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Original Article

RAPD Fingerprinting and Genetic Diversity of *Salmonella* Spp. Isolated from Broiler and Layer Flocks in Karbala, Iraq

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Abstract

Salmonellosis in poultry is one of the most significant bacterial infections causing mortality, reduced production, and serious economic losses. This study aimed to study the molecular diversity among *Salmonella* isolates and investigate the epidemiological spread of these bacteria in broiler and layer chicken flocks in five different farms in Karbala, Iraq, using random amplified polymorphic DNA (RAPD) polymerase chain reaction (PCR). In total, 217 cloac a swabs were collected from the farms, out of which 129 and 88 swabs were taken from broiler and layer chickens. The samples were screened by PCR for *S. enterica* subsp. *enterica* using primers specific for the *invA* gene. Afterward, RAPD-PCR with uniplex or multiplex octamer primers was applied to genotype the isolates. The incidence rate of *Salmonella* infections in broilers and layers was estimated to be 27.9% and 12.5%. The uniplex primers P2 and P3, along with the multiplex primers yielded discriminatory patterns. Moreover, the RAPD typing showed a diverse range of banding patterns of *Salmonella* spp. Dendrograms created through GelJ software revealed various *Salmonella* genotypes in broilers and layers. The RAPD-PCR could be used as an accurate and fast tool to identify genetic relatedness among *Salmonella* spp. The obtained results would assist researchers in epidemiological studies and controlling salmonellosis in poultry fields.

Keywords: Chicken, RAPD-PCR, *Salmonella*

1. Introduction

Salmonellosis in poultry is one of the most significant bacterial infections causing mortality, reduced production, and serious economic losses. This infectionis caused by *Salmonella* species as Gram-negative bacteria belonging to the *Enterobacteriaceae* family. More than 2,600 serovars of *Salmonella* can cause serious infections in a wide range of hosts and are distributed worldwide (1, 2). Two species of *S. enterica* and *S. bongori* have been recognized within the genus *Salmonella* (3). They can infect birds during various stages of the production chain, including live production in which they can induce infections by clinical symptoms or asymptomatically (4). *Salmonella* in poultry can be categorized into two groups based on the diseases they cause. The first group includes host-adapted poultry containing the pathogenic and non-motile *S. Pullorum* and *S. Gallinarum* (5), and the second group, known as paratyphoid, contains *S. Typhimurium* and *S. Enteritidis* (5). Furthermore, other *Salmonella* serovars (e.g., *S.* Hadar, *S.* Infantis, and *S.* Newport) isolated from poultry are associated with *Salmonella* outbreaks through direct contact with live birds (6).

Isolates of *Salmonella* serovars have been differentiated in the epidemiological studies using

different molecular typing tools (7), such as ribotyping and pulsed-field gel electrophoresis (8-10). Nevertheless, some of the molecular assays have been shown to have little value for strain typing in the epidemiological surveys owing to limitations on facilities availability, ease of use, and cost-effectiveness (7). Generally, two main methods have been used for DNA typing. The first method includes restriction fragment length polymorphism (RFLP) that depends on the use of restriction enzymes to cut DNA specifically to produce DNA patterns called fingerprints. The second method is random amplified polymorphic DNA (RAPD)-based polymerase chain reaction (PCR) that uses short oligonucleotide primers of arbitrary octamer sequences to amplify discrete regions of the genome (11). In comparison with RAPD, RFLP-PCR has low discriminatory potential due to its inability to generate different banding patterns. In the other words, the discriminating power of RFLP is poor due to the limited genome region tested, compared with other techniques, such as RAPD (12).

Numerous researchers agree that RAPD has many advantages in that it is simpler, quicker, and more economic, compared to other genetic typing methods (13, 14), and no prior knowledge of the genomic sequences of the organism is required (15).Despite the widespread use of the RAPD technique for genetic fingerprinting, no specific primer is available for discrimination. Sometimes the primers are inadequate to discriminate genetic variances among the related and unrelated strains (16). Therefore, the current study aimed to use five uniplex primers along with multiplex primers containing all five primers in the RAPD test to analyze the epidemiology of *Salmonella* spp. at the molecular level in broiler and layer chicken flocks in five separate farms located in Karbala, Iraq.

