ISOLATION AND IDENTIFICATION OF *ESCHERICHIA COLI, SALMONELLA* SPP., AND *YERSINIA* SPP. FROM RETAIL MEAT PRODUCTS IN BOLU

by

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ABSTRACT

ISOLATION AND IDENTIFICATION OF *ESCHERICHIA COLI* , *SALMONELLA* SPP., AND *YERSINIA* SPP. FROM RETAIL MEAT PRODUCTS IN BOLU

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Meat and meat products are the major source of foodborne infections and the most important link between food-producing animals and humans. When animals are slaughtered, bacteria from the hide, the gut or the processing environment may contaminate the surfaces of meat. This occurs despite of washing animals before slaughter, various treatments to clean carcasses during processing, and programs to keep the environment clean. Some of these

organisms are spoilage bacteria while others are pathogenic to humans. The aim of the research is to isolate and identify pathogenic bacteria such as *Escherichia coli*, *Salmonella* spp., and *Yersinia* spp. in the retail meat products. Between November 2007 and May 2008, a total of 225 retail meat products (poultry meat, meat, ground beef) were collected from butcher shops and delicatessens in Bolu. Any presence of *E. coli, Salmonella* spp. and *Yersinia* spp. in meat samples were analyzed according to "The International Organization for Standardization" methods. Especially, Most Probable Number (MPN) technique in a standard ISO procedure was used to identify for *E. coli*. Enumeration of *E. coli* in foods could be acquired at low concentrations by MPN technique. Out of 225 samples, the results were found to be positive for *Salmonella* spp. in 50 (22.2%) and *Yersinia* spp. in five (2.2%) samples. *E. coli* was examined from 168 of 225 samples, and was found to be positive in 90 (53.5%) samples. Among *Salmonella* species, *S.* Typhimurium was found to exist in 22 (9.8%) samples, *S. bongori* in 20 (8.9%), *S. enterica* subsp. *diarizonae* in eight (3.5%) samples. Among *Yersinia* species, *Y. mollaretii* was found to exist in three (1.33%), *Y. intermedia* in one (0.44%), *Y. pseudotuberculosis* in one (0.44%) sample.

Keywords: Meat products, *Salmonella* spp., *Yersinia* spp., *Escherichia coli,* isolation, MPN technique, identification.

ÖZET

BOLU İLİNDE PERAKENDE SATILAN ET ÜRÜNLERİNDEN *ESCHERICHIA COLI, SALMONELLA* VE *YERSINIA* TÜRLERİNİN İZOLASYONU VE TANIMLANMASI

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Et ve et ürünleri gıda kaynaklı enfeksiyonların asıl kaynağı ve insan ve besin üreten hayvanlar arasında en önemli bağlantıdır. Hayvanların kesimi esnasında; deriden, bağırsaktan ya da işlem yapılan çevreden gelebilecek bakteriler etin yüzeyini kontamine edebilirler. Bu durum kesimden önce hayvan yıkanmış, işlem esnasında karkas üzerinde çeşitli temizleme muameleleri yapılmış ve çevreyi temiz tutma programları uygulanılmış olsa bile

oluşmaktadır. Bu organizmaların bazıları bozulmaya sebep olan bakteriler iken, diğerleri de insanlar için patojendirler. Çalışmanın amacı, perakende et ürünlerinden *Escherichia* coli, *Salmonella* ve *Yersinia* türleri gibi patojen bakterileri izole etmek ve adlandırmaktır. Bu çalışmada Kasım 2007 ile Mayıs 2008 ayları arasında Bolu ilindeki çeşitli kasap ve şarküterilerden toplam 225 adet perakende et ürünleri (tavuk eti, et, kıyma) toplanmıştır. Et örneklerinde *E. coli, Salmonella* ve *Yersinia* türlerinin varlığı "Uluslararası Standardizasyon Örgütü" nün yöntemleri doğrultusunda analize tabi tutulmuştur. Özellikle, *E. coli*' nin adlandırılmasında standart ISO prosedüründen En Muhtemel Sayı (EMS) yöntemi kullanılmıştır. EMS yöntemi ile gıdalarda düşük konsantrasyondaki *E. coli* miktarı tayin edilebilmektedir. Bu 225 örnekten, 50' sinde (%22.2) *Salmonella* türleri ve beşinde (%2.2) *Yersinia* türleri tespit edilmiştir. 225 örneğin 168' inde *E. coli* varlığı araştırılmıştır ve 90' nında (%53.5) *E. coli* bulunmuştur. İzole edilen 50 *Salmonella* türünün, 22' si (%9.8) *S.* Typhimurium, 20' si (%8.9) *S. bongori*, sekizi (%3.5) *S. enterica* subsp. *diarizonae* olarak adlandırılmıştır. *Yersinia* türlerinden, *Y. mollaretii* üç örnekte (%1.33), *Y. intermedia* bir örnekte (%0.44), *Y. pseudotuberculosis* bir örnekte (%0.44) bulunmuştur.

Anahtar Kelimeler: Et ürünleri, *Salmonella* türleri, *Yersinia* türleri, *Escherichia coli,* izolasyon, en muhtemel sayı yöntemi, tanımlama.

To my dear family…

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1. INTRODUCTION

Foodborne infection occurs by the consumption of food and water contaminated with pathogenic enteric bacteria and viruses. It is necessary that the cells of enteropathogenic bacteria and viruses remain alive in food or water during consumption. Viable cells even if present in small numbers have the potential to establish and multiply in the digestive tract to cause illness (Ray B. 2004a).

A pathogen is an organism that can cause cellular damage by establishing in tissue, which results in clinical signs with an outcome of either morbidity or mortality. Specifically, a pathogen is characterized by its ability to replicate in a host, and by expressing specific virulence determinants to allow a microbe to establish within a host for transmission to a new susceptible host. Pathogens could be classified as zoonotic, geonotic, or human origin based on their transmission patterns and movement between the different hosts and vectors. Zoonotic diseases are characterized by transmission of infective agents from animals to humans; geonotic diseases are acquired from soil, water, or decaying plant materials; and human origins are exclusively transmitted from person to person. For example *Escherichia coli* O157:H7, *Staphylococcus aureus, Salmonella enterica* serovar Typhimurium, *Salmonella enterica* serovar Enteritidis, *Campylobacter jejuni, Yersinia enterocolitica, Mycobacterium tuberculosis* as zoonotic pathogens; a

geonotic pathogen is *Listeria monocytogenes*; and pathogens of human origin are *Salmonella enterica* serovar Typhi, *Vibrio cholerae, Shigella* spp., and Hepatitis A (Bhunia K.A. 2008).

There are many different bacterial pathogens that have been associated with foodborne disease. Despite this large number of bacterial pathogens, they generally cause disease by a limited number of mechanisms, such as via a preformed toxin, the production of a toxin in the intestine, invasion of the intestinal epithelial cells, or some other process (Acheson D.W.K. 2004).

Foodborne pathogens that are ubiquitous in nature are generally found in soil, water, animals and plants. Recontamination of processed food also usually occurs and contributes to foodborne outbreaks and illnesses. Food animals and poultry are the most important reservoirs of foodborne infection. Thereby, meat, milk, or egg products may carry *Salmonella enterica, Campylobacter jejuni, Listeria monocytogenes, Yersinia enterocolitica*, or *E. coli* O157:H7. However, the presence of pathogens in ready-to-eat (RTE) product is a serious problem since those products generally do not receive any further treatment before consumption. Most of the recent foodborne outbreaks resulted from consumption of undercooked or processed ready-to-eat meats (hotdogs, sliced luncheon meats, and salami), dairy products (soft cheeses made with unpasteurized milk, ice cream, butter, etc.), or minimally processed fruits (apple cider, strawberries, cantaloupe, etc.) and

vegetables (sprouts, lettuce, spinach, etc.) (Bhunia K.A. 2008). Following slaughter and dressing the carcasses of animals and birds contain many types of microorganisms, bacteria predominantly coming from the skin, hair, feather, gastrointestinal tract, etc. Normally, carcasses of the animals contain average of 10^{1-3} bacterial cells. Besides, chilled raw meat and ground beef contains microorganisms especially from the equipments used during processing, personal air and water (Ray B. 2004a).

Despite of cooking kills the pathogens, cooked meat may become recontaminated by food handlers during processing or from bacteria harbored in the environment (Mustapha *et* al. 2005).

The most common contaminated foods resulted with human salmonellosis include poultry, beef, pork, eggs, milk, seafood, and fresh produce such as sprouts (Bandekar *et* al. 2008). Raw ground meat is a good medium for the rapid growth of microorganims (Sırıken B. 2004a). Besides, meats are the most perishable of all important foods. They include an abundance of all nutrients required for the growth of bacteria, yeasts and molds, and an adequate quantity of these constituents exist in fresh meats in available form (Jay J.M. 1992a). Ground beef is made from meat, trimmings, and fat. The knives of meat grinder may be the source of contamination if not properly sanitized or washed (Bhunia K.A. 2008). Studies around the world have shown that *Salmonella, E. coli, S. aureus, Listeria* spp., *Campylobacter, Yersinia* and other

pathogenic bacteria are often present in ground beef (Sırıken B. 2004a). Ground beef provides a greater surface area, which itself accounts in part for the increased flora. It should be taken back that as particle size is reduced, the total surface area increases with a consequent increase in surface energy (Jay J.M. 1992b). A variety of these food products, predominantly poultry and other types of meat products are the main important sources of human *Salmonella* infection (Çetinkaya *et* al. 2008). Poultry products contaminated with *Salmonella* can occur at multiple steps along the food chain, which includes production, processing, distribution, retail marketing and handling / preparation (Meng *et* al. 2006).

