grams
Ethanol (95%) 100 ml

They were mixed until dissolved completely. Where used 10ml of solution A were poured into 100ml of solution B.

Acid alcohol:-

Concentrated HCL 3g
Ethanol (95%) 97ml

sLugol’s iodine:

Iodine 5g
Potassium iodine 10g
Distilled water 100ml
Potassium iodine and iodine were dissolved in 10 ml of distilled water then completed to 100 ml.

3.5.1.2. Hydrogen peroxide (H$_2$O$_2$):-

It was obtained from the British Drug House Chemical (BDH) company, England. It was prepared as 3% aqueous solution and used for catalase test.

The enzyme catalase mediates breakdown of hydrogen peroxide into oxygen and water.

3.5.1.3. Oxidase reagent: (Tetramethyl-p-phenylene diamine dihydrochloride).

This was obtained from Hopkin and Williams, London. It was prepared fresh as 1% aqueous solution. Filter paper was impregnated in the solution and used for oxidase test.

3.5.1.4. Phenol red:-

This reagent prepared by dissolving 0.002g of phenol red in 1000ml of distilled water.

3.5.1.5. Nitrate test reagent:-

This reagent composed of two solutions:

**Solution A:** 0.8% Sulphanilic acid in 5N - acetic acid. It was prepared by mixing 0.4ml of sulphanilic acid in 50ml of 5N – acetic acid.

**Solution B:** 0.5% Dimethyl-α naphthylamine in 5N-acetic acid. Mixed and the complete reagent was used to detect nitrate reduction.
3.5.1.6. Methyl red reagent:-

It was prepared by dissolving 0.04 grams of methyl red in 40ml ethanol and diluted with distilled water to 100ml. It was used in methyl red test.

3.5.1.7. Kovac’s reagent:-

It was composed of 5g of P-dimethyl amino-benzaldehyde, 75ml isoomylalcohol and 25ml concentrated hydrochloride acid. The aldehyde was dissolved in alcohol by the addition of the acid. The reagent was stored at 4°C in dark bottle. It was used for detection of indole production.

3.5.2. Indicators:-

3.5.2.1. Andrade’s:-

It was prepared by dissolve 5g Acid fuchsine in 1000ml distilled water and 150ml of alkali solution (N-NaOH). Was added mixed and allowed to stand at room temperature with frequent shaking for 24h and used for different purposes.

3.5.2.2. Zinc Powder: -

It was obtained from British Drug House (BDH). It was used for nitrate reduction test in 5mg/ml culture.

3.6. Solutions:-

3.6.1. Normal saline (0.85%):-

It was prepared by dissolving 8.5gram sodium Chloride in 1liter distilled water.

3.6.2. Phosphate buffer saline (FBS):-
It was prepared by dissolving 8g of Sodium chloride (NaCl), 1.15g of Disodium hydrogen phosphate (Na2HPO4) and 0.2g of Potassium dihydrogen phosphate (KH2PO4) in on liter of distilled water.

3.7. Sterilization:-

3.7.1. Sterilization of equipments:-

Glassware such as McCartney, bijou and universal bottles and glass beads were sterilized in the autoclave at 15pounds pressure for 15minutes (121°C For 15 minuets).

Petri-dishes, graduated pipettes, tubes and glass beads were sterilized in the hot air oven at 160°C for 90 minutes.

3.7.2. Sterilization of Culture media:-

Motility medium, nutrient broth, peptone water, O-F medium and nitrate broth were sterilized at 115°C for 20minutes. Blood agar medium, Campylobacter selective medium, MacConkey’s agar medium, Nutrient agar medium, and Campylobacter enrichment broth, were sterilized at 121°C for 15 minutes.

3.7.3. Sterilization of sugars:-

Sugars were sterilized by tyndalization.

3.7.4. Sterilization of solutions:-

Normal saline, phosphate buffered saline, phenol saline and distilled water was sterilized by autoclaving at 115°C for 10 minutes.
3.8. Incubation condition:-

All plate are incubated at 42°C-37°C at microaerophilic by anaerobe Jars. These were used with Campy-gas –generating Kits (oxoid). Which contains 5% Oxygen,1% Carbon-dioxide and 85% Nitrogen.

