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# Isolation and identification of Escherichia coli and Salmonella from meat and liver of local and imported chicken

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[فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ وَلَا تَعْجَلْ بِالْقُرْآنِ مِن قَبْلِ أَن يُقْضَىٰ إِلَيْكَ وَحْيُهُ وَقُل رَّبِّ زِدْنِي عِلْمًا]

صدق الله العلي العظيم

سورة طه /الاية ١١٤

***Dedication:***

*TO*

the Popular Crowd

Iraq

## ***Supervisor certification***

I certify that this study, entitled "***Isolation and identification of Escherichia coli and Salmonella from meat and liver of local and imported chicken***" was prepared under my supervision at college of veterinary medicine / university of Al-Qasisiya in partial fulfillment of the requirement for Bachelor's degree in Veterinary Medicine and surgery.

***Assit. Prof.***

Huda Abdal-Hadei Ali Al-Nasrawi

***Supervisor***

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By the name of God first who deserves all thanks & pleasure for granting me with well strength & help with which this dissertations has been accomplished peace & prayer on my Bachelor. Guide prophet Mohammed & his household.

We would like to gratefully thank my supervisor Assit. Prof. Dr. Huda Abdal-Hadei Ali Al-Nasrawi for his invaluable guidance & guidance penetrating support. Also we thank very much our teachers & our friends.

## ***Certificate of Instructor***

We certify that Ameer Mansour Mohammed has completed the fulfillment of his graduation project entitled "***Isolation and identification of Escherichia coli and Salmonella from meat and liver of local and imported chicken***" for the year 2016/2017 under our construction.

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**Summary**

The objective of this study was to screen for two major food-borne pathogens, *Escherichia coli* and *Salmonella* spp. from meat and liver samples of local and imported chickens obtained from different local market in Al-Qasim city in Babil governorate during the period from October 2016 to March 2017.

A total of 110 meat and liver samples (fresh and frozen) of local and imported chickens were used in this study and each sample was placed in peptone water. They were transported to the laboratory in a cooler and then seeded on selective media appropriate for each organism. Colonies were identified using conventional microbiological methods.

In this study, *E. coli* was found in higher rates in meat and livers samples from local and imported chickens. In samples of local chickens, the identification rate of *E. coli* was 41.4%, *Salmonella* spp. was 4.2% and both pathogens *E.coli* and *Salmonella* spp. was 2.8%. The detection rates of *E.coli*, *salmonella* spp. and both pathogens in all samples of imported chicken were 35%, 2.5% and 2.5% respectively.

The results showed, out of 35 fresh meat samples of local chickens, 14(40%) were positive for E.coli, 3(8.5%) were positive for salmonella spp. and 2(5.7%) were positive for *E.coli and Salmonella* spp.; For the liver samples collected from local chicken, out of 35 samples were 15(42.8%) showed positive result for *E.coli* , while *Salmonella spp.* did not diagnosed in all liver samples of local chickens.

The percentage of bacterial isolate from 20 frozen meat samples of imported chickens ,8(40%) were positive for *E.coli*, whereas *Salmonella spp.* did not isolated from all meat samples .While the detection rates in 20 frozen liver samples of imported chicken were 6(30%) for E.coli , 1(5%) for *Salmonella spp* and 1(5%) for both pathogens . These results showed a high degree of contamination in

meat and liver samples from local chicken as compared to those from imported chicken.

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## *1: Introduction*

Food-borne pathogens are the leading cause of illness and death in developing countries. In developed countries, food-borne pathogens are responsible for millions of cases of infectious gastrointestinal diseases each year, costing billions of dollars [Iyer etal.2013]. *Salmonella* spp. is the most important bacterial pathogens of poultry, where infection causes significant economic losses in poultry rearing and food industries. Losses are also including high mortalities in addition to growth retardation (Shivaprasad ,2000), in addition, human gastroenteritis as a result of infection with poultry-associated Salmonellae is a well-known food-borne zoonosis and of health burden (Crump et al.,2011).