A characteristic feature of *Salmonella* is its wide host range, including mammals, birds and cold-blooded animals in addition to humans (Orji *et* al. 2005). Infection of human can be acquired through direct contamination with carrier domestic or wild animals or through the consumption of contaminated foods or water (Egan *et* al. 2006). Human salmonellosis usually causes fever, diarrhea, abdominal pain and nausea. The infection may be so serious with severe dehydration and sometimes death occurs (Norrung *et* al. 2008).

Yersiniosis is caused by *Y. enterocolitica* that affects firstly young children and symptoms are dominated by diarrhea. In older children and adults abdominal pain and fever may be seen. Some complications may occur such as joint pains or spread of bacteria

to the blood stream can result (Norrung *et* al. 2008). Pork, ground beef, and poultry are major reservior of *Yersinia enterocolitica*-like species (Sulakvelidze A. 2000).

E. coli is inhabitant enteric tract of humans and warm-blooded animals. The presence of *E. coli* in foods is an indicator of direct or indirect fecal contamination. Additionally, it is an indicator of the possible presence of enteric pathogens in water, shellfish, dairy products and other foods (Gonzalez *et* al. 2003). Recent evidence suggests foodborne *E. coli* may, indirectly via host fecal flora, be an important cause of urinary tract infections (Schroeder *et* al. 2004).

Foodborne disease is a main problem worldwide, and foodborne pathogens result as numerous sufferings and deaths (Bhunia K.A 2008). Recent developments in food production and processing techniques and the subsequent changing trends in food consumption have existed in the emergence of new hazards (Collison *et* al. 2001).

It is a fact that meat products usually contain pathogenic microorganisms that cause foodborne diseases such as *Escherichia coli*, *Salmonella* spp. and *Yersinia* spp. The aim of the research is to isolate and identify such pathogenic microorganisms in retail meat products. The present research may hold up particular significance in identifiying the distribution of patterns of *Yersinia* and *Salmonella* species, and identifying *E. coli* with Most Probable

Number (MPN) technique in retail meat products from butcher shops and delicatessens in Bolu.

1.1. *Escherichia coli*

1.1.1. History

In 1885, Austrian pediatrician Theodar Escherich isolated *Escherichia coli* from the feces of a child. It was originally called as "*Bacterium coli* commune" (Welch R.A. 2006).

1.1.2. Description of the Genera

The genus *Escherichia* include five species: *E. coli, E. hermannii, E. fergusonii, E. vulneris* and *E. blattae* (Scheutz *et* al. 2005).

Escherichia coli is the type species of the genus *Escherichia* which in turn is the type genus of the family *Enterobacteriaceae* (Donnerberg M.S. 2005). *E. coli* is gram-negative, nonspore forming, straigth rods. Approximately they are 0.5 µm in diameter and 1.0-3.0 µm in length. They can be motile by peritrichous flagella or nonmotile (Welch R.A. 2006).

E. coli is the most abundant facultative anaerobe having both a respiratory and a fermentative type of metabolism (Scheutz *et* al. 2005). They are chemoorganotrophic. They grow optimally at 37˚C

and at pH 5.5-8.0, best growth occur at neutrality (Welch R.A. 2006).

Escherichia produce both acid and gas from the fermentation of D-glucose and also from the most fermentable carbonhydrates. Methyl-red test is positive but non-acetoin production. Usually citrate is negative. They do not produce H_2S. Indole is utilized. Hydrolysis of urea is negative. *Escherichia* species reduce nitrates (Scheutz *et* al. 2005).

E. coli can be separated from *Salmonella* and *Shigella* firstly by lactose fermentation. Besides *E. coli* strains ferment D-mannitol, D-sorbitol, L-arabinose, maltose, D-xylose, trehalose and D-mannose (Welch R.A. 2006).

The use of microbial enzyme profiles to detect indicator bacteria is an attractive option as existing method (Hafiz *et* al. 2003). Various studies on the *E. coli* ß-glucuronidase (ß-GUR) enzyme have identified 4-methylumbelliferyl-ß-D-glucuronide (MUG) as providing a reliable, convenient, differential test for *E. coli*. The use of MUG to detect ß-GUR activity in both coliform selective broth and solid media allows a 24 h presumptive detection of *E. coli.* The assay is based on the examination that 96-97 % of *E. coli* strains produce this enzyme (Doğan *et* al. 2002). The MUG substrate is cleaved by ß-glucuronidase (ß-GUR) enzyme to a fluorescent final product and this reaction is easily determined by a long wave UV lamp at 366 nm (Çakır *et* al. 2002).

The MUG method has been criticized for sometimes giving false negative and false positive results. In addition to *E. coli,* some strains of *Salmonella, Shigella, Enterobacter* and *Klebsiella* are determined to be ß-GUR positive and therefore these strains are responsible for false positive results in *E. coli* analysis. On the contrary, some strains of *E. coli* such as *E. coli* O157:H7 serotype do not possess this enzyme and exactly give false negative results. False positive results may be eliminated easily by an indole test. While *E. coli* is ß-GUR and indole positive, others such as, *Salmonella, Shigella, Enterobacter* and *Klebsiella,* are indole negative. Lastly, the indole test is easy to apply and can be used directly in LST+MUG broth cultures (Anonymous 1996).

1.1.3. Natural Habitats

Escherichia coli commonly lives in the gastrointestinal tract of humans and animals. They are commensals of the intestinal tract. Other *E. coli* strains (without commensals) are pathogens for humans and animals (Welch R.A. 2006).

Escherichia coli can be found secondarily in soil and water. This presence of *E. coli* in the environment is usually thought to the fecal contamination occurrence. But there is evidence to consider that *E. coli* may replicate freely in tropical fresh water (Welch R.A. 2006).

Other *Escherichia* species' habitats are; *E. blattae* is in the hind-gut of cockroaches, *E. fergusonii, E. hermannii* and *E. vulneris* are found especially in the extraintestinal sites of warm-blooded animals (Scheutz *et* al. 2005).

1.1.4. Pathogenesis

Escherichia coli is found normally in the bowel flora of healthy individuals (Bopp *et* al. 1999). Its presence in raw foods is thought an indication of direct or indirect fecal contamination. Direct fecal contamination occurs during the processing of raw foods of animal origin because of poor personal hygiene of food handlers. Indirect fecal contamination occurs through sewage and polluted water (Ray B. 2004b). Certain strains may cause extraintestinal and intestinal infections in immunocompromised healthy individuals. Such as urinary tract infections, bacteremia, meningitis and diarrheal diseases (Bopp *et* al. 1999).

E. coli has several completely identified components that contribute to its ability to cause disease: pili, a capsule, endotoxin and two exotoxins (enterotoxins) (Levinson *et* al. 2002a).

E. coli is usually subdivided serologically or according to the presence of virulence factors to identify and characterize epidemiologically pathogenic strains. All serotyping include somatic (O), capsular (K), and flagellar (H) antigens (Holt *et* al.

1994a). Because of these different genotypes and their disease-causing abilities lead to categories of *E. coli* often referred to as "pathotypes". There are eight pathotypes; six of them (Enterotoxigenic *E. coli*–ETEC, Enteropathogenic *E. coli*–EPEC, Enterohemorrhagic *E. coli*–EHEC, Enteroaggregative *E. coli*–EAEC, Diffusely Adherent *E. coli*–DAEC, Enteroinvasive *E. coli*-EIEC) are intestinal and two of them (Meningitis-associated *E. coli*–MAEC, Uropathogenic *E. coli*-UPEC) are extraintestinal currently recognized (Welch R.A. 2006).

1.1.4.1. Enterotoxigenic *E. coli* (ETEC)

A frequent cause of diarrhea in both humans and animals, causing 800.000 deaths from 600 million cases of human diarrhea, majority in children under the age of five are resulted in the worldwide. In animals they cause economically important diarrheal disease includes neonatal calves, pigs and lambs (Welch R.A. 2006).

ETEC also causes watery diarrhea, sometimes it can be life-threatening. Infants and children under the age of five are mostly affected in endemic areas. And also ETEC exposure is one of the most common causes of traveler's diarrhea in endemic areas (Welch R.A. 2006).

The pathogenesis of ETEC infection as it is currently understood includes pilus-mediated adherence and toxin-mediated fluid secretion (Donnenberg M.S. 2005).

1.1.4.2. Enteropathogenic *E. coli* (EPEC)

They are currently known as those diarrheagenic *E. coli* strains that cause attaching and effacing lesions on intestinal epithelium but which lack Shiga-toxins (Welch R.A. 2006). EPEC strains were first identified in the 1940s as the cause of devastating outbreaks of nosocominal and community-acquired neonatal diarrhea, but now rarely cause disease in the developed world. EPEC infections are principally spread by person to person and hospitals continue to be a source of infection (Donnenberg M.S. 2005). They cause watery diarrhea, may be included mucus but not blood. Symptoms are vomiting, fever, malaise and dehydration (Welch R.A. 2006). Additionally, disease caused by EPEC may be protracted resulting in weight loss, malnutrition, and death (Donnenberg M.S. 2005).

1.1.4.3. Enterohemorrhagic *E. coli* (EHEC)

They cause disease of the large intestine that may present watery diarrhea with bloody stools, resulted as the ulcerations of the bowel. Several days later, it becomes severe and life-threating hemolytic uremic syndrome (HUS). HUS is composed of triad of

hemolytic anemia, thrombocytopenia and renal failure (Welch R.A. 2006). Kidneys are the most vulnerable target organs, any tissue can become ischemic from capillary and larger vessel thrombosis. The brain (strokes), eyes (blindless), and colon (ischemic bowel) are other organs usually affected (Donnenberg M.S. 2005). Occurrence of EHEC disease in humans is by ingestion of contaminated beef or foods with cattle feces (Welch R.A. 2006). The low infectious dose of EHEC strains is estimated to be less than 100 organisms (Donnenberg M.S. 2005).

EHEC plays an important role for renal injury. And also especially the children under five are the victims of EHEC disease (Welch R.A. 2006).

