

Isolation and Identification of *Salmonella Spp.* Bacterial Pathogens from Frozen Chickens Meat in Diyala Province

Ayat Jasim Mohammed¹, Mahmood Ahmed Khadim², Shaymaa Jabbar Hassoon³,

College of Veterinary Medicine, University of Diyala, Iraq Department of internal and preventive veterinary medicine¹

College of Veterinary Medicine, University of Diyala, Iraq, Department of Microbiology^{2,3}

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Abstract

Background: Chicken meat is widely used by humans all over the world. Frozen chicken (meats) are produced at meat processing plants as well as at home and has been identified as the main cause of food-borne Salmonellosis.

Aims: The aim of this study was to examine the major food-borne pathogen *Salmonella spp.* from frozen chicken meat samples of local and imported chickens obtained from the different local markets in Diyala Province/Iraq. Eighty samples were taken from a local market to detect *Salmonella spp.* forty frozen samples of local chicken meat and 40 frozen samples of imported chicken meat were used in this study

Results: Primary samples were cultured and placed in peptone water. Using traditional microbiological procedures, colonies were discovered. salmonella spp. was found at higher rates in meat samples from the local market. The identification rate of *Salmonella spp.* were 7.5% and 17.5% of imported frozen chicken meat and local frozen chicken meat, respectively.

Conclusions: hygienic standards of local frozen chicken meat is critical as *Salmonella spp.* was higher in local frozen chicken meat compared with imported meat

Key word: *Salmonella spp.*, Local, Imported, Chicken, Meat.



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Introduction

Salmonella species are responsible for outbreaks of human gastroenteritis and enteric fever all over the world [1]. It has frequently been identified as the etiologic agent of food-borne outbreaks, and this bacterial species is a major pathogen in the food industry [2]. *Salmonella* microorganisms affect the broilers chickens then through the food chain it can transmit to humans to cause human salmonellosis [3].

Meat's poultry are one of the mainly Sources of food products in the entire world, Poultry meat's popularity and its significant economic and dietary factors can be attributed to its nutritional qualities, such as its low-fat content, high protein content, and favorable fatty acid composition, specifically unsaturated. It is easy to prepare poultry meat at home and is generally used in fast-food restaurants. Poultry meat is also not subject to religious restrictions [4]. In many under developed nations, poultry products consistently have the highest

rate of salmonellosis [5]. Most Salmonellosis in humans is due to the intake of unhygienic poultry meat[6], [7].

In both industrialized and developing nations, food-borne illnesses are one critical problem [8]. This diseases are a widespread public health concern [9], The World Health Organization (WHO) estimates that each year, one in ten individuals contract a food-borne illness, losing 33 million years of healthy life. Most illnesses caused by food-borne infections are diarrhoeal disorders, which affect 550 million individuals per year. One of the four major causes of diarrheal illnesses worldwide is thought to be a salmonella infection [10].

In this research, the present study aimed at isolating *Salmonella* spp. from imported frozen chicken meat, and local frozen chicken meat from different markets in Diyala province.

Materials and methods

1- Sample preparation

In our study, 80 frozen chicken meat samples were collected, 40 samples being local and 40 samples being imported from different Diyala markets. Upon arrival of these frozen samples to the College of Veterinary Medicine, University of Diyala, they were immediately transferred to the Microbiology Laboratory. These samples were prepared for the cultivation and isolation of salmonella. A stomacher bag with 225 ml buffered peptone water was used to homogenize 25 grams of each sample and incubated for 16 to 20 hours at 37°C. Sample incubate at 37 °C for 20–24 hours in 10 ml of selenite cystine broth.

2- Culturing

On XLD agar plates, cultures were cultured and incubated at 37 °C for 24 hours, and then subcultures were performed on XLD and SS agar to get pure cultures. A typical Salmonella colony was observed on XLD (red center with a black border) and SS (with a black center). Also, the colonies of Salmonella were confirmed by traditional biochemical tests, which include Simmons citrate, Indole, urease, TSI, Voges-Proskauer glucose, and methyl red [11].

Results

The results of this study (Table 1) show a total of 7,5% and 17,5% of Salmonella spp. were isolated from imported frozen chicken and local frozen chicken, respectively.