2. Materials and Methods

2.1. Sample Collection

A total of 217 cloaca cotton swabs were collected from chickensaged12 to 47daysthat were suspected to have salmonellosis. Out of these 217 swabs, 129 and 88 swabs were obtained from broiler and layer hens, respectively. The birds were bred in five farms. Three broiler and two layer chickens were situated in two different locations (Al-Husseinia and Al-Zubeilia) inKarbala Governorate, Iraq (Table 1) during the period from August to November 2020. The cotton swabs were inoculated into 10 ml of peptone water and incubated at 37°C for 18 to 24 h.

Table 1. Breeds, numbers, ages, and locations of chicken from which cloaca swabs were collected.

Farm	Location	Breed	Age/day	No. of Birds	No. of swabs
A	Al-Husseinia	Broiler	12-45	129	41
B	Al-Husseinia	Broiler	13-45		44
C	Al-Husseinia	Broiler	14-45		44
D	Al-Zubeilia	Layer	12-47	88	44
E	Al-Zubeilia	Layer	13-47		44

2.2. Bacterial Isolation and Identification

After the cloaca swabs were inoculated into peptone water, the growth was sub-cultured onto different selective and differential bacteriology media containing MacConkey's agar and Salmonella-Shigella (SS) agar which were used for the isolation, cultivation, and differentiation of *Salmonella* spp.(17).The isolates were then identified as *Salmonella* spp., based on the morphological characters, color formation, and biochemical tests.

2.3. Molecular Diagnosis

2.3.1. Genomic DNA Extraction

Genomic DNA was extracted from the bacterial growth according to the protocol of G- spin™ Genomic DNA Extraction Kit (Intron). Shortly, 1-3 colonies of overnight bacterial growth were suspended in 1 ml of phosphate-buffered saline (PBS) and spun at 13,000 rpm for 1 min using a centrifuge to obtain the bacterial pellet. Subsequently, 300 µl of G-buffer solution was added to the pellet, mixed well, and incubated at 65°C for 15 min. The tube was mixed every 5 min during the incubation to aid cell lysis. Afterward, 250 µl of the

binding buffer was added and mixed well by pipette at least 10 times. Following the loading of the cell lysate onto the column, it was centrifuged at 13,000 rpm for 1 min. Subsequently, 500 µl of the washing solution A was added to the column and spun for 1 min at 13,000 rpm. In total, 500 µl of the washing buffer B was added to the column after removing the flow-through and centrifuged according to the procedure mentioned above. The flow through was removed, and the centrifugation step was repeated. Eventually, the G-spin™ column was placed in a clean 1.5 ml Eppendorf tube, and 100 µl of Elution buffer was added directly onto the membrane, incubated at room temperature for 1 min, and then centrifuged for 1 min at 13,000 rpm to obtain the DNA extract.

2.3.2. Conventional PCR

The *Salmonella* isolates obtained from the five farms were confirmed using PCR. A thermal cycler was applied to amplify the *invA* gene-specific for *Salmonellaenterica* subsp. *enterica*. A preliminary trial was conducted before running the final PCR to optimize the conditions for obtaining the best amplification for the *invA*gene (data is missing).The PCR reaction mixture composed of 1× AccuPower® PCR PreMix (Bioneer, Korea), 10 µM forward primer (5'-ATCGCACCGTCAAAGGAAC-3'), 10 µM reverse primer (5'-TGAAATTATCGCCACGTTCG-3'), approximately20-25 ng/µl DNA sample, and deionized distilled water (ddH_2O) up to the final reaction volume (25 µl). The PCR cycling conditions involved initial denaturation at 95ºC for 3 min and 1 cycle, followed by 35 denaturation cycles at 95ºC for 3 sec, annealing at 55ºC for 35 sec, and extension at 72ºC for 55 sec. The final elongation was done for 5 min and 1 cycle at 72ºC, followed by holding at 12ºC. The PCR amplicons were resolved by electrophoresis on 1%agarose gel in 1× Tris-borate EDTA (TBE) buffer and contained 5 µg/ml Ethidium Bromide to stain the bands. Eventually, the bands in the gel were visualized under UV light using the Gel imaging system, and the photographs were captured.