1.1.4.4. Enteroaggregative *E. coli* (EAEC)

EAEC strains was first recognized as a cause of diarrhea in 1987, and are defined by their aggregative pattern of adherence to tissue culture cells. They form two-dimensional clusters when they adhere to cells in vitro, to glass slides, to plastic, or to intestinal mucosa (Donnenberg M.S. 2005). EAEC causes a watery diarrhea, sometimes with abdominal cramps but no fever and blood (Welch R.A. 2006).

EAEC has been associated with acute and chronic diarrhea and abdominal colic in young children in Germany and also four

outbreaks of gastroenteritis in the UK (Scheutz *et* al. 2005). Thus, the disease is reported as a persistent, seemingly chronic watery diarrhea (Welch R.A. 2006).

1.1.4.5. Diffusely Adherent *E. coli* (DAEC)

The epidemiology and pathogenesis of the DAEC are unclear. They may cause diarrhea in less than one year old children (Welch R.A. 2006).

1.1.4.6. Enteroinvasive *E. coli* (EIEC)

These organisms are pathogenetically related to *Shigella* species so much. EIEC causes invasive inflammatory colitis and dysentery with blood and mucus stools accompanied by fever and severe cramps. EIEC and *Shigella* invade intestinal epithelium, mostly in the large intestine (Welch R.A. 2006).

1.1.4.7. *E. coli* in human Extraintestinal Infections (EXPEC)

EXPEC strains firstly belong to pathogenic clones of a limited number of O : K : H serotypes, generally with fimbriae, siderophores, capsules, O antigens, serum resistance and toxins often alpha-hemolysin. The most common infections contain urinary tract infections (UTIs) ranging from uncomplicated to the

febrile to invasive, pyelonephritis, neonatal and postneurosurgical meningitis and septicemia (Scheutz *et* al. 2005).

Two different pathotypes of EXPEC are meningitis-associated *E. coli*, MAEC (neonatal septicemia / meningitis *E. coli*) and the uropathogenic *E. coli*, UPEC (urinary tract and blood-stream *E. coli*) (Welch R.A. 2006).

UPEC is heterogenous group of clones that causes cystitis, pyelonephritis and urosepsis. Nearby the hospital-acquired *E. coli* infections, UPEC strains are principal causes of morbidity and mortality (Welch R.A. 2006).

MAEC along with Group B *Streptococci* is the most common cause of neonatal meningitis. It is a severe disease having a high mortality rate and possible long-term neurological problems in survivors. There are a few number of *E. coli* serotypes caused with this disease but more than 80% of the strains express K1 capsule. So, it is generally considered that the newborn acquires the K1 strain from its mother during passage through the birth canal (Welch R.A. 2006).

1.2. *Salmonella* spp.

1.2.1. History

The *Salmonella* are present in the intestines of animals and have evolved with their hosts. Therefore, *Salmonella* infection is an ancient disease. There was confusion between typhus and typhoid until the mid-1800s, in spite of investigators had differentiated these two diseases. Many rumors were present about typhoid in the past such as ileal ulcers associated with typhoid or the Peyer's patches were inflamed in typhoid, etc. “Typhoid fever” was used firstly in 1829 by French physician Pierre Charles Alexandre Louis (1787-1872) (Ellermeier *et* al. 2006).

In 1850 William Jenner (1815-1898) explained the differences between typhus and typhoid as to symptomology and epidemiology. William Budd (1811-1880) completed that typhoid fever was spread by the fecal-oral route in 1873. Karl Eberth (1835-1926) noticed rod-shaped organisms in the spleens and lymph nodes of typhoid patients in 1880 in Germany and was accepted with discovering the serovar Typhi organism (Ellermeier *et* al. 2006).

In 1885 Theobald Smith (1859-1934), working under American veterinarian Daniel E. Salmon (1850-1914), isolated *Salmonella choleraesuis* from the pig's intestine. Thus, French bacteriologist Joseph Leon Marcel Lignieres (1868-1933) suggested in 1900 that the group of bacteria represented by the swine-cholera organism

should be termed "*Salmonella*" in honor of Salmon. The work of P.B. White was extended by Fritz Kauffmann. Kauffmann F. (1899-1978) started to establish serological analysis of *Salmonella* in the 1940s (Ellermeier *et* al. 2006).

1.2.2. Description of the Genera

The *Salmonella* are facultatively anaerobic, gram-negative, rod-shaped bacteria in the family *Enterobacteriaceae* (Ellermeier *et* al. 2006). The cells are typically 0.7-1.5×2.0-5.0 µm (Popoff *et* al. 2005). They optimally grow at 37°C (mesophile) and at pH 6.5-7.5 (neutrophile) and at a water activity of 0.995 (Ellermeier *et* al. 2006).

Salmonellae are usually motile (peritrichous flagella). Nitrates are reduced to nitrites. Most Salmonellae are aerogenic. They produce the gas from D-glucose. And also hydrogen sulfide is usually produced on triple-sugar iron agar. Indole is negative. So tryptophan is not oxidatively deaminated. As a carbon source, citrate is usually utilized. Urease is not hydrolyzed. Decarboxylation of lysine and ornithine reactions are usually positive (Popoff *et* al. 2005).

Salmonella are chemoorganotrophs, having both fermentative and oxidative metabolism (Ellermeier *et* al. 2006). They usually D-

mannitol, D-mannose, L-rhamnose, D-sorbitol, trehalose and D-xylose (Holt *et* al. 1994c).

In the Kauffmann-White scheme, *Salmonella* spp. were serotyped according to their O (somatic) antigens, Vi (capsular) antigens, H (flagellar) antigens. Because of the basis of biochemical tests serovars were further divided (Holt *et* al. 1994b). The O antigens are outer polysaccharides of the cell wall and used for subdividing Salmonellae into groups A-I. The H antigens have two forms, phases 1 and 2. The Vi antigens are antiphagocytic and are an important virulence factor for *S.* Typhi, moreover, they are used for serotyping *S.* Typhi in clinical laboratory (Levinson *et* al. 2002b). All of the *Salmonella* serovars were depend on two species; they were *Salmonella bongori* which includes less than 10 serovars and, *Salmonella choleraesuis* which includes 2500 serovars. Also *Salmonella choleraesuis* was divided into six subspecies phenotypically and genetically (Holt *et* al. 1994b). But there was a confusion about naming between the serotype Choleraesuis and the genus *Salmonella choleraesuis.* So that, *Salmonella choleraesuis* was replaced into "*Salmonella enterica*" (Popoff *et* al. 2005). *Salmonella* nomenclature was shown in Table 1 (Ellermeier *et* al. 2006).

Table 1. *Salmonella choleraesuis* Nomenclature

Subsp.	Approved nomenclature	Widely accepted nomenclature
I	*S. choleraesuis* subsp. *choleraesuis*	*S. enterica* subsp. *enterica*
II	*S. choleraesuis* subsp. *salamae*	*S. enterica* subsp. *salamae*
IIIa	*S. choleraesuis* subsp. *arizonae*	*S. enterica* subsp. *arizonae*
IIIb	*S. choleraesuis* subsp. *diarizonae*	*S. enterica* subsp. *diarizonae*
IV	*S. choleraesuis* subsp. *houtenae*	*S. enterica* subsp. *houtenae*
V	*S. choleraesuis* subsp. *indica*	*S. enterica* subsp. *indica*
VI	*S. choleraesuis* subsp. *bongori*	*Salmonella bongori*

Furthermore, serovars of *Salmonella enterica* subsp. *enterica* are written in not italicized. Serovars are written in Roman letters and the first letter is capitalized. Serovars of other species are manifested by their antigenic formulae. Since only *Salmonella enterica* subsp. *enterica* strains are named, different kinds of shortened versions have become acceptable, such as *Salmonella enterica* serovar Typhimurium, *Salmonella* Typhimurium or serovar Typhimurium (Ellermeier *et* al. 2006).

1.2.3. Natural Habitats

The *Salmonella* primarily inhabit the intestinal tracts of animals. *Salmonella enterica* subsp. *enterica* lives in warm-blooded animals, whereas all other *S. enterica* subsp. and *S. bongori* are commensals of cold-blooded animals and chiefly the environment (Ellermeier *et* al. 2006). In Table 2, the *Salmonella enterica* subsp. *enterica* serovars can be divided into three groups; “host adapted” infect one host but cause a disease in others, “host restricted” infect only a single host and at last “generalists” infect a variety of animals, also kind of disease differ in different hosts (Ellermeier *et* al. 2006).

Table 2. Host range of *Salmonella enterica* subsp. *enterica* serovars

Classification	Serovar	Natural host	Rare host
Host restricted	Typhi	Humans	None
	Paratyphi A and C	Humans	None
	Sendai	Humans	None
	Abortusovis	Ovines	None
	Gallinarum	Poultry	None
	Typhisuis	Swine	None
	Abortusequi	Equines	None
Host adapted	Cholerasuis	Swine	Humans
	Dublin	Bovines	Humans, ovines
Generalist	Typhimurium	Humans, poultry, swine, bovines, rodents	None
	Enteritidis	Humans, poultry, rodents	Swine, bovines

1.2.4. Pathogenesis

The most *Salmonella* infections are occured by eating contaminated foods from animal origins such as meat, poultry, eggs and dairy products. Other infections are resulted by other animals, fruits, and vegetables that contaminated with animal feces (Ellermeier *et* al. 2006).

Salmonella infections are pathogenic for humans and many animal species. They cause typhoid fever, enteric fevers, gastroenteritis and septicemia (Holt *et* al. 1994b).

