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Investigation of *salmonella* spp. by conventional and molecular methods in chicken carcasses in karbala province, Iraq

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***Abstract*— This study aimed to detect the presence of Salmonella spp. in chicken carcasses from a meat processing company in Karbala city, as well as in street foods, using both PCR and conventional methods, 100 samples were collected from five areas in Karbala city which include city center, Al-Hindiya, Al-Hussainiya, Al-Hur, and Ain Al-Tamr. 24 samples were taken from each region, the study begning from september 2024 to March 2025. The samples included three types of chicken: locally slaughtered, imported, and randomly slaughtered. Eight samples were taken from each type. The samples included the thigh and breast parts of the chicken. PCR and conventional culturing methods were used to confirm the isolates and determine the specificity and sensitivity of two assay;Statistical analysis revealed statistically significant differences between the isolation rates in the different areas and chicken species studied, and Using the conventional method, Salmonella spp. were detected in 21 out of 125 samples. The bacteria were isolated from chicken thigh and breast samples collected from five different areas within Karbala city, using selective culture media. Isolation rates varied across the different locations and types of chicken meat. The highest rates were observed in the Hindiya and Husseiniya areas, each with an isolation rate of 25%, while the lowest rate was found in the Ain al-Tamr area, where it did not exceed 4.1%, while the highest positivity rate was recorded in Al-Hussainiyah district, at 20.83% .In conclusion: The results highlight the potential of the PCR molecular technique as a valuable alternative for detecting Salmonella spp. in chicken, thanks to its high specificity and rapid processing—making it especially useful for ensuring the distribution of safe products to consumers .**

***Keywords* — Salmonella, chicken, culture, PCR.**

INTRODUCTION

THE Salmonellosis is a zoonotic disease with a worldwide distribution. Salmonella spp. infections can cause small outbreaks in the population; between 60% and 80% of cases are sporadic; sometimes large outbreaks occur in hospitals, daycare centers, nursing homes, and restaurants (1).

The food products commonly associated with outbreaks were fish (22%), water (20%), and cattle (14%). According to the data, Salmonella spp was the bacteria that caused the most outbreaks, accounting for 20% of the total reported outbreaks (2).

Previous studies conducted in the city of Iraq have reported a 10.3% incidence of Salmonella spp. in street foods and market stalls (3).

The surveillance of this pathogen at all stages of the food processing chain is an important element in investigating the epidemiology of salmonellosis. To protect the health of its buyers, the national and international market requires that all consumer products be free of pathogens such as Salmonella spp. (4)

Major efforts have been made in the area of prevention and control of foodborne diseases by industries and entities responsible for control. Many countries, such as the United States, Canada, and Colombia, have established of its "zero tolerance" legislation for this pathogens (5). Based on this legislation, it is necessary to implement rapid and highly sensitive techniques for industry control before releasing food onto the market. The implementation of molecular methods is necessary, since conventional microbiological techniques take 4 to 6 days to detect and identify Salmonella spp. (6).

Advances in biotechnology have allowed the development of various alternative methods, which offer advantages in terms of efficiency, sensitivity, and reduced detection time. These rapid methods, because they are based on the determination of nucleic acids, have the potential to be highly specific. This is the case with the polymerase chain reaction (PCR) technique a molecular technologies based on in vitro DNA amplification

(7). RThe latter has the advantage of simultaneously performing the amplification and detection processes in the same vial and determining the amount of DNA synthesized at each stage of the reaction (8).The objective of this study was to determine the presence of Salmonella spp. in chicken carcasses from a meat processing company operating a karbala city and in street foods, using the PCR technique and the conventional method.