2.3.3. RAPD-PCR

The RAPD-based PCR was applied in this study to genotype the *Salmonella* cloaca isolates collected randomly from five poultry fields. Initially, the appropriate primer concentration and proper PCR annealing temperature were chosen by optimization of reaction and cycling conditions, and trials were also performed to select the best octamer oligonucleotide primers (available commercially, table 2).

Table 2. Primers used in the RAPD test

No.	Primer name	Symbol used	Sequence 5′-3′	%G-C	Tm ºC
1	AP-7	P1	GTGGATGCGA	60	32
2	P5	P2	AACGCGCAAC	60	32
3	OPP-16	P3	CCAAGCTGCC	70	34
4	OPE-20	P4	AACGGTGACC	60	32
5	OPE-4	P5	GTGACATGCC	60	32

The RAPD-PCR reaction mixture consisted of 5 µl of the commercial Master Mix (Pioneer, Korea), 3 µl of genomic DNA template of approximately 20-25 ng/µl, 10 or 20 pmol of octamer primers P1, P2, P3, P4, and P5, or the primer mixture containing all of the five primers together, (up to 25 µl of ddH_2O). The PCR program included initial denaturation for 5 min at 95ºC and succeeded by 35 cycles of denaturation at 94ºC for 1 min, annealing at36°C or 40°C for 1 min, and extension at 72ºC for 1 min. The final extension was conducted at the same temperature for 7 min, followed by holding at 12ºC.

2.3.4. *In silico* Analysis of RAPD-PCR Data

In silico analysis was performed to analyze and compare the results of RAPD-PCR using the free GelJ software (Version2.0, Spain) for MacBook computers. Only specific and thick bands were included to obtain an accurate analysis. The GelJ program was later developed and updated at the Department of Mathematics and Computer Science in the University of La Rioja, Spain, by Heras, Domínguez (18). The program, a Java application, was designed to analyze DNA fingerprint images. This software employed several methods to compute migration models, generate

dendrograms, compare banding patterns from different experiments, and support the database.

3. Results

3.1. Prevalence of *Salmonella*

Salmonella spp. was isolated from 47 (21.7%) samples out of 217 cloaca swabs taken from the chickens. In total, 36 (27.9%) *Salmonella* spp were isolated from 129 swabs taken from broilerchicken farms located in Al-Husseiniain Karbala governance, Iraq. However, *Salmonella* spp(11 isolates, 12.5%) were identified out of 88 samples collected from layer chickenfarmslocated inAl-Zubeilia regionin Karbala governance, Iraq.

3.2. Molecular Identification by PCR

The PCR reaction showed successful amplification for the specific *invA* gene,which showed a band of nearly 282 bp on the agarose gel. Figure 1 reveals some isolates collected from different farms which were confirmed as *S. enterica* subsp. *enterica* paralleled to the positive control.

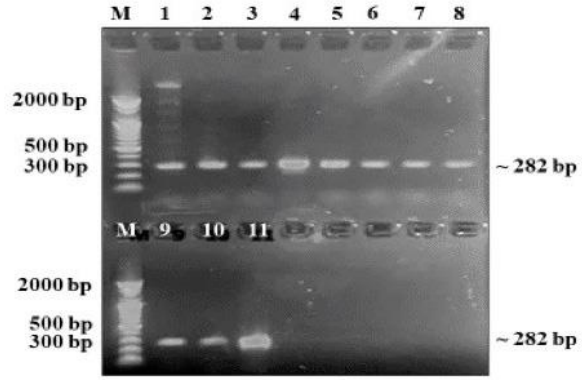

Figure 1. Agarose gel electrophoresis shows bands of ~ 282 bp representing the *invA* gene amplified partially by PCR using a primer pair specific for the detection of *S. enterica* subsp. *enterica*. M: 100 bp DNA size marker, lanes 1-10: the PCR amplicons of the tested samples, and lane 11: the positive control.