Salmonellosis is occured by the fecal contamination of water and food. It can be passed person to person by direct contact. In developing countries, its probability is higher because of the poor hygiene (Ellermeier *et* al. 2006). Non-typhoidal *Salmonella* strains usually cause an intestinal infection showing by diarrhea, fever, abdominal cramps. Every year, 1.000 deaths from one million to four million cases of illness are caused by non-typhoidal salmonellosis in the United States (Bopp *et* al. 1999).

Typhoid fever is a serious systemic infection caused by serovar Typhi. In the United State it is rare but it is reported approximately 400 cases every year. Causative agent is mostly about foreign travel. Humans are the only reservoir and may be they are the carriers. It has a low infectious dose and its incubation period takes so long time. Transmission of typhoid fever is through person to person contact, also food and water contaminated fecally (Bopp *et* al. 1999).

Salmonella serovars are ubiquitous that are responsible for foodborne infections, especially serovar Typhimurium (Popoff *et* al. 2005). *S.* Typhimurium is invariably the most commonly found foodborne serovar throughout the world (Jay J.M. 1992c). They

have low infective doses but they are effective causing to clinical symptoms (Popoff *et* al. 2005).

In 2000, *Salmonella* Typhimurium and *Salmonella* Enteritidis were the most common serotypes that caused human salmonellosis accounting for 42% of all laboratory cases. In humans, nontyphoidal *Salmonella* infections are most often associated with food products such as food of animal origin including meat, poultry, eggs, or dairy products can become contaminated with *Salmonella* (Pegues *et* al. 2005).

Serovars of *Salmonella* may be adapted to one particular host strictly, may be ubiquitous or may be unknown pathogenicity (Popoff *et* al. 2005).

1.3. *Yersinia* spp.

1.3.1. History

In 1894, Alexandre Yersin had first isolated the plaque bacillus. In 1944, Van Loghem proposed that the transfer of *Pasteurella pestis* and *Pasteurella pseudotuberculosis* to his newly defined genus *Yersinia*, named later then Alexandre Yersin. In 1964, Frederiksen further inserted *Pasteurella X* (syn. *Bacterium enterocoliticum*), which previously had been explained by Schleifstein and Coleman (Aleksic *et* al. 1999).

1.3.2. Description of the Genera

Members of the genus *Yersinia* are nonspore forming, gram-negative, straight rods to coccobacilli, 0.5 to 0.8 µm in width and 1 to 3 µm in length. They are motile with peritrichous flagella under 30˚C except *Yersinia pestis* which is always non motile. Colonies are translucent to opaque, 0.1 to 1.0 mm in diameter after 24 hours. They grow optimally at 28-29˚C and at pH 7.2-7.4 (Bottone *et* al. 2005).

Yersiniae are facultatively anaerobic having both a respiratory and fermentative type of metabolism. Chemoorganotrophic. Oxidase negative, catalase positive and indole production varies among species (Holt *et* al. 1994c). Phenotypic characteristics are differed by the temperature and usually more characteristics are expressed by cultures incubated at 25-29˚C than 35-37˚C (Bottone *et* al. 2005). Usually methyl-red positive. Voges-proskauer and citrate tests are negative at 37˚C but these tests vary at 25-28˚C. They reduce nitrates. Cells usually are lysine decarboxlyase and arginine dihydrolyze negative, ornithine decarboxylase positive except *Yersinia pestis, Yersinia pseudotuberculosis* and *Yersinia rohdei.* H_2S is not produced. Urea is usually hydrolyzed except *Yersinia bercovieri, Yersinia pestis* and *Yersinia ruckeri.* They ferment D-glucose and other carbonhydrates with the production of acid but little or no gas (Holt *et* al. 1994c).

Taxonomic studies in the 1980s, the genus *Yersinia* include 10 species; *Yersinia pestis, Yersinia pseudotuberculosis, Yersinia enterocolitica, Yersinia frederiksenii, Yersinia intermedia, Yersinia kristensenii, Yersinia bercovieri, Yersinia mollaretii, Yersinia rohdei* and *Yersinia aldovae*. The taxonomic status of *Yersinia ruckeri* is still uncertain (Aleksic *et* al. 1999). The *Yersinia* species are summarized in Table 3.

Table 3. *Yersinia* Nomenclature (Bottone *et* al. 2005)

New Nomenclature	Previous Nomenclature
Y. aldovae	*Y. enterocolitica*-like group X2
Y. bercovieri	*Y. enterocolitica* biogroup 3B
Y. frederiksenii	Biogroup of *Y. enterocolitica*
Y. intermedia	Biogroup of *Y. enterocolitica*
Y. kristensenii	Biogroup of *Y. enterocolitica*
Y. mollaretii	*Y. enterocolitica* biogroup 3A
Y. pestis	*Pasteurella pestis*
Y. pseudotuberculosis	
Y. rohdei	
Y. ruckeri	'Red mouth bacterium'

1.3.3. Natural Habitats

Yersiniae occur in a wide spectrum of habitats including humans, animals particularly rodents and birds, soil, water, dairy products and foods (Holt *et* al. 1994c).

The geographical distribution of *Yersinia enterocolitica* is widespread. The strains have been isolated from a broad range of animal and environmental sources (including foods), also associated with a specific host (Bottone *et* al. 2005).

Yersinia intermedia and *Yersinia frederiksenii* have been isolated in Europe, the United States, Australia and New Zealand, Israel and Japan. They have been isolated originally from fresh water, foods, unusually from nonirrigated soil, humans or animals other than fish (Bottone *et* al. 2005).

Yersinia bercovieri has been found from terrestrial ecosystems. *Yersinia mollaretii* has been found from human stool specimens, most raw vegetables, soil and drinking water (Bottone *et* al. 2005).

The epidemiology of *Yersinia pestis* is widespread and it has been isolated from all continents except Australia and Antarctica. It causes plaque which is enzootic in Africa (Central East and South), in North and South America, certain regions of Asia (Southeast Asia, Mongolia) and former USSR, India and southwestern United States and Pacific coastal area. *Yersinia pestis* remains localized in enzootic or maintenance hosts and has been isolated from more a

hundred different naturally infected species of rodents, also rarely from predatory animals such as carnivores and birds (Bottone *et* al. 2005).

Yersinia pseudotuberculosis inhabits many animal species, especially rodents and birds, soil and humans. In Japan , cats and dogs have been infected with human cases. The wild animals are thought to be the reservoir of bacteria because they are asymptomatic. Humans and animals are easily contaminated orally or by direct contact with sick or asymptomatic animals or food contaminated by the animal excretions (Bottone *et* al. 2005).

Yersinia kristensenii has been identified in Europe, the United States, Japan and Australia. They have been isolated particulary from soil, foods, and asymptomatic animals, and also from other environmental sources and rarely human infection (Bottone *et* al. 2005).

Yersinia ruckeri has been recovered mainly in the United States and Canada from fresh water ecosystems (Bottone *et* al. 2005).

1.3.4. Pathogenesis

Yersinia are important foodborne and waterborne enteric pathogens which cause acute gastroenteritis, enterocolitis and mesenteric lymphadenitis, as well as a variety of extraintestinal disorders (Sulakvelidze A. 2000). These diseases can be acquired

directly from animals but are mostly acquired through ingestion of contaminated foods (Norrung *et* al. 2008).

Yersinia enterocolitica has been known as pathogenic for chinchillas, hares, monkeys and humans. Its pathogenicity looks like the pathogenicity of *Yersinia pseudotuberculosis. Yersinia enterocolitica* causes mainly acute enteritis in children. Then, concurrent mesenteric lymphadenitis and terminal ileitis may be occur. The acute terminal ileitis and mesenteric lymphadenitis are commonly appeared in young adults (Bottone *et* al. 2005). *Yersinia enterocolitica* is transmitted by ingestion of contaminated food or water, and less commonly, by direct contact with infected animals or patients. The zoonotic reservoirs are diverse, involving rodents, rabbits, pigs, sheep, cattle, horses, dogs, and cats (Butler *et* al. 2005).

Yersinia enterocolitica is classified into five biogroups based on their pathogenicity, and ecologic and geographic distributions: 1A, 1B, 2, 3, 4, and 5. *Y. enterocolitica* has about 60 serotypes. Serogroups belong to each biogroup are presented below: 1A (O:5; O:6, 30; O:7, 8; O:18; O:46), 1B (O:8; O:4; O:13a, 13b; O:18; O:20; O:21), 2 (O:9; O:5, 27), 3 (O:1,2,3; O:5,27), 4 (O:3), and 5 (O:2,3). Worldwide, the predominant serogroups that cause most infection are O:3, O:8, O:9, and O:5,27 (Bhunia A.K. 2008).

Animals are infected by *Yersinia pseudotuberculosis* by the oral route, after passing the incubation period the bacteria are found in

mesenteric lymph nodes. So it is an intracellular parasite. *Yersinia pseudotuberculosis* causes mesenteric adenitis and chronic diarrhea. Infection evolves either in self-cure or in fatal septicemia. In a addition, humans are infected orally causing a mesenteric adenitis, which stimulates acute appendicitis or severe septicemia for the compromised host. The incidence of its infection is variable by the season and is highest in cold months (Bottone *et* al. 2005). Reservoirs of *Yersinia pseudotuberculosis* are various rodents, rabbits, deer, farm animals, and birds, including turkeys, ducks, geese, pigeons, pheasants, and canaries (Butler *et* al. 2005).

