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Investigation of contamination rates of beef and chicken meats and their products with food-Borne Salmonella spp and antibiotic susceptibility of the bacteria at Kerbela Governorate

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Abstract--This study was conducted at kerbala governorate during the period from November 2021 to March 2022. A total of 310 samples were collected from local and imported chicken meat and beef . These samples were gathered from different locations in Kerbala province and cultured on appropriate media for *Salmonella* spp cultivation and identification . Then it followed by the initial bacterial isolation process on the special and distinctive culture media for *Salmonella* spp. Results showed that rate of contamination in all examined samples was 17.4 % (in Beef and Chicken meat). Concerning local meat, and out of 20 samples of meat, 20 samples of minced meat , 20 samples of sausage and 20 samples of burger; Salmonella spp were isolated from 4(20%), 9(45%), 1(5%) and 1(5%) samples respectively. Moreover, the contamination rate of minced meat with Salmonella spp (45%) was significantly high ($p < 0.05$). However in imported beef samples, the contamination rates 2(10%) , 6(30%) , 0(0%) and 0(0%) were reported in 20 samples of meat cut, 20 minced , 20 sausage and 20 burger samples. The rate of contamination in minced meat (30%) was also significantly ($p < 0.05$) higher than others. Local Chicken meat's samples collected as follow: 25 from skin, 25 from carcass wash and

25 from liver, *Salmonella spp* were positive in 9(36%), 10(40%) and 4(16%) samples respectively. Contamination rate of carcass wash (40%) was significantly higher at ($p < 0.05$) than other samples of examined local chicken samples. Similarly, *Salmonella spp* were positive in 5(20%), 1(0.4%), and 2(8.0%) of imported chicken meat samples collected from 25 carcass skin, 25 carcass wash and 25 liver samples respectively. It was found that contamination rate in carcass wash samples (20%) was significantly ($p < 0.05$) higher than other samples. The antibiotic resistance among *Salmonella* isolates from both beef and chicken meat was evaluated through this study, and results revealed that resistance was reported in 50 (92%) of *Salmonella spp* isolates to tetracycline.

Keywords---*Salmonella*, contamination rate, beef, chicken meat, antibiotic, Kerbala.

Introduction

Salmonellae are consistently reported to be among the leading international sources of food-borne human and animals disease. *Salmonella* is a true potential pathogen for both humans and animals and cause significant economic losses worldwide, due to variety of diseases ranging from mild diarrhea to severe systemic infections like typhoid fever (Gast and Porter 2020). *Salmonella enterica* is one of the most ubiquitous enteropathogenic bacterial species on earth, and comprises more than 2500 serovars (Nair, Venkitanarayanan, and Johny 2018). The pathogenicity of each *Salmonella* strain is determined by a set of genes associated with the bacterial ability to colonize mucosa of the intestinal tract, invading host cells, replicate within these cells, and to survive by destroying the phagocytic components (Elder et al. 2016). The attachment ability of *Salmonella* has also been associated with the moisture content of meat; when carcasses are still fresh and the moisture of the skin is high, the transference from carcasses to other surfaces is more marked (Carrasco, Morales-Rueda, and García-Gimeno 2012). Foodborne diseases can be severe, especially for young children. Diarrhea causing diseases are the most common illnesses resulting from unsafe food, 550 million people falling ill each year, including 220 million children under the age of 5 years. *Salmonella* is one of the four key global causes of diarrheal diseases (WHO, 2019). Antimicrobial resistance is an increasing global problem, and the emerging antimicrobial resistance has become a public health issue worldwide. A variety of foods and environmental sources harbor bacteria that are resistant to one or more antimicrobial drugs used in human or veterinary medicine and in food-animal production (Rossi 2011). The controlling of food-borne pathogens is difficult because of their ability to surviving in processing, food and storage and improper cooking. Therefore, it is important to understand the ecology of *Salmonella enteric* and spreading of infection. The aims of this study were to determine the contamination rates in beef and chicken meats and their products with food borne *Salmonella spp* at Kerbala governorate as well as determination of the antibacterial resistance pattern of the isolates.

Materials and Methods

Study design and Specimens collection

A Cross-sectional study was performed to collect a total of 310 samples, from local and imported chickens and meat. These samples were gathered from different locations in Karbala province and cultured in appropriate media according to internationally known protocols for bacterial cultivation and identification (MacFaddin 2000). Then it followed by the initial bacterial isolation process on the special and distinctive culture media of *salmonella* such as SS agar and the followed propagation (NHS 2017).

Salmonella Isolation and identification

The *Salmonella* isolation and identification were based on the morphological examination on the culture media and microscope, as well as the biochemical tests, and molecular detection (Salm-surv and June 2010).