3.3. RAPD-PCR Optimization

The optimization experiments of RAPD-PCR were successful in amplifying polymorphic DNA fragments using a mixture of the five primers, as shown on the agarose gel, or using individual primers except for primer 1 (data is missing). However, P2, P3, and the mixed primers were chosen to be used later for the next experiments. Notably, the results also indicated that the best annealing temperature and the best primer concentration were40°C and 20 pmol, respectively.

3.4. *Salmonella* Genotyping Using RAPD-PCR

The RAPD-PCR showed that different serotypes of *S. enterica* subsp. *enterica* were present in cloacal samples either in the same farm or among different farms. Genetic heterogeneity of this bacterium was found among isolates of farms A, B, and C as well as among various farms (Figures 2 and 3). However, some degree of similarity in bands patterns did exist between isolates of farms D and E (Figures 3 and 4). Interestingly, each of the selected primers (P2, P3, or the mixed primers) was powerful in demonstrating the presence or absence of similarity in band patterns.

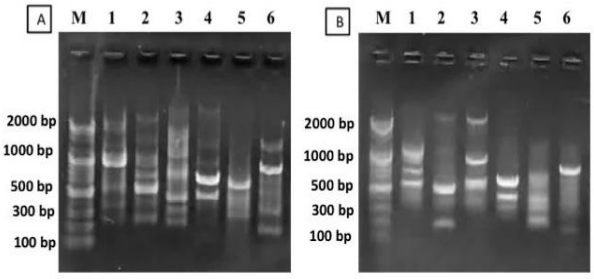

Figure 2. Agarose gel electrophoresis for the genomic DNA extracted from different *Salmonella* isolates of farms A and B amplified by PAPD-PCR. M: 100 bp DNA size marker, lanes 1,2: isolates amplified with primer P2, lanes 3,4: isolates amplified with P3, and lanes 5,6: isolates amplified with multiplex primers. A- Farm A, B- Farm B.

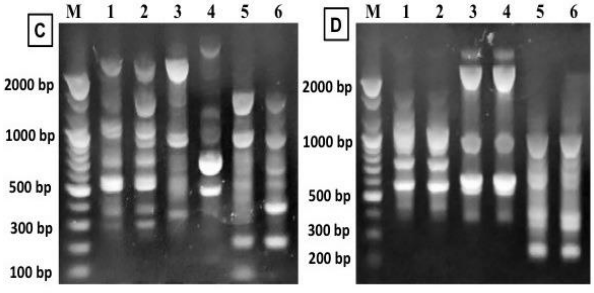

Figure 3. Agarose gel electrophoresis for the genomic DNA extracted from different *Salmonella* isolates of farms C and D and amplified by PAPD-PCR. M: 100 bp DNA size marker, lanes 1,2: isolates amplified with primer P2, lanes 3,4: isolates amplified with P3, lanes 5,6: isolates amplified with multiplex. C- Farm C, D- Farm D.

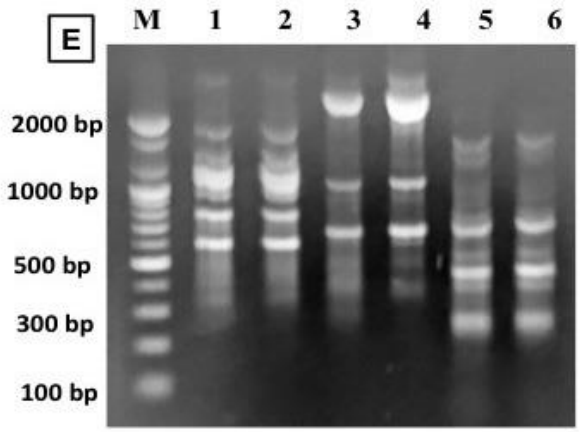

Figure 4. Agarose gel electrophoresis for the genomic DNA extracted from different *Salmonella* isolates of farm E and amplified by PAPD-PCR. M: 100 bp DNA size marker, lanes 1,2: isolates amplified with primer P2, lanes3,4: isolates amplified with P3, lanes5,6: isolates amplified with multiplex.