Yersinia pestis is a highly virulent pathogen that causes systemic infection with a high mortality rate (Murray *et* al. 2005). *Yersinia pestis* causes plaque that is particularly a disease of wild rodents. Plague is often referred to as “Black Death.” *Yersinia pestis* is transmitted through wild rodents by flea bites or ingestion of contaminated animal tissues. Later, the bacterium multiplies in fleas and blocks the esophagus and pharynx. The fleas regurgitate the bacteria when they take their next blood meal and then transmit *Yersinia pestis* to humans if other hosts aren't available. The typical bubonic form of plaque is produced within 2-8 days by the infective flea bites (Bottone *et* al. 2005). As a result of being bitten by a flea that is part of sylvatic cycle (Levinson *et* al. 2002c). If the disease is evolved untreatedly in 5-10 days to indicate septicemia in which *Yersinia pestis* may be seen in peripheral blood

smears. Secondary pneumonia may remain from hematogenous spread. The pneumonic plaque can spread from person to person, from animals to humans by droplets. Bubonic and pneumonic plaque have been transmitted to humans by the domestic cats- bubonic plaque transmits among a scratch and bite from an infected cat and pneumonic plaque transmits among face to face exposure to an infected cat (Bottone *et* al. 2005).

The urban cycle occurs by transmission of the bacteria among urban rats, with the rat flea as vector. This cycle gets started during times of poor sanitation (Levinson *et* al. 2002c).

The organism has several factors that assist in its virulence: the envelope capsular antigen, called as F-1, which protects against phagocytosis; endotoxin; exotoxin; V antigen; W antigen. Besides, other factors that contribute to the extraordinary pathogenicity of *Yersinia pestis* is group of virulence factors collectivelly called Yops (Yersina outer proteins) (Levinson *et* al. 2002c).

The pathogenicity of *Yersinia intermedia, Yersinia kristensenii, Yersinia frederiksenii, Yersinia bercovieri and Yersinia mollaretii* is unclear in humans and animals. They are considered to be more like oppurtunistic pathogens than true pathogens (Bottone *et* al. 2005). At last, *Yersinia ruckeri* causes red mouth disease in fish (Holt *et* al. 1994c).

2. MATERIALS AND METHODS

2.1. Sample Collection

The retail meat products were collected between November 2007 and May 2008 from butcher shops and delicatessens in Bolu. A total of 225 samples of retail meat products were examined; 75 of them were poultry meat, 75 of them were ground beef, 75 of them were meat samples. The samples of retail meat products were transported to the microbiology laboratory in a sterile containers and analysed at the same day.

2.2. Microbiological Analysis

2.2.1. Isolation and Identification of *Escherichia coli*

A 25 g of retail meat product was aseptically added to 225 ml of buffered peptone water (MERCK). Samples were homogenized for 2 minutes by stomacher (Interscience Bagmixer 400). Most Probable Number Technique was used to isolate for *E. coli* in foods (The International Organization for Standardization (ISO)). And as enrichment broth, Laurly Sulfate broth + 4-methylumbelliferyl-ß-D-glucuronide (MUG) was used. MPN method, is a number of tubes of liquid growth media is prepared in parallel at each dilution and results are recorded as the number of tubes in which the target organism grows, sometimes after confirmatory tests (Corry *et* al. 2006).

Beta-glucuronidase, a highly specific enzyme of *E. coli*, has a range of opportunities for the improvement of culture media for the identification of this organism. A beta-glucuronidase reaction is positive in the middle to upper 90% range of *E. coli* isolated from a variety of sources. The highly sensitive substrate 4-methylumbellifery-ß-D-glucuronide is cleaved by the enzyme and the hydrolysis product 4-methylumbelliferone shows a visible green/blue fluorescence under long-wave ultra-violet light (366 nm). The fluorogenic assay was sensitive and rapid but conventional study was time consuming and laborious.

The use of MUG in a Most Probable Number (MPN) technique for enumeration of presumptive *Escherichia coli* in food and environmental samples has been specified in a standard ISO procedure.

The three-tube MPN technique was used to determine the level of contamination of *E. coli* in samples found positive. 10-fold dilutions of up to 10^{-3} were made by transferring 0.5 ml of homogenate into tubes containing 4.5 ml of sterile peptone saline solution (0.1 % peptone and 0.85 % sodium chloride). Aseptically 1 ml of each of the prepared dilutions of the sample was pipetted into each of 10 ml of Laurly Sulfate broth + MUG of three tubes. Each of the tubes contains Durhaim tube in order to observe gas production. Then the nine tubes for each sample were incubated at 37°C for 24 h into the waterbath. A tube showing the production of

gas and turbidity were recorded as being presumptively positive. Furthermore, tubes that had turbidity and gas from Durhaim, were examined under the long wave UV lamp at 366 nm to give either flourescent light or not. At lastly, these tubes were investigated indole production. The tubes which were positive flourescence light formation, gas and indole production showed existence of *Escherichia coli.* Results were recorded as the number of tubes and then codes were occurred.

Data were entered and analyzed with SPSS 15.0 for Windows statistical program. Anova test was used for statistical analysis of the significant difference of contamination rates. An α, significant figure, of 0.05 was used for statistical significance.

2.2.2. Isolation and Identification of *Salmonella* spp.

A 25 g of retail meat product was aseptically added to 225 ml of buffered peptone water (MERCK) and homogenized for 2 minutes by stomacher (Interscience Bagmixer 400). Then they were incubated at 37˚C for 24 h as a pre-enrichment medium. To assess a suitable enrichment for cultivation of *Salmonella,* two commercially available broths and agar medium were evaluated. 0.1 ml aliquot of pre-enrichment was inoculated into 10 ml of Rappaport Vassiliadis broth (RVS) (FLUKA) and incubated at 41.5˚C. Also, 10 ml aliquot of pre-enrichment was inoculated into 100 ml

of Selenite Cystine broth (SC) (MERCK). These tubes were incubated at 37°C as a selective enrichment. After 24 h incubation, a loopful of culture from RVS broth was streaked onto both Rambach agar (Salmonella Chromogenic agar) (SCA) (FLUKA) and Salmonella-Shigella (SS) agar (MERCK) plates. Besides, a loopful of culture from SC broth was streaked onto Salmonella-Shigella agar plates. These plates were incubated at 37°C for 24 h. Following incubation, suspect colonies in red onto Rambach agar and suspect colonies of colorless, usually with black center onto Salmonella-Shigella agar were counted as *Salmonella*. Suspect colonies were selected and subcultured on SS Agar for purity and confirmed biochemically. The presumptive colonies were examined for gram-staining, Voges-Proskauer and Methyl Red (MR-VP) reaction, indole production, H_2S production on Triple-Sugar Iron agar (TSIA) (MERCK), utilization of citrate, urease and for production of each ornithine and lysine decarboxylase and also fermentation of D-glucose, D-mannitol, D-xylose, L-rhamnose, dulcitol, sorbitol, lactose and melibiose (Ellermeier *et* al. 2006). All of the tests are shown in Table 4.

Table 4. Isolation of *Salmonella* spp. from retail meat products

Tests (37˚C)	*S. enterica* serovar Typhimurium	*S. bongori*	*S. enterica* subspecies *diarizonae*
Gram stain (24h)	-	-	-
Indole	-	-	-
Methyl red	+	+	+
Voges-Proskauer	-	-	-
Simmons citrate	+	+	+
H_2S production	+	+	+
Urease	-	-	-
Lysine decarboxylase	+	+	+
Ornithine decarboxylase	+	+	+
Glucose, acid-gas	A G	A G	A G
Lactose	-	+	+
Xylose	+	+	+
Rhamnose	+	+	+
Arabinose	+	+	+
Mannitol	+	+	+
Dulcitol	+	+	-
Sorbitol	+	+	+
Melibiose	+	+	+

Symbols : +, (90-100%) and -, (0-10%).

2.2.3. Isolation and Identification of *Yersinia* spp.

A 25 g of retail meat product was aseptically added to 225 ml of sterile buffered peptone water (MERCK) and samples were homogenized for 2 minutes by stomacher (Interscience Bagmixer 400). 1 ml volume of homogenate was inoculated into tubes that contain 10 ml of Yersinia Enrichment broth (MERCK), and incubated at 30°C for 24 h. Then, a loopful of each pre-enrichment sample was streaked onto Cefsoludin-Irgasan-Novobiocin (CIN) agar (MERCK) which is highly selective against the growth of competing flora and gives characteristics colony morphology. These CIN agar plates were incubated at 30°C for 24 h. After incubation, suspect colonies of deep red center with a transparent margin, or "bull's-eye" appearance were counted as *Yersinia.* The suspect colonies were streaked onto Brain-Heart-Infusion (BHI) agar. To identify all isolates, biochemical and morphological tests were done including gram-staining, oxidase and catalase, motility (at 22°C), urease, indole production, utilization of citrate, acid reaction with methyl red and acetoin production at 37°C and for production of ornithine decarboxylase and lysine decarboxylase, nitrate reduction, and the ability to produce acid from each mannitol, L-rhamnose, L-arabinose, sucrose, cellibiose, D-raffinose, and maltose (Bottone *et* al. 2005). In Table 5, the tests given above are summarized.

Table 5. Isolation of *Yersinia* spp. from retail meat products

Tests (37˚C)	*Y. mollaretii*	*Y. intermedia*	*Y. pseudotuberculosis*
Gram stain (24h)	-	-	-
Oxidase (24h)	-	-	-
Catalase (24h)	+	+	+
Indole	-	-	-
Methyl red	+	+	+
Voges-Proskauer	-	-	-
Simmons citrate	-	-	-
H_2S production	-	-	-
Urease	[+]	[+]	+
Lysine decarboxylase	-	-	-
Ornithine decarboxylase	+	+	-
Motility (22˚C)	-	+	+
Nitrate reductase	+	+	+
Arabinose	+	+	+
Raffinose	-	d	-
L-Rhamnose	-	d	+
Cellibiose	+	+	-
Sucrose	+	+	-
Mannitol	+	+	+
Maltose	d	+	+

Symbols: +, (90-100% positive); -, (0-10% positive); d, (11-89% of strains are positive); [+], (76-89% positive).