Characteristics of bacterial culture

All samples were inoculated for 24 hours at 35°C-37°C on *Salmonella* Shigella agar and Xylose Lysine Deoxycholate agar. Colonies on *Salmonella* Shigella agar were shown Colorless colonies with black centers if H₂S is produced. However, on Xylose Lysine Deoxycholate agar the colonies show clear colonies with black centers (Salm-surv and June 2010).

Susceptibility test for antimicrobials using disk diffusion (DD) method (CLSI 2020)

Step 1: Preparation of inoculum

Few colonies of the fresh isolate were selected from XLD agar and suspended with BHI broth medium to make direct colony suspension and compared visually with McFarland standard 0.5%.

Step 2: Culturing of Petri-dishes

A sterile cotton swab was inserted into the direct suspension and squeezed well on the inner wall of the tube in order to remove excess fluid. Then Muller-Hinton agar plate was then inoculated using the streaking method across the whole agar surface more than three times.

Step 3: Application of the antibiotic discs

The discs were placed over equal distances between each disc on the agar plate with a size of 90 mm and 200mm. Then, incubated in an inverted position at 37°C.

Step 4: Reading the Results

The inhibition zone diameter was calculated after incubation for 18 hours.

Results

Isolation, cultivation and characterization of *Salmonella*

All samples were cultured on Salmonella-Shigella agar, and the colonies were circular, smooth, convex and pale in color with a black center. Then sub-cultured by using selective media Xylose-Lysine-Deoxycholate (XLD) agar for the confirmation of the xylose fermentation, lysine decarboxylation and production of hydrogen sulfide, while this bacterium appeared on XLD agar as a small red colony with black center Figure(1). Then Biochemical tests were implemented such as the Urease test that revealed the inability of *Salmonella* to urea hydrolysis. In addition to that, the ability of *Salmonella* to ferment certain sugars and produce the hydrogen sulfide gas was tested by using the TSI test Figure(2), the microscopic examination shows salmonella under microscope with red color as shown in Figure(3).



Figure 1. Isolated *Salmonella* on SS agar and XLD agar. (1) shows the positive result of *Salmonella* isolates on an SS agar plate. (2) Shows the positive. *Salmonella* isolation XLD agar plate.

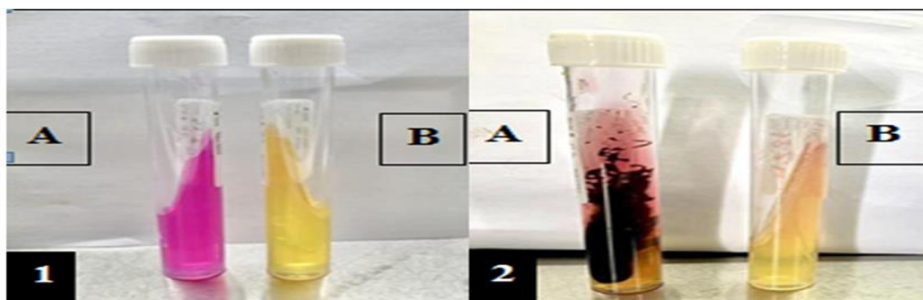


Figure 2. Isolation result of *Salmonella* on Urease test, and TSI test. (1. A) show the positive result of the Urease test, (1. B) show the negative result, (2. A) showing the positive result of the TSI test, and (2. B) negative result.

Contamination rate of *Salmonella* spp from red meat

our result illustrated in table 1 indicate that total of 80 sample of local fresh red meat were collected from different location of Karbala province and as follow 20 sample of meat, 20 sample from minced meat, 20 sample of sausage and 20 sample of burger, and that salmonella spp were isolated from 4(20%), 9(45%), 1(5%) and 1(5%) sample respectively. The contamination rate of minced meat with salmonella spp (45%) was significantly high ($p < 0.05$) from other source of examined meat sample.

Similarly salmonella spp were isolated from 2(10%), 6(30%), 0(0%) and 0(0%) of imported meat collected from 20 meat cut, 20 minced, 20 sausage and 20 burger sample respectively. Once again, that contamination rate of minced meat with salmonella spp 30% was found significantly high ($p < 0.05$) than other source

Table 1: Isolation of *Salmonella* spp .from local markets and imported meat

Type of meat	No. Sample	Meat cut		minced		sausage		burger		Total positive No (%)	Chi-Square (χ^2)
		No. Sample examined	No. Sample positive	No. Sample examined	No. Sample positive	No. Sample examined	No. Sample positive	No. Sample examined	No. Sample positive		
local	80	20	4 (20.00%)	20	9 (45.00%)	20	1 (5.00%)	20	1 (5.00%)	15 (18.75%)	12.78**
imported	80	20	2 (10.00%)	20	6 (20.00%)	20	0 (0.00%)	20	0 (0.00%)	8 (10.00%)	8.261**
* ($P \leq 0.05$), ** ($P \leq 0.01$).											