3.5. *In silico* Analysis

In silico analysis, data were consistent with the preliminary observations of the RAPD tests performed on randomly selected *Salmonella* isolates from the five farms (Figures 5, 6, 7 and 8). In this regard, a distant phylogenetic relationship (50%) was observed between the isolates, particularly those from farms A and B, and roughly 66% among the isolates from farm C. The compiled dendrogram clearly showed that various *Salmonella* spp. infected the broiler chicken farms (Figure 5). However, some degree of similarity was observed between some of the isolates collected from farms D (more than 75%) and E (85%). The analysis of isolates taken from farms D and E together demonstrated different scores of relatedness between the isolates. It is worth mentioning that, the highest relatedness rate was 85% between two isolates from the same farm.

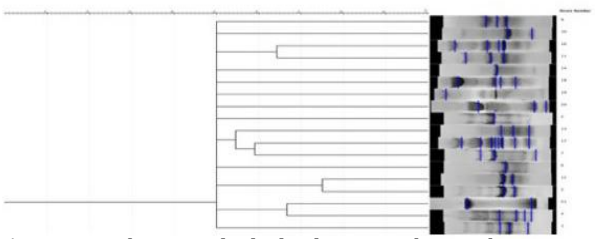

Figure 5. The complied dendrogram shows the genetic diversity of *Salmonella* spp. isolated from broiler farms (A, B, and C). Numbers 2-21 represent all isolates presented in Figure 6 that were tested with primers P2, P3, and multiplex.

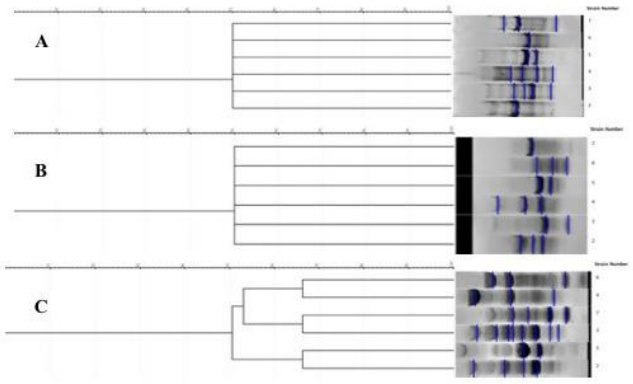

Figure 6. Dendrograms show genomic analysis of electrophoresed DNA extracted from different *Salmonella* isolates of broiler farms A, B, and C. The DNA was amplified by PAPD-PCR. Numbers 2-7 represent isolates tested: 2, 3 isolates tested with primer P2; 4, 5 with P3; 6,7 with multiplex.

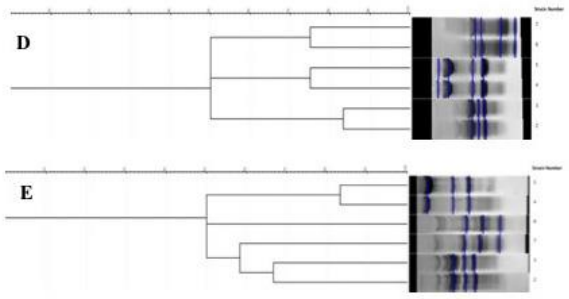

Figure 7. Dendrograms show genomic analysis of electrophoresed DNA extracted from different *Salmonella* isolates of layer farms D and E. The DNA was amplified by PAPD-PCR. Numbers 2-7 represent isolates tested: 2, 3 isolates tested with primer P2; 4, 5 with isolates tested with P3; 6, 7 isolates tested with multiplex.