2.3. List of culture media and applied tests in isolation of Bacteria

Brain Heart Infusion Agar.....(Oxoid CM0375)

Buffered Peptone Water Broth.....(Merck 1.07228)

D-(+)-Cellibiose.....(Sigma-Aldrich C7252)

D-(+)-Glucose monohydrate.....(Merck 1.08342)

D-(-)-Mannitol.....(Merck 1.05982)

D-(+)-Melibiose.....(Fluka 63630)

D-(-)-Raffinose.....(Merck 1.07419)

D-Sorbitol.....(Fluka 85532)

D-(+)-Xylose.....(Merck 1.08689)

Dulcitol.....(Merck 1.05990)

Griess' Reagent for Nitrite.....(Fluka 03553)

Lactose monohydrate.....(Merck 1.07657)

Laurly Sulfate Broth + MUG.....(Oxoid CM0980)

L(+)-Arabinose.....(Merck 1.01492)

L-(+)-Rhamnose Monohydrate.....(Fluka 83650)

L-Lysine monohydrochloride.....(Merck 1.05700)

L-Ornithine monohydrochloride.....(Merck 1.06906)

Maltose.....(Merck 1.05910)

Methyl-Red Voges-Proskauer Broth.....(Fluka 69150)

Nitrate Broth.....(Fluka 72548)

Rambach Agar.....(Fluka 84369)

Rappaport Vassiliadis Broth.....(Fluka 17173)

Salmonella-Shigella Agar.....(Merck 1.O7667)

Selenite-Cystine Broth.....(Merck 1.07709)

SIM Medium.....(Merck 1.05470)

SIMMONS' Citrate Agar.....(Merck 1.02501)

Sucrose.....(Merck 1.07651)

Triple Sugar Iron Agar.....(Merck 1.03915)

Urea Broth.....(Fluka 51463)

Yersinia Agar (CIN) + CIN Supplement.....(Merck 1.16434 +1.16466)

Yersinia Selective Enrichment Broth.....(Merck 1.16701)

3. RESULTS AND DISCUSSION

3.1. *Escherichia coli*

This research has emphasized data indicating retail meats are an important reservoir of *E. coli.* In this study, a total of 168 from 225 retail meat products were tested to the analysis of *E. coli. E. coli* was found in 90 (53.5%) of the 168 meat products. Distribution of these meat samples: *E. coli* was positive in 49 (87.5%) of the 56 poultry meats, 27 (48.2%) of the 56 ground beef and 14 (25%) of the 56 meat samples. The *E. coli* level in the positive poultry samples is presented as MPN/g in Table 6. Between 3.6 X 10^2 and 4.6 X 10^4 MPN/g with a mean of 3.6 X 10^3 MPN/g of *E. coli* was found from positive samples. Table 7 shows the level of *E. coli* in the positive ground beef samples as MPN/g. Between 3.6 X 10^2 and 2.4 X 10^4 MPN/g with a mean of 1.3 X 10^3 MPN/g of *E. coli* was counted from positive samples. In Table 8, *E. coli* level in the positive meat samples is presented as MPN/g. Between 3.6 X 10^2 and 2.4 X 10^4 MPN/g with a mean of 6.4 X 10^2 MPN/g of *E. coli* was estimated from positive samples.

Table 6. Distribution of *E. coli* species isolated by Most Probable Number Technique from poultry meat

Number of Samples	Codes	Calculations of bacteria
1	3 1 0	4.3×10^3
2	3 1 0	4.3×10^3
3	3 0 0	2.3×10^3
4	3 2 1	1.5×10^4
5	3 1 0	4.3×10^3
6	3 2 0	9.3×10^3
7	2 0 0	9.2×10^2
9	3 0 0	2.3×10^3
10	3 1 0	4.3×10^3
11	3 0 0	2.3×10^3
12	3 1 1	7.5×10^3
13	3 0 0	2.3×10^3
14	3 2 0	9.3×10^3
15	1 0 0	3.6×10^2
16	3 1 0	4.3×10^3
18	3 2 1	1.5×10^4
19	1 0 0	3.6×10^2
20	1 0 0	3.6×10^2
21	2 1 1	2.0×10^3
22	3 0 0	2.3×10^3

23	1 0 0	3.6×10^2
24	1 1 0	7.4×10^2
25	1 0 0	3.6×10^2
26	1 0 0	3.6×10^2
29	2 0 0	9.2×10^2
31	2 0 0	9.2×10^2
32	2 0 0	9.2×10^2
33	1 0 0	3.6×10^2
34	3 1 0	4.3×10^3
35	1 0 0	3.6×10^2
36	1 0 0	3.6×10^2
37	1 0 0	3.6×10^2
39	3 3 1	4.6×10^4
40	2 1 0	1.5×10^3
41	2 2 0	2.1×10^3
42	3 1 1	7.5×10^3
43	3 2 0	9.3×10^3
44	3 1 1	7.5×10^3
45	3 0 0	2.3×10^3
46	3 0 0	2.3×10^3
47	3 1 0	4.3×10^3
48	3 1 0	4.3×10^3
49	3 1 1	7.5×10^3

50	1 0 0	3.6×10^2
51	1 1 0	7.4×10^2
52	2 0 0	9.2×10^2
53	3 0 0	2.3×10^3
54	3 0 0	2.3×10^3
55	2 0 0	9.2×10^2
Total	49	**Average:** 3670.714

Table 7. Distribution of *E. coli* species isolated by Most Probable Number Technique from ground beef

Number of Samples	Codes	Calculations of bacteria
1	3 0 0	2.3×10^3
2	3 2 0	9.3×10^3
5	1 0 0	3.6×10^2
6	3 3 0	2.4×10^4
7	2 0 0	9.2×10^2
9	3 1 0	4.3×10^3
10	3 0 0	2.3×10^3
11	1 0 0	3.6×10^2
12	1 0 0	3.6×10^2
13	3 0 0	2.3×10^3
22	1 0 0	3.6×10^2
30	1 0 0	3.6×10^2
31	2 0 0	9.2×10^2
32	1 0 0	3.6×10^2
33	2 1 0	1.5×10^3
37	2 2 0	2.1×10^3
40	2 2 0	2.1×10^3

41	2 1 0	1.5×10^3
42	3 2 1	1.5×10^4
43	2 0 0	9.2×10^2
44	1 0 0	3.6×10^2
46	1 0 0	3.6×10^2
49	1 0 0	3.6×10^2
50	3 0 0	2.3×10^3
52	1 1 1	1.1×10^3
53	1 0 0	3.6×10^2
56	2 0 0	9.2×10^2
Total	27	**Average :** 1381.79

Table 8. Distribution of *E. coli* species isolated by Most Probable Number Technique from meat

Number of Samples	Codes	Calculations of bacteria
1	3 3 0	2.4×10^4
2	1 0 0	3.6×10^2
3	2 0 0	9.2×10^2
4	1 0 0	3.6×10^2
12	3 0 0	2.3×10^3
14	1 0 0	3.6×10^2
15	1 0 0	3.6×10^2
33	1 0 0	3.6×10^2
35	1 0 0	3.6×10^2
36	2 0 0	9.2×10^2
37	1 1 0	7.4×10^2
50	1 0 0	3.6×10^2
53	3 1 0	4.3×10^3
54	1 0 0	3.6×10^2
Total	14	**Average :** 643.9286

The Laurly Tryptose broth + MUG in a most probable number assay was superior for *E. coli* detection. The fluorogenic assay was sensitive and rapid but conventional study was time consuming and laborious. The present study was undertaken to developed specific and more efficient assays for *E. coli* by using the fluorogenic substrate MUG to detect GUR positive bacteria.

Improved *E. coli* testing methods have shortened the time required to obtain an *E. coli* count substantially. MUG was not found to be inhibitory or stimulatory effect to the growth rate of *E. coli.* In this case, the MUG method was more sensitive, especially in foods with lower levels of *E. coli.* Feng and Hartman (1982) emphasized that even the presence of one viable cell initially would produce fluorescence within 20 h and, when initial inocula of about 10^4 cell ml^{-1} were used, fluorescence was observed within 4 h in LST+MUG medium. Moreover, the MUG method was evaluated to be a rapid, accurate, simple to perform and less expensive method, with no interference from food products (Robison B. 1984, Moberg *et* al. 1988).

Autofluorescence of some glass tubes and a negative ß-GUR reaction of some enteropathogenic strains of *E. coli* and some false reading of the fluorogenic reaction give disadvantages of the MUG method (Poelma *et* al. 1987). This technique is preferable to the current ISO method, because enumeration of *E. coli* in foods could be acquired at low concentrations. Also, the analysis time was

reduced from 6-10 days to maximum 48 h, and labour and media savings were the other advantages. MUG method can confidently be used for routine analysis of foods (Dogan *et* al. 2002).

In the present study, data were analysed by SPSS 15.0 for Windows statistical program and showed in Table 9. Descriptives is another frequenlty used SPSS procedure, and descriptive statistics are designed to give us information about the distributions of our variables.

Table 9. Analyses of the data by SPSS statistical program - Descriptives

Product type	N	Mean	Std. Deviation	Std. Error	95% Confidence Internal for Mean	
					Lower Bound	Upper Bound
Poultry meat	56	3670.71	6755.075	902.685	1861.69	5479.74
Ground beef	56	1381.79	3906.705	522.055	335.56	2428.01
Meat	56	643.93	3246.921	433.888	-225.60	1513.46
Total	168	1898.81	5019.690	387.278	1134.22	2663.40

Anova identifies whether the mean of one group differs significantly from the mean of another group or groups. In all instances a significance value will be calculated identifying the likelihood that a particular outcome is or is not reliable. The smaller the *p* value, the greater the likelihood that the findings are valid. Scientists have generally accepted that if the *p* value is less than 0.05, then the result is considered statistically significant. When the significance level falls far below 0.05 (e.g. 0.001, 0.0001, etc.) the smaller the value the greater confidence the researcher has that his or her findings are valid. Table 10 shows interpretation of *E. coli* data from meat samples according to one-way Anova. In the present study, *p* value for "between groups" was found to be equal to 0.003. Therefore, our results were statistically significant, valid and reliable.