Contamination rate of *Salmonella* spp from chicken

our result illustrated in table 2 indicate that total of 75 sample of local chicken were collected from different location of Karbala province and as follow 25 sample of skin, 25 sample from carcass wash and 25 sample of liver and that salmonella spp were isolated from 9(36%), 10(40%) and 4(16%) sample respectively. The contamination rate of carcass wash with salmonella spp (40%) was significantly high ($p < 0.05$) from other source of examined chicken sample. Similarly salmonella spp were isolated from 5(20%), 1(0.4%), and 2(8.0%) of imported chicken collected from 25 carcass skin, 25 carcass wash and 25 liver sample respectively. that contamination rate of with salmonella spp 30% was found in carcass skin significantly high ($p < 0.05$) than other source

Table 2: Isolation of salmonella spp .from local markets and imported chicken

Type of meat	No .Sample	Carcass skin chicken		Carcass wash		Liver		Total positive No (%)	Chi-Square (x 2)
		No.sample examined	No.sample positive	No.sample examined	No.sample positive	No.sample examined	No.sample positive		
Local	75	25	9 (36.00%)	25	10 (40.00%)	25	4 (16.00%)	23 (30.66%)	9.316 **
Imported	75	25	5 (20.00%)	25	(4.00%) 1	25	2 (8.00%)	8 (10.66%)	4.973 *
* (P≤0.05), ** (P≤0.01).									

Evaluation of antibiotic susceptibility test

This study was also conducted to highlight the current antibiotic-resistant profile of 54 *Salmonella* isolates in order to screen the prevalence of antibiotic resistance in the *Salmonella* population in the beef and chicken, and the effect of this on public health. All 54 isolates were tested for their susceptibility to 10 antimicrobial drugs and classified as resistant, and susceptible, Figure (3).

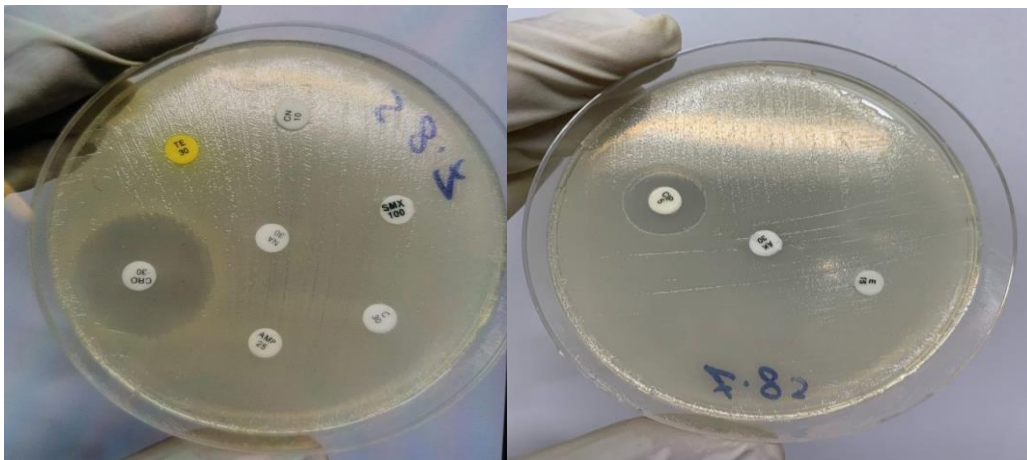


Figure 3: This figure demonstrates the extreme resistance of some *Salmonella* isolates according to the antibiotics susceptibility test

The prevalence of susceptibility to each antibiotic tested is presented in Table (3). From the total positive isolates 54 (17.4%) the resistant pattern is as follows: tetracycline 50 (92.6%), ampicillin 52 (96.3%), nalidixic acid 46 (85.2%), erythromycin 44 (81.4%), sulfamethoxazole 39(72.3%), ciprofloxacin 47 (87%), chloramphenicol 16 (29.6%), and susceptible for ceftriaxone 52 (96.2%), showed significant differences ($p<0.01$) as summarized in Table (4).

Table3: Evaluation of antibiotic resistance among *Salmonella* isolated from beef and chicken

Antibiotic	S	R	P-value
	F(%)	F(%)	
Ampicillin	2(3.7%)	52 (96.3%)	P<0.01
Gentamicin	41(76%)	13(24%)	
Erythromycin	10(18.5%)	44(81.4%)	
Tetracycline	4(7.4%)	50(92.6%)	
Ciprofloxacin	7(13%)	47(87%)	
Amikacin	39(72.3%)	15(27.7%)	
Sulfamethaoxazole	15(27.7%)	39(72.3%)	
Chloramphenicol	38(70.4%)	16(29.6%)	
Nalidixicacid	8(14.8%)	46(85.2%)	
Ceftriaxone	52(96.2%)	2(3.8%)	

F=Frequency=resistants'=susceptible,Chi-squaredtest.