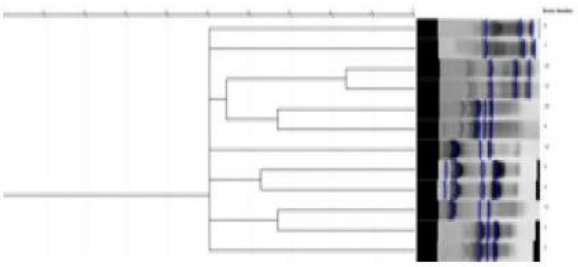

Figure 8. Complied dendrogram shows genetic diversity of *Salmonella* spp. isolated from layer farms (D and E). Numbers 2-14 represent all isolates shown in Figure 7 and tested with primers P2, P3, and multiplex.

Figure 9 presents compiled dendrogram involving all the *Salmonella* spp. isolated from broiler and layer chicken from the five farms. Based on the generated

dendrogram, the isolates were classified into six clusters (A to F) of75% similarity. Some *Salmonella* spp. isolated from different farms and chicken breeds were placed in the same cluster, suggesting the presence of close genetic relatedness between broiler and layer isolates of different farms. For example, clusters B, C, and F included isolates originating from layer and broiler chickens. On the other hand, clusters A and E involved isolates of the same chicken breed. While cluster A contained isolates from broilers of farms A and B, cluster E comprised strains from layer chickens of farms D and E.

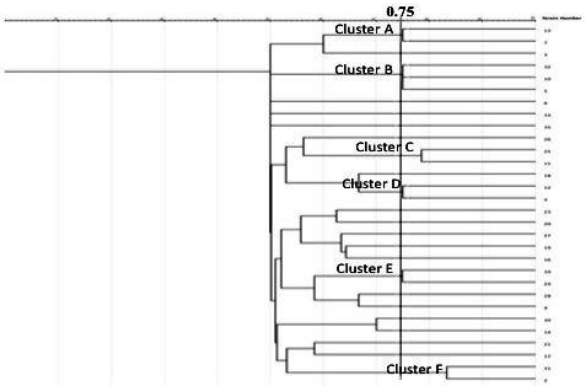

Figure 9. Complied dendrogram showing genetic diversity of *Salmonella* spp. isolated from broiler and layer chickens farms. Numbers 2-35 represent all of the isolates tested with primers P2, P3, and multiplex.

4. Discussion

In this study, 217cloaca cotton swabs were collected from three broiler chicken farms (n=129 swabs) and two layer chicken farms (n=88 swabs).*Salmonella* spp. were isolated from 47 (21.7%) samples out of the total 217 cloaca swabs. A higher incidence rate of 27.9% was reported in the broiler, compared to layer flocks (12.5%). These isolates were identified as *S. enterica* subsp. *Enterica* through the partial amplification of the *invA* gene using PCR with specific primers. In another study, the incidence rate of *Salmonella* isolated from cloaca swabs collected from Basrah province, Iraq, was reported to be 19%, and no significant difference was reported between the layer and broiler swabs (19). An incidence rate of 26% in cloacal swabs and cecal samples were observed in another study carried out in Al-Qadisiyah province, Iraq (20).The results of another study conducted by Taha, Ahmed (21) indicated that 19% of the chicken carcasses imported from different countries were contaminated with *Salmonella* spp.

Determination of genetic relatedness among *Salmonella* spp. isolates is critical for the epidemiological surveillance, control of infections, and outbreaks of these bacteria. The use of the RAPD fingerprinting technique, as a promising alternative typing tool, could characterize isolates of *S.* Gallinarum (7) and differentiate between strains of *S.* Typhi, *S.* Enteritidis, and other *Salmonella* isolates (22, 23).RAPD fingerprinting has proved to be a powerful epidemiological tool for *Salmonella* serovars typing (7). Therefore, this technique was used in the present study to determine the genetic diversity of *Salmonella* isolated from broiler and layer chicken at several farms.