Table 10. Interpretation of data according to one-way Anova

ANOVA (one-way)	Sum of Squares	df	Mean Square	F	Sig.
Between groups	2.8E+008	2	139487216.67	5.858	0.003
Within groups	3.9E+009	165	23811959.567		
Total	4.2E+009	167			

One-way Anova generates a significance value indicating whether there are significant differences within the comparisons being made. In this research, between data there were meaningful differences. Means for groups in homogeneous subsets are displayed in Table 11. Data which were analysed with statistical program showed that both meat and ground beef were put in Group 1, and poultry meat was put in Group 2.

Table 11. Interpretation of data according to Duncan[a] Test

Product type	N	Subset for alpha = 0.05	
		1	2
Meat	56	643.93	
Ground beef	56	1381.79	
Poultry meat	56		3670.71
Sig.		0.425	1.000

Coliform, fecal coliform or *E. coli* is thought the most important and compulsory measure of microbiological quality of food and food related products in terms of hygiene. All of them are used as indicators of fecal pollution. Especially, *E. coli* is usually preferred as an indicator because it is specific and most reliably

reflects fecal origin (Hafız *et* al. 2003). The contamination of the foods with *E. coli* include a variety of ways; bowel rupture during evisceration, indirect contamination with tainted water, handling and packaging of finished products (Schroeder *et* al. 2004).

The microbiological quality of the raw meat, other ingredients, personal hygiene and any contamination during the process determine the microbiological quality of final product. According to studies, ground meat is a good medium for the growth of microorganisms and causes foodborne infections. In one survey, *E. coli* from 50 ground beef were counted at the average of 4.6 X 10^1 and also from 50 raw meat were determined at the average of 1.2 X 10^3 (Elmali *et* al. 2005). Davidson *et* al. (2000) reported that *E. coli* was found at the level of 4.8 X 10^3 from ground beef. Above 30 samples of chicken were studied, and *E. coli* was detected in 93.3% of samples. To sum up chicken by-products show high microbiological contamination levels and high incidence rates with pathogenic microorganisms (Capita *et* al. 2002a). Our results were similar to Capita *et* al. (2002a). We found *E. coli* positive in 49 (87.5%) of 56 chicken samples. As a consequence, chicken meat was more frequently contaminated than meat and ground beef.

In Turkey, Sırıken B. (2004a) reported that *E. coli* was positive in 30% of ground beef samples using MPN method. In our study, *E. coli* was found a higher prevalence rate as 48.2% of ground beef samples.

In this research, the differences in the microbial numbers of the poultry meat, ground meat and meat were observed. The results can be changed depending on the technique used to slaughter animals, contaminations during evisceration of the internal organs, abattoir hygiene, conditions of storage and personal hygiene.

3.2. *Salmonella* spp.

Salmonella infections are seen worldwide and constitute an important public health problem in Turkey as well as in many parts of the world (Erdem *et* al. 2005). Several studies indicated that salmonellosis in humans was caused by consumption of contaminated foods. A variety of food products, especially poultry and other types of meat products are the most important sources of human *Salmonella* infection.

In this research, a total of 225 retail meat products were tested to the analysis for *Salmonella* spp. In 50 samples, *Salmonella* spp. was found in a prevalence of 22.2%. All 50 isolates could be serotyped as 22 (9.8%) of these were *S. enterica* serovar Typhimurium (*S.* Typhimurium), 20 (8.9%) of them were *S. bongori*, eight (3.5%) of them were *S. enterica* subsp. *diarizonae*. It is displayed in Table 12.

Table 12. Distribution of *Salmonella* species in 50 positive isolates

Salmonella species	Number of Strains	Percentage (%)
S. Typhimurium	22	9.8
S. bongori	20	8.9
S. enterica subsp. *diarizonae*	8	3.5
Total	50	22.2

The occurrence of *Salmonella* isolates in these three different samples analyzed has been summarized in Table 13, Table 14 and Table 15. In 75 poultry meat samples; 20 (8.88%) strains were identified as *S.* Typhimurium and two (0.88%) as *S. bongori*. From 75 of ground beef samples; one (0.44%) sample was typed as *S.* Typhimurium, ten (4.44%) as *S. bongori* and five (2.22%) as *S. enterica* subsp. *diarizonae*. Of 75 meat samples; one (0.44%) sample was identified as *S.* Typhimurium, eight (3.55%) as *S. bongori* and three (1.33%) as *S. enterica* subsp. *diarizonae*.

Table 13. Distribution of 22 *Salmonella* species isolated from poultry meat

Salmonella species	Number of Strains	Percentage (%)	Number of Samples
S. Typhimurium	20	8.88	2, 4, 8, 10, 19, 21, 22, 29, 36, 40, 41, 43, 44, 45, 48, 49, 51, 52, 53, 57
S. bongori	2	0.88	1, 62

Table 14. Distribution of 16 *Salmonella* species isolated from ground beef

Salmonella species	Number of Strains	Percentage (%)	Number of Samples
S. Typhimurium	1	0.44	2
S. bongori	10	4.44	2, 5, 7, 23, 27, 33, 55, 63, 69, 75
S. enterica subsp. *diarizonae*	5	2.22	2, 16, 37, 39, 60

Table 15. Distribution of 12 *Salmonella* species isolated from meat

Salmonella species	Number of Strains	Percentage (%)	Number of Samples
S. Typhimurium	1	0.44	40
S. bongori	8	3.55	6, 16, 22, 26, 27, 44, 54, 57
S. enterica subsp. *diarizonae*	3	1.33	57, 59, 66

A total of 110,229 of raw meat samples (bovine, porcine, poultry) were subjected to the analysis for *Salmonella* spp. over the three-year period. The prevalence rate of *Salmonella* spp. that was found to be positive was 1.0%. The percentage of samples contaminated with *Salmonella* decreased over the three-year from 1.2% in 2002 to 0.9% in 2004. There was no seasonal trend during isolation period of *Salmonella* from any of the meats or meat products. Recovery of the organism was the highest for poultry meat at 3.1%. These results showed that poultry meats were generally higher risks than other meats for *Salmonella.* Besides, *S.* Typhimurium was the most prevalent serotype isolated in that

survey (Egan *et* al. 2006). Our results were consistend with data mentioned above.

In Nigeria, Orji *et* al. (2005) studied with 120 samples of poultry and 96 samples of raw beef. They found 46 *Salmonella* spp. out of the 120 poultry samples and 24 *Salmonella* spp. out of the 96 raw beef samples. Eight isolates from 46 positive samples and two isolates from 24 positive samples were serotyped as *S.* Typhimurium like our findings. Other isolates that were found to be positive for *Salmonella* spp. were identified into six different serotypes such as *S.* Paratyphi A, *S.* Typhi, *S.* Enteretidis, *S.* Gallinarum, *S.* Pullorum, *S.* Agama.

Prevalence of *Salmonella* vary may be attributed to several factors; including the initial numbers of *Salmonella* carriers entering the factory, slaughterhouse sanitation, the level of processing of the poultry, possible cross-contamination at retail level and variations in the isolation methods employed to detect the pathogen in individual studies (Baumagartner *et* al. 1992, Uyttendaele *et* al. 1998). Moreover, reasons for differences in the distribution of *Salmonella* serotypes are not simply understood, it may be attributed to seasonal or demographic factors or the prevalence of a particular species in different product types (Ziprin R.L. 1994).

In the U.K., Humphrey *et* al. (2006) studied with cattle, sheep, chicken and pig meat samples to isolate *Salmonella* spp. They

reported that *S.* Typhimurium was identified from cattle, sheep and pigs in 15%, 6%, 71% respectively. But no *S.* Typhimurium was isolated from chickens in the research. This result is not agreement with our data obtained from chicken. Nearby, *S. enterica* subsp. *diarizonae* was isolated from only sheep in 66%. Also, this research showed us that *S.* Typhimurium was the predominant species isolated from the meat products like our study.

Kozacinski *et* al. (2006) reported that *Salmonella* spp. were positive in 15.39% of chicken breasts without skin, in 9.52% of chicken breasts with skin and in 10.53% of ground chicken meat. Bacteriological analysis of chicken meat samples showed that chicken breasts without skin contained higher number of bacteria. Authors emphasized indicative of contamination and inadequate hygienic conditions in the production and processing of poultry meat.

Cardinale *et* al. (2003) showed a high frequency of isolation of *Salmonella* spp. in chicken carcasses. They detected 96 (32%) positive out of 300 samples for *Salmonella* spp. Eight different *Salmonella* serotypes were isolated. It was demonstrated that the most important factor leading to *Salmonella* spp. disease outbreaks were improper personal hygiene.

Compared to the our research, many authors found a higher prevalence of *Salmonella* in some countries: 50% in the U.S. (Schlosser *et* al. 2000), 50% in Japan (Kudaka *et* al. 2006), 57% in

Thailand (Padungtod *et* al. 2006) and 49% in Spain (Capita *et* al. 2003). Meanwhile, many authors found a lower prevalence of *Salmonella* than we found in some countries such as 0.24% in Turkey (Çetinkaya *et* al. 2008), 5.4% in Australia (Mayrhofer *et* al. 2004), 3.94% in USA (Logue *et* al. 2004), 6.5% in Albania (Beli *et* al. 2001), 20% in Botswana (Gashe *et* al. 2006) and 16.4% in India (Bhandare *et* al. 2007). At lastly, all previous studies showed that the serotypes isolated can vary geographically.

In the present study, two different selective enrichment broths and selective agar medium were used; broths were Selenite Cystine broth (SC) and Rappaport Vassiliadis broth (RVS), solid medium were Salmonella-Shigella agar (SS) and Rambach agar (Salmonella Chromogenic agar) (SCA). We observed that both of the selective enrichment broths in the research gave good results. SS agar, one of the solid medium used in the research, was highly selective. However, the selectivity of SCA was insufficient for isolation of *Salmonella*. Most of *Salmonella* spp. were isolated from SS agar.