3.9. Preparation of material for bacteriological examination:-

3.9.1. Methods:-

3.9.1.1. Primary Isolation:-

Method of culturing was done according to FDA (2001), FAO (1992) and Tran (1998) 25g of sample were cut and weighted in the sterile plastic bag by the balance the lab table were sterilized carefully by alcohol and flame and so the instruments used in the experiment sensitive balance and the blender) then 100ml of the already made enrichment broth was added. Mixed in blender which was sterilized after each sample mixing. The mixture was filtered with cheese cloth in 150 conical flask and put in an anaerobic jar supported by Campy gas generation kits and the jar was put in incubator at 42°C for 48hour.

Then the culture was streaked onto Skirrow’s agar plates, a small drop 0.1ml of the broth was placed at the edge of the plate using the bubbler pipit then agar plates were incubated at 42°C for 48 hours, under microaerophilic conditions which were achieved by anaerobic jar with campy gas generation Kits (oxoid).

3.9.1.2. Sub culturing of primary isolation:-

Typical and well isolated colony was picked by means of wire loop and streaked onto a surface of fresh solid medium plate or inoculated into a fresh liquid medium.
3.9.1.3. Examination of cultures:-

All Cultures on semi-solid media were examined with the naked eye for growth, colony characteristic and changes in the medium.

For liquid media turbidity, change of color, formation of pellicle or sediment and accumulation of gas in case of carbohydrates media were observed.

3.9.1.4. Purification of cultures:-

This was obtained by sub-culturing of typical and well isolated colony on blood agar plate. Growth was checked for purity by examining smears stained by Gram’s Method under the microscope.

3.9.1.5. Preservation of isolates:-

The isolates which were confirmed as pure were preserved on serum agar slants. This was performed by inculcation of these media with the culture of pure isolate and incubation at 37°C for 48hours prior storage at 4°C.

3.10. Staining Techniques:-

3.10.1. Preparation of smears:-

This was done by spreading a loop full of liquid culture or an emulsified colony on a clean dry glass slide. The smears were allowed to dry in air and then fixed by gentle flaming.

3.10.2. Staining methods:-

3.10.2.1. Gram stain:-

This was done according to Cowan (1985). Gram – positive bacteria appeared purple, while Gram-negative bacteria stained red.
3.11. Identification of isolated bacteria:

3.11.1. Cultural characteristic:

The growth characteristic of bacterium in artificial media is examined such as colony shape, size, colour and haemolysis.

3.11.2. Primary tests:

The purified bacterium was identified according to by Cowan and Steel (1985). Gram stain, acid fastness, morphology and other tests specified below.

3.11.2.1. Motility tests:

The tested strain was inoculated into the motility medium using straight wire and incubated at 37°C for 48 hours. Motile bacteria spread away from the inoculated line.

3.11.2.2. Microaerophilic growth:

The tested organism was streaked onto Skirrow’s agar plate and Campylobacter Agar plate and incubated at 42°C for 48 hours under microaerophilic conditions which were achieved by anaerobic jar with campy gas generation Kits growth of organism indicates positive result.

3.11.2.3. Aerobic Growth:

The tested organism was streaked onto Skirrow’s and Campylobacter Agar plate and incubated at 42°C for 48 hours under normal air condition.
3.11.2.4. Oxidase test:-

The organism was grown in a medium free from glucose and nitrate. A filter paper was placed in a Petri-dish and 2-3 drops of fresh 1% (Tetra methyl- P-phenylene diamine dihydrochloride were dropped on the paper. The tested organism was picked using sterile bent glass rod and rubbed on the impregnated filter paper. Positive reaction was indicated by formation of dark purple colour within 10 seconds.

3.11.2.5. Catalase test:-

A drop of 3% hydrogen peroxide was placed on clean slide; one to two colonies of the tested organism grown in nutrient agar was placed on the drop of the hydrogen peroxide solution using wooden stick. Production of air bubbles indicates activity.

3.11.2.6. Oxidation Fermentation test of Carbohydrate:-

Two tubes containing Hugh and Leifson’s medium were inoculated with the tested organism by stabbing with straight wire. The medium in one of the inoculated tubes was covered with a layer of sterile liquid paraffin oil to a depth of one cm. the tubes were incubated and examined daily for 1-4 days. Change of colour of Andrade’s reagent to purple in both tubes indicates a fermenting organism; purple colour in the open tube only meant that organism was oxidative.