Infection with Salmonella is the most frequent food-borne gastrointestinal disease transmitted from animals to humans mainly through water, meat, eggs and poultry [Riyaz-Ul-Hassan et al., 2004].Salmonella infection is world-wide food-borne zoonosis and poultry products and byproducts are the common source of infection. Poultry associated Salmonellae are the most frequently reported human zoonoses in the European Union which can cause relatively vast economic damage due to chronic effects of the infections (EFSA, 2007). Moreover, food borne Salmonella out-breaks can lead to severe economic losses to poultry producers as a result of regulatory actions, market restrictions or reduced consumption of poultry products [Waltman et al.,1998].

As meat consumption around the world increases concerns and challenges to meat hygiene and safety also increase. These concerns are mostly of a biological nature and include bacterial pathogens such as *E. coli* O157:H7, *Salmonella* spp. and *Campylobacter* in raw meat and poultry, and *Listeria monocytogenes* in ready-to-eat processed products, while viral pathogens are of major concern in foodservice (Harakeh et al 2005) . Hence, these two pathogens are a major cause of concern and were therefore selected for our study. This study was

aimed to investigate the load of bacterial contaminant in meat and liver of local and imported chickens with *E. coli* and *Salmonella* spp.

## *2: Literature Review*

### *2-1: Food borne disease challenge*

Today there is an increasing concern over food borne pathogens spreading from farm animals to human populations. Epidemiological data have demonstrated that a significant source of drug-resistant food borne infections in humans is the acquisition of resistant bacteria originating from animals. This source of infection has been demonstrated through several different types of food borne disease follow-up investigations, including laboratory surveillance, molecular subtyping, and outbreak investigations (Holmberg et al., 1984).

More studies have confirmed that using antimicrobial drugs in poultry increases the risk of selecting for resistant food borne pathogens, and that these pathogens can then be transferred to humans through direct contact with either contaminated food or animals (Van den Bogaard et al., 2001). Due to the lack of alternative strategies, most attempts to control gastrointestinal tract microflora in chickens have so far relied on the use of broad-spectrum antibiotics. However, the recent and widening concern over disseminating antibiotic resistance genes has led to bans on the prophylactic use of many antibiotics in a number of countries. In indigenous chicken, the diet and the environment affect the microbial status of the gastrointestinal tract.

Dirty litter and other animal management parameters affect microbial composition of the chicken gastrointestinal tract by providing a continuous source of bacteria through ingestion (Apajalahti et al., 2004). Raw retail chicken meats are potential vehicles for transmitting food borne diseases. Additionally, these retail chicken meats are often associated with direct hand-to-mouth

exposure to enteric pathogens and cross-contamination of the kitchen environment and ready-to-eat foods (Zhao et al., 2001)

Many infections are transmitted through food and cause illness ranging from mild gastroenteritis to severe illness requiring hospitalization. The task of providing accurate information on trends in specific food borne pathogens capable of causing syndromes is at the hands of researchers (Pinner et al., 2003). Salmonella spp. and E. coli are prominent food pathogens. Factors influencing the occurrence of food borne illnesses are complex and include human population increase, poverty, changing life-styles-including more adventurous eating, more convenience foods, less time devoted to food preparation; ever-evolving technologies for food production, processing, distribution, and emergence of newly recognized microbial pathogens (Jianghong et al., 2002).

## 2-2: *Salmonella spp.:*

Salmonellosis is one of the most common and widely distributed food borne diseases. It constitutes a major public health burden and represents a significant cost in many countries. Millions of human cases are reported worldwide every year and the disease results in thousands of deaths (WHO, 2005). Salmonella infections are mainly asymptomatic in poultry, but are associated with widespread human illness from this source. Therefore, there is continuing interest in finding ways of preventing flock infection and hence contamination of poultry products with Salmonella (Saeed et al., 1999). Pullorum disease, (S. pullorum) and fowl typhoid (S. gallinarum) are two classic and distinctive diseases of poultry that have received considerable attention because of their economic impacts (Snoeyenbos, 1994).