Cardoso *et* al. (2006) explained that the selectivity of RVS and SC broth for isolation of *Salmonella* were equal. The selectivity of SS agar was the best for growth of *Salmonella* spp. In Norway, Alvseike *et* al. (2000) studied with lamb meat and faeces from sheep for isolation of *Salmonella* spp. They emphasized that RVS and SC broths were the most efficient selective enrichment broths. However, the efficacy of the RVS broth and the toxicity of the SC

broth have resulted in the RVS broth becoming the most commonly used enrichment medium for food analysis. Therefore SC broth has been used more often when analysing clinical specimens, and this broth also produced the best results from the faecal samples in their study. Schönenbrücher *et* al. (2008) recorded the same interpretation about selective enrichment broths. They explained that RVS broth was the most suitable enrichment media, besides SC broth was less advisable for the investigation of meat products. We observed that these selective enrichment broths were the most efficient broths for cultivation of *Salmonella* spp. from meat products in the research.

The importance of *Salmonella* as a zoonotic pathogen has meant that there have been many investigations of contamination incidences with common foods. *Salmonella* have been known for over 100 years and the importance of various transmission pathways is much better known. But there is no suspicion that the infection with or carriage of *Salmonella* and other pathogen in red meat animals represents a substantial risk to human health, which is exacerbated by poor handling in commercial and domestic kitchens (Humphrey *et* al. 2006).

Consequently, implementation of good cooking techniques and good kitchen and personal hygiene during preparation are necessary.

3.3. *Yersinia* spp.

The occurrence of *Yersinia* spp. in meat have been investigated in several countries. There are very few reports about the incidence of *Y. enterocolitica* in different foodstuffs in Turkey. In this study, *Yersinia* spp. were found in five (2.2%) out of 225 meat products. Table 16 and Table 17 display distribution of *Yersinia* species in five positive isolates. Among these positive meat samples, one (0.44%) sample was identified as *Y. intermedia,* one (0.44%) as *Y. pseudotuberculosis* and three (1.33%) as *Y. mollaretii*.

Table 16. Distribution of *Yersinia* species in five positive isolates

Yersinia species	Number of Strains	Percentage (%)
Y. pseudotuberculosis	1	0.44
Y. intermedia	1	0.44
Y. mollaretii	3	1.33
Total	5	2.2

Table 17. Distribution of five *Yersinia* species isolated from retail meat products

Yersinia species	Number of Strains	Number of Samples	Product Type
Y. pseudotuberculosis	1	22	Poultry meat
Y. intermedia	1	19	Ground beef
Y. mollaretii	3	8 41 44	Ground beef Poultry meat Ground beef
Total	5		

Capita *et* al. (2002b) reported 26 (65%) poultry carcasses harboured *Yersinia* spp.: 20 (50%) contained as *Y. enterocolitica,* four (15%) as *Y. frederiksenii,* two (5%) as both *Y. enterocolitica* and *Y. frederiksenii*. Sırıken B. (2004b) showed that *Yersinia* spp. were recovered from 20 of 61 (32.8%) ground beef; 17 (27.9%) as *Y. enterocolitica*, two (3.3%) as *Y. intermedia,* one (1.6%) as *Y. frederiksenii.* Nortje *et* al. (1999) reported of the 51 ground beef samples, 15.7% contained *Y. enterocolitica.* Those studies had higher incidence for isolation of *Yersinia* spp. than the present study. Differences between these findings might be due to several

factors such as isolation methods, number of analyzed samples, types of samples, season and geographical location. These factors may cause an increase or decrease in the prevalence of *Yersinia* spp.

The direct isolation of *Yersinia* spp. in foods and environmental samples, even on selective media, is seldom successful. While selective enrichment media are not selective enough, they contain agents which inhibit the growth of some pathogenic strains (Korkeala *et* al. 2003).

Simonova *et* al. (2007) reported that the highest incidence of isolates of *Y. enterocolitica* was found in pigs (3.3%) while in cattle and poultry their incidence was low (0.5% and 0.2%) respectively. Also they showed that pigs are mainly reported as the reservoir of *Y. enterocolitica*, this has already been corroborated by a number of studies. Mayrhofer *et* al. (2004) studied with 90 pork, 91 beef and 21 turkey meat samples for isolation of *Y. enterocolitica*. The prevalence of *Y. enterocolitica* was positive in 39 pork, 29 beef and ten turkey meat samples. Pork meat samples had a high prevalence rate (43.3%) to *Y. enterocolitica*. Therefore pork meat was thought to be an important source of yersiniosis, and pork was more frequently contaminated than beef. Boer *et* al. (1982) and Khalafalla F.A. (1990) emphasized that the contamination levels of *Yersinia* in live poultry is low, and scalding destroys yersiniae. As above mentioned, the existence of these bacteria in poultry carcasses is

attributed to a post-scalding contamination and to multiplication during refrigeration storage. Therefore, hygiene while the processing of poultry carcasses is an important factor with regard to *Yersinia* spp.

Meat and meat products are important sources of foodborne infections. Occurrence of zoonotic pathogens in raw meat is variable, although most often 1% and 10%, depending on the organisms, geographical factors, farming and meat production practices (Norrung *et* al. 2008).

4. CONCLUSION

The results of the study indicate that the retail meat products create a potential hazard to consumers and this might be due to a combination of the low quality of beef carcass used, poor manufacturing processes (poor temperature control) during processing and storage, inadequate cleaning and disinfection of both equipment and surfaces such as floors or poor personel hygiene and use of untrained personel.

In this research, out of 225 retail meat products, the results were found to be positive for *Salmonella* spp. in 50 (22.2%), *Yersinia* spp. in five (2.2%) samples. *Escherichia coli* was detected in 90 (53.5%) from 168 retail meat samples. The incidence of *Escherichia coli, Salmonella* spp. and *Yersinia* spp. may be considered remarkable.

Foodborne diseases are an important public health problem in most countries. Foodborne diseases have been reported to be mainly associated with poor handling as well as lack of awareness with regard to safety of food.

Bacterial hazards are a major concern in the production of food of animal origin. Poultry and poultry products are the frequent vehicles of the bacterial species. Increase in demand for meat without the infrastructure for proper sanitary handling may lead to transfer of pathogenic organisms from the animals to the consumer. Meat poses a direct food-poisoning hazard when

consumed in a raw or undercooked form. However, raw meat poses an indirect food-poisoning hazard through cross-contamination of cooked meats and of other foods that are not cooked before consumption.

Most *E. coli* are harmless inhabitant of the intestinal tract, and only a small percentage of strains are considered pathogenic. Pathogenic *E. coli* strains cause a variety of diseases including gastroenteritis, dysentery, hemolytic uremic syndrome (HUS), urinary tract infection (UTI), septicemia, pneumonia, and meningitis. Meats are a common source of *E. coli* contamination, which may be acquired during slaughter through fecal contact.

Salmonella are present in the intestinal tract of birds, reptiles, turtles, insects, farm animals, and humans. Poultry are a major source for human foodborne salmonellosis. Human salmonellosis is generally foodborne and is contracted through consumption of contaminated food of animal origin such as meat, milk, poultry, and eggs. *Salmonella enterica* causes gastroenteritis, typhoid fever, and bacteremia. Poultry, egg, meat, dairy products, and fruits and vegetables serve as vehicles of transmission. *Salmonella* Typhimurium causes serious illness in children, and immunocompromised individuals, often resulting in systemic infection. In healthy individuals, symptoms include fever, diarrhea, abdominal pain, and sometimes vomiting.

Salmonella spp. have been found to be associated with illness diagnosed in many animals, including cattle with clinical signs of acute onset of fever and diarrhea. Among the enteric pathogens, *Salmonella* are considered to be the cause of the largest number of outbreaks, cases, and fatalities that result from foodborne infections. Humans become infected with *Salmonella* primarily through fecal contamination of food products or water. Cooked meats can become infected with *Salmonella* through contact with other products or surfaces that are already contaminated and pose a greater risk to consumers as they will undergo no further processing prior to consumption.

Yersinia pseudotuberculosis causes gastrointestinal disorders, septicemia, and mesenteric adenitis. Children are more susceptible than adults to foodborne yersiniosis showing acute enteritis. Symptoms include severe abdominal pain at the lower quadrant of the abdomen mimicking appendicitis, diarrhea, nausea, vomiting, and fever. It also causes enterocolitis, mesenteric lymphadenitis, and terminal ileitis. *Y. pseudotuberculosis* is transmitted through food, and pig serves as the primary reservoir. The pathogenicity of *Yersinia intermedia* and *Yersinia mollaretii* is uncertain.

The poor hygiene and sanitation prevailing in the abattoirs as well as the shops encourage microbial contamination, survival and growth. High concentrations of poultry, slaughtering and processing equipment and cooling devices can be the causes of

significant bacterial contamination, and also of a shorter meat shelf life. Besides, a significant risk of bacterial contamination and an increase in the number and species of bacteria depend on the specific part of analysed chicken meat, mode of packaging and storage after distribution to the market.

The present study has emphasized that the presence of *Escherichia coli, Salmonella* spp. and *Yersinia* spp. which are responsible for foodborne illness in the retail meat products indeed poses a serious risk to consumers' health. To limit this risk, good hygienic practices and good cooking of meat are necessary.

Consequently, education of the meat retailers' community which run the traditional meat shops, in terms of the importance of hygienic and sanitary precautions would go a long way towards providing wholesome and safe meat to the consumers. Besides this, some efforts should be made for consumers to be informed and follow the basic instructions regarding storage temperature, cooking and prevention of contamination and cross-contamination.

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