3.11.3. Secondary tests:-

3.11.3.1. Nitrate reduction test:-

Inoculated nitrate broth was incubated up to five days. Gas formation was indicated by replacements of medium in the inverted Durham’s tube. Ten 1ml of
nitrate reagent solution A was added followed by 1ml of nitrate reagent solution B. Positive reaction was indicated by the development of red colour in one minute. To tube not showing red colour within 5 minutes, powdered zinc was added, the development of red colour indicated that nitrate was not reduced indicate a negative test. The absence of red colour with zinc powder is indicative of a positive reaction which results from a complete reduction of nitrate to ammonia.

3.11.3.2. Methyl Red test:-

Glucose phosphate broth was inoculated with the test organism and after incubation two to three drops of 0.02% methyl red indicator were added. Positive reaction was indicated by the development of red colour.

3.11.3.3. Indole test:-

The organism was grown in peptone water and after 48 hours incubation; 0.5ml of Kovac’s reagent was added. Positive result was indicated by formation of red ring.

3.11.3.4. Urease test:-

Christensen’s medium was cultured with test organism and incubated at 37°C. Positive result was indicated by the development of red colour.

3.11.3.5. Hydrogen sulphide production (H₂S):-

Tested organism was streaked on sugar iron agar slop, incubated at 37°C and observed for the blackening of the medium for up to 7 days.
3.11.3.6. Fermentation of sugars:-

Bijou bottles containing the various peptone water sugars were cultured with the test organism. Positive reactions were indicated by changes of colour to purple due to acid production. Durham’s tubs were also examined for gas production.

3.12. Storage and Labeling of bacterial Isolates

Each strain of bacteria isolate and identified carefully labeled and preserved on serum agar slants, Dorset egg medium or cooked meat medium.
CHAPTER FOUR

RESULTS

In the hundred samples cultured in Campylobacter selective media, 75 samples showed growth while the 25 samples were negative to any bacterial growth. The isolates did not contain *Campylobacter spp*.

4.1 Laboratory investigation:

4.1.1. Growth character and isolated bacteria:

From hundred specimens seventy five of the isolated were able to grow on Campylobacter selective media after incubation at 42°C for 48 hours in microaerophilic condition.

4.1.2. Aerobic growth:

From hundred specimens seventy five of the isolated were able to grow on Campylobacter selective media after incubation at 42°C for 48 hours in normal air condition.

During this study, more than one bacteria were isolated from the 75 samples of culture of the raw chicken meat, the bacteria isolated were Klebsiella spp, Enterobacter spp, Pasteurella haemolytica, and Listiria spp.

The prevalence and frequency of the bacteria isolated are given in table (1).

4.2. Klebsiella spp:

The organism appeared, in stained smears as gram negative, rods, it was non motile, on spore forming, and non acid fast and non capsulated.
On primary isolation the cultures in Campylobacter Agar Base showed shiny large mucoid colonies grey in colour, with irregular edge.

In Nutrient Agar Growth showed translucent colonies, flat, and yellowish to white colour.

And in MacConkey Agar the bacteria grow well on the plate after 48 hours incubation at 37°C under microerophilic condition. Figure (1)

All *Klebsiella spp* isolate grow well on Campylobacter selective media after incubation at 42°C for 24hours in normal air condition.

**Biochemical reaction:**

**Primary biochemical tests for *Klebsiella spp*:**

The cultures were positive to catalase, oxidase negative, urease negative, ferment carbohydrates with gas production. The results of primary biochemical test of *Klebsiella spp* were illustrated in table (2).

Secondary Bio chemical tests: all isolates, were negative to urease production, positive to VP test at 37°C, negative to H2S from TSI, and negative to In table (3) indole.

**In the Sugars fermentation test:**

All examined isolates fermented carbohydrates and produced acid with gas from glucose. Figure (2)

The result of secondary Biochemical testes of *klebsiella spp* was illustrated in table (5).
4.3. *Enterobacter spp*:

The organism appeared, in stained smears as gram negative, rods. occurred singly short chin was not uncommon, they were motile and non spore forming.