MATERIALS AND METHODS

The study was descriptive and the study was conducted at the Biological Research Lab affiliated with the Faculty of Veterinary Medicine and Animal Science of the University of karbala, One hundred and twenty samples were collected from five areas in Karbala city: the city center, Al-Hindiya, Al-Hussainiya, Al-Hur, and Ain Al-Tamr. Twenty-four samples were taken from each area. The samples included three types of chicken: locally slaughtered, imported, and randomly slaughtered. Eight samples were taken from each type. The samples included the thigh and breast parts of the chicken, Isolation and identification were performed according to conventional protocols previously described (9). The samples were sterilely ground and then transferred to nutrient broth for bacterial growth, Twenty-five g of each sample were weighed and inoculated into 225 ml of peptone water, which was incubated at 37°C for 24 hours. From this, 1 ml of each sample was inoculated into 10 ml of Rappaport broth and tetrathionate (Oxoid, Basingstoke, UK). They were subsequently cultured on Salmonella-Shigella-SS, and xylose-XLD agar (Oxoid, Basingstoke, UK), which were incubated at 37°C for 24 hours. Suspect colonies were identified using conventional biochemical tests and serological tests using the routine Kauffman-White scheme (10) After incubation, suspected colonies exhibiting Salmonella characteristics, such as black colonies on SS agar and XLD medium or were observed, For DNA extraction, 25 g of each sample was weighed and inoculated into 225 ml of peptone water, which was incubated at 37°C for 24 hours. From this, 50 µl of each sample was transferred to microcentrifuge tubes containing the extraction and lysis solution (11). The tubes were then placed in a water bath for 15 minutes at 87°C and cooled for one minute to 18°C. The tubes were then centrifuged at 8,000 rpm for one minute to recover the purified DNA (Addbio bacterial Kit, Korea).

A pair of primers specifically designed to amplify the invA gene characteristic of Salmonella bacteria was used. The sequences of these primers were obtained from a previous study conducted by (12). These primers were chosen for their ability to produce a specific amplicon size and high efficiency, ensuring accurate detection of Salmonella in the samples studied. INV A gene forward: 5-GTG AAA TTA TCG CCA CGT TCG GGC AA -3 and INV A gene reverse Reverse: 5´ TCATCG CAC CGT CAAAGG AAC C -3´, The final reaction mixture was prepared in a volume of 25 µL, with 12.5 µL of the ready-made Master Mix (from Promega USA), 1 µL of forward primer (concentration 10 pmol/µL), 1 µL of reverse primer (concentration 10 pmol/µL), and 3 µL of DNA template extracted from samples suspected of containing Salmonella. The volume was then made up to 25 µL using nuclease-free water. The reaction tubes were placed in a thermal cycler after being set according to the following program: initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 1 minute, final extension at 72°C for 5 minutes, and finally storage at 4°C until analysis.

After preparing agarose gel (1.5%) and adding ethidium bromide dye (0.5 µg/ml), the samples were loaded with a DNA ladder (100 bp ladder) and electrophoresis was run at 70 V for 1 hour. The results were detected using a UV lamp, where fluorescent bands appeared at the expected lengths of the invA gene.

STATISTICAL ANALYSIS

Data were collected using a standardized form and tabulated in an MS Excel® spreadsheet. This program's data analysis tools were used to compile descriptive statistics. The data were then transferred to SPSS® v. 13. To analyze the significance of the differences found in the compared methods (PCR and conventional methods), the chi-square test (13) was used with a significance level of ($P< 0.05$).

RESULT & DISCUSSION

Occurrence of Salmonella spp. by culturing

According to the chi-squared analysis, the techniques used to detect Salmonella spp. in chicken showed a significant difference ($p < 0.001$). Table 1 shows the distribution of Salmonella spp. isolates by chicken type and isolation method used; the conventional method revealed a total of 21/125 positive cases for Salmonella spp. Salmonella bacteria were isolated from chicken thigh and breast samples collected from five different areas in Karbala city using selective culture media. The results showed variations in isolation rates between the different areas and the chicken species studied. The highest isolation rate was recorded in the Hindiya and Husseiniya areas, with each reaching 25%, while the lowest rate was recorded in the Ain al-Tamr area, where it did not exceed 4.1%. Regarding chicken species, the highest isolation rate was observed in locally slaughtered chicken at 25%, followed by imported chicken at 17.5%, and then randomly slaughtered chicken at 10%. Statistical analysis revealed statistically significant differences between the isolation rates in the different areas and chicken species studied. The identity of the bacterial isolates was confirmed through morphological and biochemical characteristics Table 1.