Discussion

Contamination Rates

Percent's of infected samples with *Salmonella* that collected from local markets in the current study were (7.4%) meat cut, (16.6%) minced, (1.8%) sausage, (1.8%) burger, respectively. Contamination rate of meat cut was similar to (Saad, M.S., et al 2011) who reported (8%) in Egypt, and was higher than (Mezali and Hamdi 2012) who reported (5.5%) in Algeria local markets. Number of salmonella isolates in minced meat was lower than (Zaiko et al. 2021) who documented (18.8%) in minced meat in Moscow, but it's a higher than what found by (Terentjeva et al. 2017) in Latvia. Salmonellosis' rate in sausage was lower than what found by (Ed-dra et al. 2017) and (Abd El Tawab et al. 2015) who report's (15%) and (2.5%) in Morocco and Egypt respectively. Frequency of salmonella in burger was higher than (El-tawab et al. 2015) who found (0%) in Egypt, while it's lower than (Ejo et al. 2016) who revealed (2.9%) in Ethiopia. Current study data about salmonella isolation from imported meat reporting (3.7%) meat cut, (11.1%) minced, (0%) sausage, (0%) burger, respectively. And these of meat cut isolation were a higher than (Sehgal apoorva. 2018) who found (2%) in Egypt, and were lower than (Abdal et al. 2016) who found (40%) in Al- Diwaniya City. And these of minced meat was comparable with (Kusumaningrum et al., 2012) who found (12.5%) of minced meat in Indonesia, and were a higher than what found by (Moustafa et al., 2014) in Egypt. And were in sausage lower than what found by (Ertas, et al. 2014) and (Hegazy 2016) who found (4%) and (10%) in Turkey and Egypt respectively. As well as in burger was similar with (Shaltout et al. 2017) who found (0%) in Egypt, but was lower than (Shaltout et al. 2017) who found (23.3%) in Assiut City. *Salmonella's* Isolates which collected from local markets in the current study were; (16.6%) skin, (18.5%) carcass wash and (7.4%) liver, respectively, Skin contamination was lower than (Taha et al., 2015) who found (19%) in Kurdistan, Iraq. But in carcass wash were higher than (Rivera-Pérez et al., 2014) that found (10%) in California, United States. As well as the isolation rate of liver was higher than (Taib et al., 2019) who found (4%) in

Duhok, Iraq. *Salmonella* Isolates which collected from imported chickens in the current study were: (9.2%) skin, (1.8%) carcass wash and (3.7%) liver, respectively. , skin contamination was higher than (Taib et al., 2019) who found (8%) in Duhok, Iraq. And in carcass wash were a higher than (Naik et al. 2015) report's (10%) in India. And in liver was lower than (A. A. Ahmed and Khudor 2019) who found (80%) in Basra, Iraq . Isolation rate difference might come from many reasons such as the difference in prevalence between the different geographical regions, study design and the meat and poultry industry being poorly managed in many parts of Iraq, where biosecurity and disease prevention are still lagging and do not receive valuable attention from breeders and slaughters.

Evaluation of antibiotic susceptibility test

The resistance rate of tetracycline, ampicillin, nalidixic acid, sulfamethoxazole, and ciprofloxacin in the current study table (4-6) was agreed with the results of other studies (Hameed et al., 2014; Harb et al. 2018; Hassan and Alhatami 2019; Saleem et al., 2021) among poultry and meat samples in Al-Hilla province, the Middle Euphrates, and in Thi-Qar governorate respectively. Furthermore, the resistance rate of erythromycin (81.4%) among *Salmonella* isolates in the current study was compatible with the results of studies in other countries such as Turkey and Egypt (Yildirim et al. 2011); (Abd-Elghany et al. 2015). While the resistance rate of ceftriaxone (3.8%) in this study was in accordance with (Sodagari et al., 2015) results in Iran.

These findings support that the increased antibiotic resistance in zoonotic bacteria could be due to indiscriminate and unrestricted use of antimicrobial agent whether in treatment or prevention in a poultry and cattle farms due to the lack of a definitive diagnosis. Moreover as a growth promoter in the poultry industry, this means that these rates of resistance changed according to the source of isolate and the antibiotic selective pressure and many other factors affecting the antibiotic resistance pattern among poultry and cattle beyond the geographic factor (Ja et al. 2017).

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