In Campylobacter Agar Base, Colonies were small grey colour shiny raised regular of arranged droplets like.

In Nutrient Agar. Colonies were small flat, white to creamy in colour. Figure (3).

In MacConkey Agar the colonies were smooth, shiny and yellow in colour. All *Enterobacter spp* isolates grow well on Campylobacter selective media after incubation at 42°C for 24 hours in normal air condition.

**Biochemical reaction:**

**Primary biochemical tests:**

All *Enterobacter spp* tested were positive to catalase, oxidase negative, utilized carbohydrates by fermentation with gas production.

The results of primary biochemical test of *Enterobacter spp* were illustrated in table (3).

**Secondary Bio chemical tests:**

All *Enterobacter spp* isolate were positive to (vages-proskaller) VP test at 37°C, and negative for indole, Fermented of glucose with gas production, and positive for urease test. Figure (4).

In Sugars all examined isolates fermented carbohydrates with acid production. The result of secondary Biochemical testes of Enterobacter spp was illustrated in table (6).
4.4. - *Pasteurella haemolytica. (Type A. haemolytica):*

The organism appeared, in stained smears as gram negative, rods, non motile, non spore forming and non capsulated.

In Campylobacter Agar Base colonies were small, mucoid, shiny, grayish to green in colour, with odors irregular of arrange, moist round edge surrounded with a narrow zone of complete haemolysis. Figure (5)

In Nutrient Agar colonies were small, flat, moist, irregular edge, Creamy to white colour, no odors.

In MacConkey Agar colonies were smooth, shiny and pink colour. All *Pasteurella haemolytica*. Showed grow well on Campylobacter selective media in normal air condition.

**Biochemical reaction:**

**Primary biochemical tests:**

All strains tested were catalase positive, oxidase positive, O.F positive, non motile fermented carbohydrates with gas production. Figure (6).

The results of primary biochemical test of Pasteurella haemolytica were illustrated in table (4).

Secondary Bio chemical tests: All isolate were negative of urease production, negative of H2S production, reduced nitrate and negative for indole.

**Sugars fermentation test:**

All examined isolates fermented carbohydrates produced acid with gas from glucose.
The result of secondary Biochemical tests of Pasteurella haemolytica spp was illustrated in table (9).

4.5 .Listeria spp:

The organism appeared, in stained smears as gram positive short rods, occurring singly or in clumps, motile, non spore forming and acid fast.

In Campylobacter Agar Base colonies were bright, small, mucoid, shiny, white to grayish in colour, with acetone odors, raised and regular of arrange, circular with entire edge. Figure (7)

In Nutrient Agar small coloni, flat, mucoid, Creamy to white colour with odors.

In MacConkey Agar colonies grow well under microerophilic condition. Campylobacter selective media support the growth of Listeria spp. in normal air.

Biochemical reaction:

Primary biochemical tests:

All strains tested were catalase positive, oxidase negative, fermented carbohydrates with gas products.

The results of primary biochemical test of Listeria spp were illustrated in table (7).

Secondary Bio chemical tests:

All isolate were negative of urease production, negative of H2S production reduced nitrate and negative for indole, positive vp test.
Sugars fermentation test:

All examined isolates attacked carbohydrates by fermentative and produced acid with gas from glucose.

The result of secondary Biochemical testes of Listeria spp was illustrated in table (8).

Table (1): Percentage and Frequency of the bacteria isolated from chicken carcasses.

<table>
<thead>
<tr>
<th>Bacteria isolated species</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Klebsiella spp</em></td>
<td>41</td>
<td>55%</td>
</tr>
<tr>
<td><em>Enterobacter spp</em></td>
<td>13</td>
<td>17%</td>
</tr>
<tr>
<td><em>Pasteurella haemolytica</em></td>
<td>12</td>
<td>16%</td>
</tr>
<tr>
<td><em>Listria spp</em></td>
<td>9</td>
<td>12%</td>
</tr>
<tr>
<td><em>Campylobacter ssp</em></td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Negative isolation</td>
<td>25</td>
<td>25%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100</strong></td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>
Table (2): Results of primary biochemical test of *Klebsiella* spp.