Table 1. occurrence of Salmonella spp. in local, imported and random slaughter chicken by culture assay

Location	Local slaughter		Imported chicken		Random slaughter		Total	
Culture	No. of examined	No. positive	No. of examined	No. positive	No. of examined	No. positive	No. of examined	No. of positive
City center	8	2 (25%)	8	0(0%)	8	1 (12.5%)	24	3 (12.5%)
Alhindyia	8	5 (62.5%)	8	1 (12.5%)	8	0 (0)	24	6 (25%)
Al-Hussenyia	8	1 (12.5%)	8	5 (62.5%)	8	0 (0)	24	6 (25%)
Al-Hur	8	1 (12.5%)	8	1(12.5%)	8	3 (37.5%)	24	5 (20.8%)
Ain-Altumor	8	1 (12.5%)	8	0 (0)	8	0	24	1 (4.1%)
Total	40	10 (25%)	40	7 (17.5%)	40	4 (10%)	120	21 (17.5%)
Statistical analysis	X2= 18.22; DF=8; P value= 0.019							

Table 2.occurrence of Salmonella spp. in local, imported and random slaughter chicken by PCR assay

Location	Local slaughter		Imported chicken		Random slaughter		Total	
	No. of examined	No. positive	No. of examined	No. positive	No. of examined	No. positive	No. of examined	No. of positive
City center	8	2 (25%)	8	0(0%)	8	0 (0%)	24	2 (8.3%)
Alhindyia	8	3 (37.5%)	8	1 (12.5%)	8	0 (0)	24	4 (16.67%)
Al-Hussenyia	8	1 (12.5%)	8	4 (50%)	8	0 (0)	24	5 (20.83%)
Al-Hur	8	1 (12.5%)	8	1(12.5%)	8	2 (100%)	24	4 (16.67%)
Ain-Altumor	8	1 (12.5%)	8	0 (0)	8	0	24	1 (4.1%)
Total	40	8 (20%)	40	6 (15%)	40	2 (5%)	120	16 (13.3%)
Statistical analysis	X2= 13.267; DF=8; P value= 0.102							

Occurrence of Salmonella spp. by using PCR assay

According to the chi-squared analysis, the techniques used to detect Salmonella spp. in chicken showed non significant difference ($p > 0.001$). Table 2 shows the distribution of Salmonella spp. isolates by chicken type and isolation method used; the PCR assay revealed a total of 16/125 positive cases for Salmonella spp. Salmonella bacteria were detected in chicken thigh and breast samples collected from five areas in Karbala using PCR technique. The results showed that the total number of positive isolates was 16 out of 120 samples tested (13.3%). The highest positivity rate was recorded in Al-Hussainiyah district, at 20.83% (five isolates out of 24 samples), followed by Al-Hindiyah district and Al-Hur with the same percentage (4 isolates out of 24 samples). The lowest positivity rate was recorded in Ain Al-Tamr district (4.1%, one isolate out of 24 samples). In terms of chicken type, the highest positivity rate was observed in locally slaughtered chicken (20%, eight isolates out of 40 samples), followed by imported chicken (15%, six isolates out of 40 samples), and then randomly slaughtered chicken (5%, two isolates out of 40 samples). Statistical analysis using the chi-square test showed no statistically significant differences between the different areas (P value = 0.102). It is worth noting that all positive isolates were confirmed through culture and biochemical testing for the Salmonella spp. table 2.