Salmonella enterica-associated gastroenteritis is an important food borne human disease. Most serotypes are capable of infecting a variety of animal species, including humans. There is considerable variation with time and geographical location in serotypes commonly

associated with human Salmonellosis notably S. enterica serovar Typhimurium and S. enterica serovar Enteritidis (Cormican et al., 2002), serotype Typhimurium is responsible for various disease manifestations, usually in the form of mild gastroenteritis with low mortality, but it can cause septicemia with high mortality (Salvatore et al., 2004). The level of contamination of chicken and chicken products with pathogens associated with gastroenteritis such as Salmonella spp. is significantly increasing in many countries , For example Salmonella serotypes were isolated from 22.0% of broiler flocks, and from 15.3% of the layer flocks in The Netherlands (Dufrenne et al., 2001). The most important cause of Salmonellosis has been attributed to broiler chickens and layer hens (Wegener et al., 2003).

### *2-2-1: Salmonella characteristics, nomenclature and habitat*

Salmonella is a Gram-negative facultative anaerobic rod-shaped bacterium in the family of Enterobacteriaceae, also known as enteric bacteria. Salmonella is a motile bacterium with the exception of S. gallinarum and S. pullorum and they are all nonsporeforming. There is a widespread occurrence of Salmonellosis in animals, especially poultry (FDA, 1998). There are over 2500 serotypes, of Salmonella (WHO, 2005). Different strains of Salmonellae have been identified, and these are placed into groupings called serovars on the basis of their antigens (Snoeyenbos, 1994). The latest nomenclature, which reflects recent advances in taxonomy (Popoff, 2001), in the genus Salmonella consists of only two species: S. enterica and S. bongori (Cooper, 1994). Salmonella enterica is divided into six subspecies, which are distinguishable by certain biochemical characteristics (Brenner et al., 2000). Strains of Salmonella are classified into serovars on the basis of extensive diversity of lipopolysaccharide (LPS) antigens (O) and flagellar protein antigens (H) in accordance with the Kauffmann–White scheme

Salmonellae have a wide range of hosts. Although primarily intestinal bacteria of animals and birds, Salmonellae are widespread in the environment and commonly found in farm effluents, human sewage and in any material subject to faecal contamination and are

transmitted to humans by contaminated foods of animal origin ( Hohmann, 2001). Some serovars show remarkable host specifity for instance Salmonella typhi and Salmonella gallinarium are strictly found in humans and birds respectively (Jorgensen, 2001). Epidemiological and bacteriological evidence indicate that these animals may transmit the infection to human Handeland et al., 2002)

### *2-2-2: Salmonella isolation, manifestation and pathogenesis of infections*

The most commonly used media selective for Salmonella are Salmonella-Shigella (SS) agar, bismuth sulfite agar, Hektoen enteric (HE) medium, brilliant green agar, xyloselysine-deoxycholate (XLD), and MacConkey agar. All these media contain both selective and differential ingredients (Edwards and Ewing, 1972).

*Salmonella* organisms are a etiological agents of diarrhoeal and systemic infections in humans, most commonly as secondary contaminants of food originating from the environment, or as a consequence of septicaemia in food animals (EU, 1992). Onset of the illness is usually 6 - 48 h. The infective dose is 15–20 cells; which depends upon ageand health of host, and strain differences among the members of the genus. Acute symptoms include nausea, vomiting, abdominal cramps, diarrhea, fever, and headache, which may last for 1 to 2 days or may be prolonged. Chronic consequences include arthritic symptoms that may follow 3 - 4 weeks after onset of acute symptoms (FDA, 1998). The infections are caused by Salmonella serovars (e.g., Typhimurium). About 12-24 hours following ingestion of contaminated food (containing a sufficient number of Salmonella), symptoms appear (diarrhea, vomiting, fever) and may last 2-5 days usually before spontaneous cure. Salmonella infections vary with the serovar, the strain, the infectious dose, the nature of the contaminated food, and the host status.