<table>
<thead>
<tr>
<th><em>Klebsiella</em> spp</th>
<th>Biological test</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 41 isolate</td>
<td>Oxidase</td>
</tr>
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<td>Result</td>
<td>-ve</td>
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</tbody>
</table>

*Oxidation fermentation test of carbohydrates.*

Table (3): Results of primary biochemical test of *Enterobacter* spp.

<table>
<thead>
<tr>
<th><em>Enterobacter</em> spp</th>
<th>Biological test</th>
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<tbody>
<tr>
<td>No. 13 isolate</td>
<td>Oxidase</td>
</tr>
<tr>
<td>Result</td>
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</tbody>
</table>

*Oxidation fermentation test of carbohydrates.*
Table (4): Results of primary biochemical test of *Pasteurella haemolytica* spp.

<table>
<thead>
<tr>
<th>Pasteurella haemolytica spp.</th>
<th>Biochemical test</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 12 isolate</td>
<td>Oxidase</td>
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<tr>
<td>Result</td>
<td>+ve</td>
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With Gas
### Table (5) Secondary Biochemical tests of *Klebsiella spp* isolate

<table>
<thead>
<tr>
<th>Isolates</th>
<th>SBiochemical test</th>
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<tbody>
<tr>
<td>No. 41</td>
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<tr>
<td></td>
<td>VP</td>
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<td></td>
<td>*test at 37°C</td>
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<tr>
<td></td>
<td>H2Sproduction</td>
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<td></td>
<td>Urease</td>
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<td></td>
<td>Malonate</td>
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<td>Gas from glucose</td>
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<td></td>
<td>Adinoitol</td>
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<td>Arabinose</td>
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<td>Rafinose</td>
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* Voges- proskauer test (vp) (Barrit method).

### Table (6) Secondary Biochemical tests of *Enterobacter spp* isolate

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<tr>
<th>Isolates</th>
<th>Biochemical test</th>
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<td>No. 13</td>
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<td></td>
<td>VP</td>
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<td></td>
<td>*test at 37°C</td>
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<td>Urease</td>
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<td></td>
<td>Glucanate</td>
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<td>Malonate</td>
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<td>Gas from glucose</td>
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<td>Adinoitol</td>
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Table (7): results of primary biochemical test of *Listeria spp.*

<table>
<thead>
<tr>
<th><em>Listeria spp.</em></th>
<th>Biochemical test</th>
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<tr>
<td>NO 12 isolate</td>
<td>oxidase</td>
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<tr>
<td></td>
<td>Catalase</td>
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<td>Motility</td>
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<td></td>
<td>Glucose utilization</td>
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<tr>
<td></td>
<td>O.F *</td>
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Table (8): Secondary Biochemical testes of *Listeria spp* isolate.

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<td>Urease</td>
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<td>Vp test</td>
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<td>Gas from glucose</td>
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### Table (9) Secondary Biochemical testes of *Pasteruella haemolytica* spp isolate

<table>
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<th>Isolate</th>
<th>Biochemical test</th>
</tr>
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<tbody>
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<td>Xylose</td>
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Figure (1): Growth of Klebsiella spp. In MacConkey Agar medium after 24 hours incubation at 37°C under microaerophilic condition.
Figure (2): Klebsiella spp positive result of Sugars fermentation test (right bottle). Change of colour of indicator to purple.
Figure (3) Growth of Enterobacter spp on Nutrient Agar after 24 hours incubation at 37°C under microerophilic. Appears yellowish to white in colour.

Figure (4). Enterobacter spp positive result for urease test (right tube). Colour of indicator change to red.
Figure (5) Growth of Pasteurella haemolytica. (Type A. haemolytica) on Campylobacter Agar Base after incubated at 42°C for 48 hours under microaerophilic conditions. Colonies were small, mucoid, shiny, grayish to green in color.
Figure (6) Pasteurella haemolytica. Utilized carbohydrates by fermentation (right tube). Change of colour of indicator to purple.

Figure (7): Growth of Listeria spp on Campylobacter Agar Base after incubated at 42°C for 48 hours under microaerophilic condition. Colonies were bright, mucoid, white to grayish in colour.
CHAPTER FIVE

DISCUSSION

The microbiological safety of food products is of extreme important to industry, public health officials, regulatory agencies, and the public. Little information’s are available regarding the occurrence of pathogens in poultry meat products in the retail market place in Khartoum north.