Specificity and Sensitivity test of Salmonella detection by culture and PCR assay

The results showed that the PCR test had a sensitivity of 76.19% (95% confidence interval, CI, 52.83% to 91.78%), indicating its ability to detect 76.19% of true positive samples confirmed by bacterial culture. The test's specificity was 100% (95% confidence interval, CI, 96.34% to 100%), confirming the absence of any false positives. The positive predictive value was 100% (95% confidence interval, CI, 79.41% to 100%), indicating that all positive PCR results were true. The negative predictive value was 95.19% (95% confidence interval, CI, 90.21% to 97.70%), indicating that the test was accurate in ruling out negative cases. Table 3: Specificity and Sensitivity assay for detection of Salmonella spp. via culture and PCR assays

Table 3. Specificity and Sensitivity of culture and PCR assay

Test Results	Salmonella Present (Culture)	Salmonella Absent (Culture)	Total
Positive (PCR)	TP = 16	FP = 0	16
Negative (PCR)	FN= 5	TN= 99	104
Total	21	99	120

DISCUSSION

This determination of Salmonella spp. in chicken products using molecular biology techniques, such as PCR, is the first field study conducted in an area such as districts of karbala, which is the main supplier of chicken in Iraq. The chicken production industry is an important sector in the country.

The importance of producing safe products lies in the ability to market them with a certain degree of certainty about their origin and sanitary quality, which translates into a reasonable level of consumer confidence in the products they purchase. Furthermore, it increases the likelihood of successfully accessing increasingly competitive and demanding markets. The presence of pathogenic microorganisms in chicken and the diseases they cause is one of the essential and growing problems in public health, due to their increasing frequency, the emergence of new forms of transmission, the emergence of vulnerable population groups, and the socioeconomic impact they cause (14).

When comparing the results obtained in this study using the conventional method with other studies conducted in karbala and other Iraqi countries that used the same methodology, they were found to be similar to those reported by(15), who isolated Salmonella spp. in 8 (7.07%) of raw milk from the Maysan Iraq. However, they are lower than those of (16), who isolated Salmonella spp. in 8.66% of chiucken product in the Wasit region, and those of (17) in baghdad, who isolated Salmonella spp. in 10 % of Baghdad market. Additionally, this study with agreement of (18) who reported that 26% of fresh retail chicken meat samples and 39% of raw chicken carcasses were contaminated with Salmonella spp., In contrast, in Mexico, (19) isolated Salmonella spp. in 32.44% of raw meat samples, and . (20) isolated Salmonella spp. in 11% of bovine carcass samples.
Regarding the use of PCR for the rapid detection of Salmonella spp. directly from food, a comparison of the results obtained with the RT-PCR technique with other studies conducted worldwide found that they were lower than those reported by (21) and (22) who isolated Salmonella spp. in 25.5%, 11%, and 30.9% of food samples from the United States and Canada, respectively. However, they are very similar to the results obtained by (23), who isolated Salmonella spp. in 10% of raw meat samples in Japan.
The advantages of PCR in the detection and identification of bacteria that cause foodborne diseases lie in its sensitivity, specificity, and ability to process large quantities of samples in a short time (24). In this regard, this study demonstrated high specificity and excellent speed due to the short time required for the detection of Salmonella spp., which is important information for the animal processing industry that could enter the international market.

The results obtained regarding the time variable are similar to those reported by (25), who used the PCR technique to detect Salmonella spp. in cheeses within 28 hours. However, (26) reported, using the same technique in just 3 hours, the detection of this pathogen directly from chicken carcasses, without pre-enrichment. Although the RT-PCR technique is the most specific and sensitive among those currently available (27 and 28), there are significant microecological differences between the animal processing industry and street foods, such as ground beef, potatoes with meat, and salted beef. Although dilutions of these foods were performed to rule out inhibitions, Salmonella spp. were detected more frequently using the conventional method than RT-PCR. This could be due to the presence of substances in the food that have an inhibitory effect on PCR. Among the many compounds identified as having a negative effect on PCR are some polysaccharides, fats, metal ions, and proteins. Several authors have reported the existence of such inhibitors in food samples, which can act at different levels during the nucleic acid extraction and amplification process and eventually lead to false negatives.

CONCLUSION

The results are promising for the implementation of the molecular technique PCR as a valuable alternative for the detection of Salmonella spp. in chicken, due to its specificity and speed, especially when distributing safe products to the consumer market. Finally, regardless of the techniques, the high frequency of Salmonella spp. in food sold without sanitary controls in Montería is worrying and constitutes a risk to public health.

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