Salmonella pathogenesis is initiated by oral ingestion and penetration into the intestinal epithelium; induce degeneration of

enterocyte microvilli causing profuse macropinocytosis, which leads to the internalization of bacteria (Gulig, 1996).

### *2-2-3: Control of Salmonellosis*

Salmonella enterica remains one of the most important food borne pathogens of humans and is often acquired through consumption of infected poultry meat or eggs. Control of Salmonella infections in chicken is therefore an important public health issue, three types of typhoid vaccines are currently available for use: (1) an oral liveattenuated vaccine, (2) a parenteral heat-phenol-inactivated vaccine, (3) a newly developed capsular polysaccharide vaccine for parenteral use, a fourth vaccine, and an acetone-inactivated parenteral vaccine are available only to the armed forces in USA (Beal et al., 2004).

Hazards from Salmonella can be prevented by heating food sufficiently to kill the bacteria, holding chilled food below 4.4 ºC, preventing post-cooking cross contamination and prohibiting people who are ill or are carriers of Salmonella from working in food operations (Ward et al., 1997). Salmonella surveillance and control of poultry industry at slaughter should be done to identify infected flocks as regulatory procedures for food safety and security program (Veling et al., 2002).

### *2-2-4: Epidemiology of Salmonella*

Salmonellosis is one of the most common and widely distributed food- borne diseases. It constitutes a major public health burden and represents a significant cost in many countries. Millions of human cases are reported worldwide every year and the disease results in thousands of deaths. In addition to acquiring infection from contaminated food, human cases have also occurred where individuals have had contact with infected animals, including domestic animals (WHO, 2005).

Non-typhoidal *Salmonella* are important food borne pathogens that cause gastroenteritis, bacteremia, and subsequent focal infection. These bacteria are especially problematic (cause opportunistic infections) in a wide variety of immune-compromised individuals, including patients with malignancy, human immunodeficiency virus, or diabetes, and those receiving corticosteroid therapy or treatment with other immunotherapy agents. Endovascular infection and deep bone or visceral abscesses are important complications that may be difficult to treat (Hohmann, 2001).

During the last decade, antibiotic resistance and multiresistance of Salmonella spp. have increased a great deal due to increased indiscriminate use of antibiotics in the treatment of humans and animals; and the addition of growth-promoting antibiotics to the food of breeding animals (WHO, 2005). Strains of Salmonella which are resistant to a range of antimicrobials, including first-choice agents for the treatment of humans, have emerged and are threatening to become a serious public health concern (Holmberg et al., 1984).

### *2-3: Escherichia coli strains*

*Escherichia coli* strains are one of the normal bacterial floras of the gastrointestinal tract of poultry and humans. Ten to fifteen percent of the intestinal coliforms in chicken are of pathogenic serotypes (Barnes et al., 1997).

Colibacillosis is a common systemic infection caused by E. coli in poultry. The disease causes considerable economic damage to poultry production worldwide. Significant increase in appearance of drug-resistant strains of E. coli isolated from poultry has complicated the problem (Georgnaras et al., 2001).

In humans, these strains are the foremost cause of urinary tract infections (Falagas and Gorbach, 1995), as well as a major cause of neonatal meningitis, nosocomial septicemia, and surgical site infections (Thielman and Guerrant, 1999).

### *2-3-1: E. coli characteristics, nomenclature and habitat*

*E. coli* are straight rods, aerobes and facultative anaerobes; ferment most sugars producing gas but do not produce H2S on TSI agar slants (A/A with gas). They are indole positive, methyl red positive, Voges Proskaur negative, simmon's citrate negative, catalase positive and urease negative (Soomro et al., 2002). *Escherichia coli* are a commensal of the lower gastrointestinal tract of mammals. According to the modified Kauffman scheme, *E. coli* serotaxonomy is based on their antigenicity O (somatic), H (flagellar), and K (capsular) surface antigen profiles.

A total of 170 different O antigens, each defining a serogroup, are recognized currently. The presence of K antigens was determined originlly by means of bacterial agglutination tests: an *E. coli* strain that was in agglutinable by O antiserum but became agglutinable when the culture was heated, thus considered having a K antigen. A specific combination of O and H antigens defines the serotype of an isolate (Nataro and Kaper, 1998).