Table 1: Isolation of *Salmonella spp.* in the examined samples.

Sample	Salmonella +ve	Salmonella -ve	Total
Imported frozen chicken meat	3 (7.5%)	37 (92.5%)	40
Local frozen chicken meat	7 (17.5%)	33 (82.5%)	40

Initial diagnosis of a suspected bacterial isolate should be on different culture media. Salmonella spp. showed different

properties when grown on different media. First, turbidity in pre-enrichment and enrichment broths was seen. On solid

medium, Salmonella colonies appeared small, smooth, round, and transparent on SS agar with a black center because of H₂S production. Salmonella colonies

have rounded and pale appearances. In the colonies on XLD agar, there was a smooth surface and a black center.



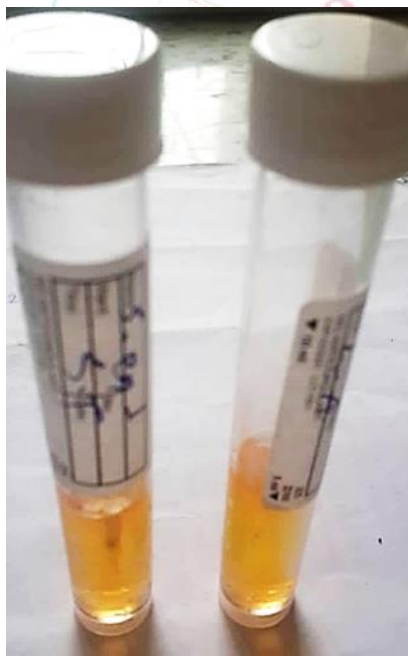
Figure: (4-2) Colonies of *Salmonella* spp. on SS agar



Figure: (4-2) Colonies of *Salmonella* spp. on XLD agar

For the detection of Salmonella, specific biochemical tests were used. All species of bacteria were catalase-positive and oxidase-negative. On TSI slants, Salmonella was glucose fermented but did not ferment lactose and sucrose with

H₂S and gas production in the majority of slants. The slants appeared red (alkaline reaction) and the bottoms were yellow (acid reaction). Salmonella spp. were indole- and urease-negative.



Urease (-)



TSI



Indol (-)

Figure: (4-3) results of biochemical test of *Salmonella* spp.

Discussion

Enterobacteriaceae bacteria are Gram-negative, rod-shaped bacteria that do not create spores, some of these are microorganisms that cause intestinal illnesses and food poisoning in humans and animals. Important genera in poultry diseases include *Salmonella* and *Escherichia coli* [12]. Salmonellosis is a severe health problem that continues to have a significant economic impact on the poultry sector in all countries [13]. Frozen chicken and raw poultry products are considered reservoirs for human infection, and human foodborne illnesses caused by *Salmonella* are often associated with consumption of the products [14]. Given the public health importance of salmonellosis, data on *Salmonella spp.* in retail meat products in Iraq are still lacking. The purpose of this study was to look at the possibility of salmonellosis in frozen chicken meat. In our study, the number of *Salmonella* species based on biochemical and bacteriological characteristics was 7 (17.5%) in local frozen chicken and 3 (7%) in imported frozen chicken. In other studies, isolated *Salmonella spp.* From Chicken, Alali et al. (2013)[15] recorded an occurrence of 27% of *Salmonella* in

chicken meat in the Russian Federation. Another study showed by Korashy and Mohammed (2003) [16] found *Salmonella* in chicken meat at a rate of 2.5% in Port-Said City. In addition, Ramya et al. (2012)[17] isolated *salmonella* from chicken meat samples, discovering that 56% of the collected samples were positive for *Salmonella* 14 out of 25. Therefore, our findings and discussions support previous studies showing the negative impact of *Salmonella* epidemics on the economy and food industry as well as human health, as local and imported frozen chicken meat is the main cause of salmonellosis in humans. The samples in this study also showed that local frozen chicken samples contained a higher proportion of imported frozen chicken isolates, which may be due to the factor of long-term freezing, which is considered to have a bactericidal effect. As reported by Jay,[18] use freezing temperatures for *Salmonella*. May be harmful, but their continued presence does not guarantee damage to microorganisms. At temperatures near freezing initially viable organisms are rapidly reduced due to freeze damage.

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