These studies provided important information’s on the occurrence of Campylobacter spp., and other bacterial pathogen in broiler carcasses offered for sale to consumers.

The present study was conducted in Khartoum north, locality to isolate the Campylobacter in poultry carcass, and other bacterial organism.

From the hundred samples which had been cultured in selective media, the 75 samples showed bacterial growth, and the other 25 samples did not show any growth, this finding may be attributed to antibiotics which were added to the media.

Moreover no Campylobacter spp was isolated (zero %) similar result has been reported by (Kozacinski et al., 2006).

Possible growth population of Campylobacter in sample taken because the freezing also reduces the number of Campylobacter in carcass. This finding agree with (Dufrenne et al., 2001), he also found that the levels of Campylobacter spp. in frozen chickens were lower than in chilled chickens and this may relate to the freeze- damaged cells encountered on frozen carcasses. (Stern and Kazmi, 1989) who said Freeze-thawing also
reduces the population of Campylobacter spp. And agree to study by Smith and Muldoon 1974, who found only 3165 samples positive, this low prevalence was probably because the method of isolation was poor until the media of Skirrow’s (1977) and Blaser et al. (1978) were devised.

The exposure of broilers to lower temperatures for extended periods of time may be a factor in decreasing Campylobacter population in broiler carcass.

The results of this study not agree to (Sanchez et al., 2002) who found that Campylobacter levels and chilled carcasses were significantly higher in immersion chilling. And with the study by (Lea et al., 1998) who stated that freezing did not eliminate the pathogen from contamination food.

In study by (Hinton et al., 2004), who stated there processing operations significantly (p<0.05) reduced the level of contamination of broiler carcasses by Campylobacter. This result did not agree to studied had been reported by Kramer et al. (2000) and Bolton et al. (1999) who isolated Campylobacter spp, from 83%(165/198) of chicken portions and 86%(290/336) of poultry at retail sale on the English market.

Other justification for this negative result from the hundred specimens may be due to the combination of scalding and picking operations which may decrease the number of Campylobacter recovered from carcasses. This finding agree with Cason et al., (1997) who said scalding may reduce carcass contamination dirt and debris from feather, feet and skin of the broilers, or may be that scald water temperatures lethal to the
bacteria (Yang et al., 2001) furthermore, the multiple tank counter-flow scald system exposes carcasses to progressively cleaner water with progressively higher temperatures (Cason et al., 1997). Immersion scalding of carcasses 58°C or 60°C water has been reported to reduce the number of Campylobacter on carcasses (Oosterom et al., 1983).

Addition of sodium chloride as found by (Omer, 2004) and antimicrobial system, such as trisodium phosphate (TSP) or acidified sodium chlorite (ASC), to the washes produces a reduction of 1-1.7 log CFU/ml in the number of Campylobacter counts. Park et al., (1991) suggested that the rearing of Campylobacter freezing poultry was not economically feasible and that reduction in incidence should be attempted at the processing stage by the addition of salt or by drying operation, in order to reduce the amount of available water, thus creating a hurdle for the growth of these organisms.

In this study, the presence of Campylobacter spp. and other bacteria, in different raw chicken samples which was examined by enrichment and direct plating to enable enumeration. agree with study made by (Fatima, 2003) and disagree with (Koenrad et al., 1996) who Saied when examining carcass rinses or intestines of poultry for the isolation of thermophilic Campylobacter which often contain large numbers, it is frequently not necessary to enrich, in fact, there are indication that some strains may predominate over other after enrichment, giving a false impression of the types or even species originally present. For instance, when examining carcass rinse using an enrichment medium with Cefoperazone, Teicoplanin, Amphotericin (C.A.T)
supplement, thermophilic Campylobacter isolated only by direct plating, and Arcobacter spp after enrichment (Atabay and Corry, 1997). Also by Atanassova and Ring (1997) who found that level of contamination of poultry meat with Campylobacter spp., mostly C. jejuni, was 50.9%.