### *2-3-2: E. coli pathogenesis*

E. coli, a natural inhabitant of the intestinal tracts of humans and warm-blooded animals, is used as an indicator bacterium because it acquires antimicrobial resistance faster than other conventional bacteria (Miranda et al. 2008). *E. coli* is responsible for three types of infections in humans: urinary tract infections (UTI), neonatal meningitis, and intestinal diseases (gastroenteritis). These three diseases depend on a specific array of pathogenic (virulence) determinants (Nataro and Kaper, 1998).

### *2-3-3: Epidemiology of E. coli*

Numerous incidents of fatal food borne diseases (FBDs) associated with pathogenic E. coli strains have been reported over a wide geographic distribution in Canada, United Kingdom, China, Argentina, Japan (Anonymous, 1995).For example, E. coli O157:H7

strains were isolated from 12 of 33 chicken samples in Seattle. This is attributed to the fact that E. coli strains can survive and multiply when stored between 0 ºC, 6 ºC and 12 ºC; and in dry foods with a wide range of water activity and pH values (Samedpour and Liston, 1994).

The impact is enormous, for instance, CDC estimates 20,000 illnesses and 250 deaths each year in the USA with 30 separate outbreaks in 1994 with the latest data indicating 62,000 illnesses, 1,800 hospitalization and 52 deaths per year (Gregory et al., 1996). *E. coli* has been isolated worldwide from poultry meat probably due to the increased usage of antimicrobials (Gladys and Olayinka ,2014). Percentage prevalence in poultry meat has been variable depending on method and media used in its isolation. 19% prevalence was observed in South Africa (Dahal, 2007)

## *3: Materials and Methods*

### *3-1: Samples preparation*

A total of 110 samples of local and imported chicken were collected from different local markets in Al-Qasim city in Babil governorate and during the period from October 2016 to March 2017 and all samples divided into two groups according to type of sample (meat and liver).

Also, a total of 70 fresh samples of a live chicken purchased from different local markets and 40 frozen samples purchased from different super markets from different trademarks. Meat and liver samples of five grams each were macerated with 10 mL of sterile peptone water and then 1 ml of sample was incubated into 9 ml of nutrient broth for enrichment and incubated overnight at $37^{o}$C

### *3-2: Identification of E. coli and Salmonella isolates*

#### ***A-Culture methods***

Bacteriological analyses were performed by plating 0.1 mL of each dilution on agar plates.

#### *Isolation of E.coli*

To detect E. coli, samples were inoculated on MacConkey's agar and Eosin Methylene Blue (EMB) agar medium was used for the purpose of observing growth of *E. coli* and incubated at 37ºC overnight (Cheesebrough, 1984).

### *Isolation of Salmonella spp*

To detect *Salmonella* spp., the samples were plated on a selective medium such as Salmonella-Shigella Agar (SS agar ), Xylose Lysine Deoxycholate agar (XLD), for 18-24 hours at 37°C (Menghistu et al 2011).

### ***B-Microscopically examination***

**Gram stain:** A pure colony was spread and fixed on the slide by drying and using a Bunsen burner flame. The slide was allowed to cool, and then flooded with crystal violet solution for 30 sec, followed with Grams iodine solution for 1 min, followed by draining excess iodine by decolorizing using acetone for at least 10 sec and then washed with water. Counter staining was done using Basic Fuchs in and allowed to stand for 30 seconds. This was followed by washing the slide and dried in the air according to Quinn et al. (1998). The slide was observed under light microscopy at X40. Short rods that stained red / pink were considered gram negative.

### ***C-Biochemical identificationfor E. coli and Salmonella***

#### *1- Indole production*

Two to five pure colonies were inoculated using a sterile wire loop in 2 ml of peptone water in bijous bottles and incubated overnight at 35$^{o}$C. 0.5 ml of Kovac's reagent was added and examined after 1minute. Presence of rose red colour on upper layer was considered positive (+), while absence of rose red or pale colour was considered negative (-)this test was done according to (Barrow and Feltham, 2003).

***2- Simmons Citrate***

Simmons Citrate agar slants in bijous bottles were stabbed using a sterile wire loop and incubated for 48h at 35$^{o}$C. Positive (+) growth for example citrate utilization produce an alkaline reaction and the medium change colour from green to blue, while no colour change (no citrate utilization) was considered negative (-),this test was done according to (Barrow and Feltham, 2003).

**3-Urease test**

Two milliliters of Urea broth base (Oxoid) in bijous bottles were inoculated with single colonies of organism and incubated for 5-6 h at 37 $^{o}$C in a water bath. Two controls were used, a negative control containing Urea broth base only and positive control containing Proteus aureus standard organism. All bijous bottles in which colour changed to pink were considered positive (+), while those that had no colour change were considered negative, this test was done according to (Barrow and Feltham, 2003).

**4-Triple Sugar Iron Agar**

TSI slopes with a butt of about 1 inch (3.5cm and 2.5cm) were inoculated by stabbing the butt and carefully streaking of slant using a sterile inoculating needle after slightly touching the center of a discrete colony on selective media. The tubes were incubated overnight at 35 $^{o}$C, this test was done according to (Barrow and Feltham, 2003).

**4: Results and Discussion**

Food-borne pathogens are very diverse in their nature and are of major concern to public health worldwide. Many high-risk pathogens that cause diseases in humans are transmitted through various food items or water. Therefore, the microbiological safety of food has become an important issue for consumers and industry and regulatory agencies (Bai et al., 2010).

In this study, culture tests showed the presence of both pathogens *E. coli* and *Salmonella* spp.in meat and liver samples of local and imported chicken. Using microbiological testing and biochemical characterization, both pathogens with varying incidence rates from the different outlets were found, with a maximum occurrence in samples of local chickens.

*E. coli* were isolated and identified from the samples after cultivation EMB agar and MC agar .The detection results of *E. coli*, samples were inoculated on MacConkey's agar showed lactose-fermenting pink colonies, while on Eosin methylene blue agar(EMB) showed yellow green characteristic metallic sheen( figure 1). Gram stained results revealed the presence of Gram-negative, pink color, small rod shaped appearance, arranged in single or paired short by optical microscopy (Quinn et al., 2002).

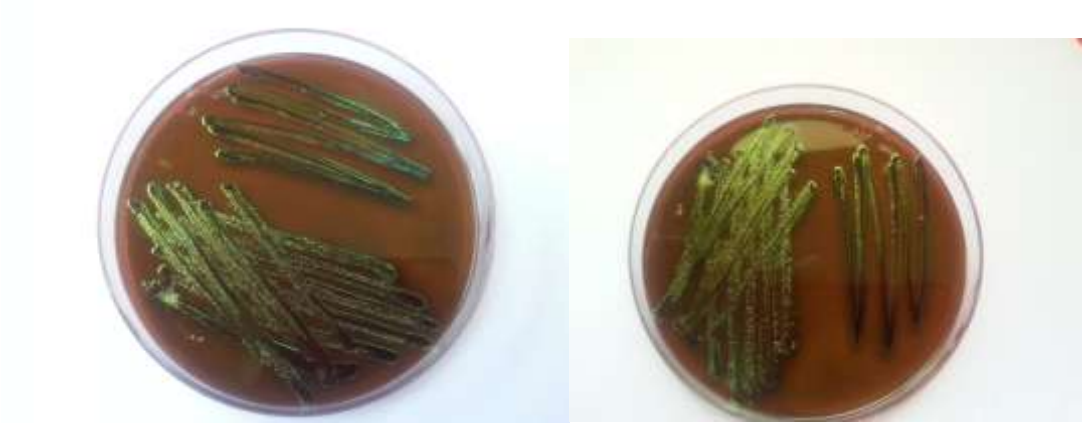

**Figure 1: Eosin methylene blue agar(EMB): Yellow green characteristic metallic sheen**

In the present study, specific media and biochemical tests which were used for the detection of *Salmonella* spp were also similarly used by a number of scientists (Tiabaijuka et al. 2003); Obtained

results revealed that all Salmonella isolates showed clear colonies with black center on XLD media(figure 2).While on *Salmonella*-Shigella agar ( SS agar) showed Opaque translucent colorless smooth round colonies (figure 3). Gram stained results revealed the presence of Gram negative straight rods cocco bacilli by optical microscopy. [Quinn et al.,2002].