In this study Klebsiella spp. was able to grow in the presence of the 10mg vancomycin representing in 41% out of the 100 chickens examined, with numbers of other bacteria found in this study confirms that raw poultry is a significant reservoir for klebsiella spp. and therefore a possible source of infection.

Also in the present study, 17% of the raw chicken sampled contained of Enterbacter spp. this result agreement with (Stolle, 1988; Nortje et al., 1990; Abu-Ruwaida et al., 1994; Alvarez-Astorga et al., 2002; Capita et al., 2002) The average number of Enterobacteria in fillets amounted to 3.62 ± 0.48 log. In addition to pathogenic bacteria, special attention in the hygienic production and storage of chicken meat is paid also to total count of aerobic mesophilic bacteria, Enterobacteria and Escherichia coli. These bacteria are considered indicators of microbiological quality. And agree with (Kozacinski et al., 2006) Enterobacteria spp. was found in 38.47% of chicken breasts without skin and in 42.85% of breasts with skin.

Also in the present study, 16% of the raw chicken sampled contained of Pasteurella haemolytica spp.

In the present study, 12% of the raw chicken sampled contained Listeria spp. This finding agrees to result by (Zivkovic et al., 1997) have isolated Listeria spp. in 27.8% of fresh chicken
samples. Ubiquity of bacteria of the genus Listeria spp. is an important factor influencing the possibility of poultry meat contamination. Presence of Listeria monocytogenes in fresh broiler meat varies from 0% to 64% (Atanassova and Ring, 1997).

Direct comparison of prevalence of these pathogens reported in different studies by (Pagotto, et al. 2001 and Heuvelink et al. 1999) there was relatively high prevalence of L. monocytogenes in raw beef, raw chicken legs, and pork chops, respectively. Other researchers have found L. monocytogenes prevalence of 1.9 to 18% in raw beef, pork, and poultry in Denmark, Switzerland, Japan, Mexico, Ireland, and Italy (Nomung et al., 1999; Inoue et al., 2000; Fantelli and Stephan, 2001; Heredia et al., 2001; Soultos et al., 2003; Busani et al., 2005).

In contrast all isolated grow well in Campylobacter Agar base medium with antibiotic supplement under microaerophilic condition but this isolated did not grow well in on Skirrow’s medium under microaerophilic condition.

The relatively high prevalence of Klebsiella spp, Enterobacter spp, Pasturella haemoletyca, and Listeria spp, in raw poultry and meat products in this study emphasizes the need for consumers to continue to be vigilant when handling these products.

Although relatively few sample of the poultry carcasses tested, in this study provides an indication of the prevalence of pathogen in products available for consumers in the retail marketplace.
RECOMMENDATION

In view of the result obtained from this study we recommend:

1- The improvement of teaching special isolation techniques to support the diagnostic methodology in order to be able to isolate Campylobacter species.

2- Culture methods have traditionally been regarded as the gold standard, however they are labor intensive, and results can take several days to obtain.

3- Although methods are available, the isolation of C. jejuni from food and environment, often complicated and expensive. More simple procedures needed that can be used under field condition.

4- The recommendation is based on the best scientific data available and will serve as a foundation for our programs designed to reduce foodborne illness and protect public health in Sudan.

5- It important to prevent cross-contamination and properly cook meat and poultry products to reduce foodborne illness.

6- Consumers should follow important tips for handling raw poultry. These tips can be summarized in three words, clean, separate and chill. Clean means to wash hands and surfaces often; separate means to keep raw meat and
poultry out of cooked foods; chill means to refrigerate or freeze foods promptly.

7- It is common for raw foods to contain pathogens, and the consumer must take some responsibility for controlling food poisoning by preventing cross-contamination in the kitchen and cooking raw foods properly.

CHAPTER Six

REFERENCES


Center for Science in the Public Interest - Nutrition Action Healthletter - Food Safety Guide - Meet the Bugs.


Dufrenne, J., Ritmeester, W., Delfgou –van Leusden, F., de Jonge , R.,(2001). Quantification of the contamination of chicken and chicken products in the Netherlands with Salmonella and Campylobacter. J.Food port.64,538-541.


Purdy, D.; Park, S.F. (1994). Cloning, nucleotide sequence and characterization of a gene encoding superoxide dismutase from