**Figure 2: XLD agar with *Salmonella* spp.: clear colonies with black centres.**

**Figure 3: *Salmonella*-Shigella agar( SS agar) with *Salmonella* spp; Opaque translucent colorless smooth round colonies**

Biochemical tests were performed to confirm *E. coli and samonlla spp*. using Gram negative staining, catalase test, indole, urase test, simmon citrate (table 1).

**Table 1: The results of biochemical tests and Microscopically examination**

| Biochemical tests | *E. coli* | *Salmonella spp.* |
|---|---|---|
| **Gram stain** | Gram-negative, pink color, small rod shaped appearance, arranged in single or paired short | Gram negative straight rods cocco bacilli |
| **Indole** | + | - |
| **Simmons citrate** | - | + |
| **Urease** | - | - |
| **Catalase** | + | + |
| **TSI** | Production of acid (yellow) slant and acid (yellow) butt, gas, without production of H2S (blackening of agar) was considered positive | Alkaline (red) slant and yellow butt (acid), gas, with H2S ((blackening of agar)) was considered positive for *Salmonella*. |

In this study, the presence of *E. coli and Salmonella* spp. in indigenous chicken demonstrates the potential for food contamination during handling and processing. The distribution of the occurrence of both pathogens in meat and liver samples of local and imported chicken from various sources is listed in Table 2.

We found a high incidence of *E. coli* in 70 fresh samples (meat and liver) collected from local chickens29(41.4%) when compared to detection rate in 40 frozen samples of imported chicken was14 ( 35%). With respect to *Salmonella*, the detection rates were 3( 4.2%) from fresh samples (meat and liver) of local chicken and 1( 2.5% )from frozen samples(meat and liver) of imported chicken, for the detection rate of both pathogens in collected samples from local chicken was 2(2.8%), while in frozen samples of imported chicken was1 (2.5%). where, The process of evisceration during slaughter of food animals is regarded as one of the most important sources of

carcass and organ contamination with pathogens (Van den Bogaard *et al*., 2001). Animal litter is now considered as a route of human exposure to antimicrobials used in food producing animals (Sakchai et al., 1999).

**Table 2: Percentage distribution of pathogens in collected samples from local and imported chicken**

| Isolates | Sources of samples | | | |
|---|---|---|---|---|
| | local chicken | | imported chicken | |
| | Positive samples/total no. | Percent | Positive samples/ total no. | Percent |
| ***E.coli*** | 29/70 | 41.4% | 14/40 | 35% |
| ***Salmonella spp.*** | 3/70 | 4.2% | 1/ 40 | 2.5% |
| ***E.coli and Salmonella spp.*** | 2/70 | 2.8% | 1/40 | 2.5% |

The results of bacterial diagnosis in meat and liver samples of local chickens shown in (table 3), out of 35 fresh meat samples, 14(40%) were positive for *E.coli*, 3(8.5%) were positive for *Salmonella* spp. and 2(5.7%) were positive for *E.coli* and *Salmonella* spp. these results were lower than recorded by (Al-Abadi et al., 2011) in Basrah city who found that the overall presence of *Salmonella spp*. was 9.2%. Moreover, (Akbarmehr, 2011) found that, the prevalence of *Salmonella spp*. in south of Iran was (8%) and in west of Iran was (9.4%).

Out of 35 fresh liver samples, 15(42.8%) showed positive results for *E.coli* , while *Salmonella spp.* did not diagnosed in all liver samples of local chickens (table 3) .Whereas, our result were agreed with Bebora *et al.*, (1994) who pointed out that Food items such as poultry products are regarded as the common source of food borne Salmonellosis and *E. coli* . The increased microbial load in local

chicks could be attributed to improper management or biosecurity measures. These results are so far in agreement with others investigators (Molbak and Neimann ,2002).

Also, the shedding of pathogens by apparently asymptomatic healthy animals is increasing concern as a source, and distribution of food borne diseases (FBDs) (Van den Bogaard *et al*., 2001).

During the slaughter of poultry birds, there can be fecal contamination of the carcasses from the gut of these birds which means bacteria present in the spilled gut content is passed on as contaminants. Of importance is the coliforms especially Escherichia coli and Salmonella. Collibacillosis and Salmonellosis have been described as the leading causes of food-borne illnesses worldwide (Panisello et al. 2000), therefore, it becomes important that ensuring consumer health concerns the greater involvement of the health sector.

**Table 3: Percentage distribution of pathogens in meat and liver samples from local chicken**

| Isolates | Species of samples | | | |
|---|---|---|---|---|
| | Meat | | Liver | |
| | Positive samples | Percent | Positive samples | Percent |
| ***E.coli*** | 14/35 | 40% | 15/35 | 42.8% |
| ***Salmonella spp.*** | 3/35 | 8.5% | 0 | 0 |
| ***E.coli and*** | 2/35 | 5.7% | 0 | 0 |

| | | | | |
|---|---|---|---|---|
| ***Salmonella spp.*** | | | | |

The percentage of bacterial isolate from 20 frozen meat samples of imported chickens were 8(40%), whereas *Salmonella spp.* did not isolated from all meat samples .While the detection rates in 20 frozen liver samples of imported chicken were 6(30%) for *E.coli* , 1(5%) for *Salmonella spp* and 1(5%) for both pathogens (table 4).

The rate of E. coli obtained is indicative that poultry meats obtained from sourced areas were unfit for human consumption in accordance with criterion of recommended limits by foreign food agencies. Poultry meat obtained from these markets should therefore be properly cooked to denature toxin produced by the organism as well as the organism such that consumption will not pose health-risks to human population (Gladys and Olayinka (2014).

**Table 4: Percentage distribution of pathogens in meat and liver samples from imported chickens**

| Isolates | Species of samples | | | |
|---|---|---|---|---|
| | Meat | | Liver | |
| | Positive samples | Percent | Positive samples | Percent |
| ***E.coli*** | 8/20 | 40% | 6/20 | 30% |
| ***Salmonella spp.*** | 0/20 | 0 | 1/20 | 5% |
| ***E.coli and Salmonella spp.*** | 0/20 | 0 | 1/20 | 5% |

# 5: Conclusions and Recommendations

## 5-1: Conclusions

1- This study has addressed an interesting subject since Salmonella is a common bacterial disease of poultry and of zoonotic concern.

2- The isolation of enteric pathogens (Salmonella spp and E. coli) in asymptomatic indigenous chicken in this study shows that they harbour foodborne pathogens which may be a source of contamination of chicken carcass and organs during the process of evisceration at slaughter; and could play a role in the spread of food borne illnesses and multidrug resistance posing a public health risk.

## 5-2: Recommendations

This study recommends that effective prevention of enteric pathogens in indigenous chicken such as Salmonella and E. coli associated with food illnesses is essential. This could be attained as follows:

1- On-farm practices that reduce pathogen carriage such as pathogen free feeds, clean water, regulated movement, increased hygiene at slaughter and poultry meat processing, consumer-education efforts to protect public health .This will minimize indigenous chicken contamination with these pathogens that can occur at multiple steps along the food chain, including production, processing, distribution, retail marketing, and handling or preparation.

2- Routine surveillance and timely reporting of antibiotic resistance patterns among enteric pathogens should become a high priority to establish possible sources of bacterial resistance and provide data that can be used to select appropriate treatment.

3- Establish a national program focusing on the identification and molecular subtyping of zoonotic food borne bacterial pathogens that could be present in retail food animals (poultry).

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