Although optimization of RAPD-PCR conditions was necessary to generate high degrees of polymorphism, somewhat similar banding patterns were observed in the current study upon using annealing temperatures of 36°C and 40°C. In addition, there was no difference between the use of 10 and 20 pmol concentration of the applied primer. A similar observation was noticed when Quintaes, Leal (23) increased the concentration of primers from 20 pmol/µl to 40 pmol/µl. It has been shown that *in silico* tools can be used to construct an optimal primer from other selected equivalent primers (11). Therefore, in this study, five octameric primers were used individually or as a mixture to amplify genomic regions of *Salmonella* spp. The RAPD primers are designed to bind randomly at variable regions to generate amplicons of great variability (15), since binding of primers at highly conserved regions may lead to the production of repeated and similar profile types (7).

The results of the present study showed that all primersexcept one were successful in amplifying many bands. However, the best primers were proved to be P2, P3, and multiplex. These primers had great discriminatory power to differentiate related and unrelated *Salmonella* isolates. Therefore, sufficient

information provided by the RAPD profiles of the aforementioned primers enabled the construction of a dendrogram of genetic relatedness. In this study, the analysis of DNA fingerprints of the gel images was conducted using the GelJ software. This tool is free and simple to use and is able to achieve precise results (18).Based on the constructed dendrograms, there was a distant phylogenetic relationship (50%) between the isolates of farm A and those of farm B, and between the isolates of farm C(roughly 66%). Compiled dendrogram made for broiler farms clearly showed that various *Salmonella* spp. infected these farms. However, some degree of genetic relationship was observed between some isolates collected from farms D (more than 75%) and E (85%). The analysis of layer chicken isolates of farms D and E together demonstrated different scores of relatedness between the isolates. It is worth mentioning that, the highest rate of relatedness was estimated at85% between the two isolates of the same farm.

The final dendrogram was constructed to involve all *Salmonella* isolates from the broiler and layer chicken from the five farms and classify the isolates with75% relatedness into six clusters (A to F). Some *Salmonella* spp. isolated from different farms and breeds were placed in the same cluster to propose the presence of a genetic relationship between broiler and layer isolates of different farms. This is exemplified by clusters B, C, and F, which included isolates that originated from layer and broiler chickens. However, clusters A and E involved isolates of the same chicken breed in different farms. This finding might suggest the circulation of these strains among various farms. Eventually, *Salmonella* isolates obtained from different farms and chicken breeds were successfully typed in this study using RAPD-PCR with either P2, P3, or multiplex primers. It could be a suitable tool for phylogenetic analysis, molecular typing, as well as polymorphism detection in multiple *Salmonella* strains. Nevertheless, further studies with other contemporary genotyping methods are recommended to clarify and confirm fingerprints and genetic relationships among various *Salmonella* serovars either in poultry or environmental samples.

Typing systems for rapid and accurate epidemiological studies are required to monitor the intra and inter spread of *Salmonella* spp. among poultry flocks. In this study, the genetic relationship between the isolates was revealed using RAPD-PCR withP2, P3, or the multiplex primer comprising five oligonucleotides together. Furthermore, the created dendrograms showed distinct clustering based on the genetic relatedness between the isolates, and no unique *Salmonella* strain was present in the five farms. Therefore, this assay is useful in tracking the transmission of strains within and among farms, as well as controlling the routes and sources of spread pathogens.

Authors' Contribution

Study concept and design: I. J. L.
Acquisition of data: T. O. H.
Analysis and interpretation of data: I. J. L.
Drafting of the manuscript: I. J. L.
Critical revision of the manuscript for important intellectual content I. J. L.
Statistical analysis: T. O. H.
Administrative, technical, and material support: T. O. H.

Ethics

The experimental procedure was approved by the Animal Care Unit of University of Baghdad, Baghdad, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Haider M, Hossain M, Hossain M, Chowdhury E, Das P, Hossain M. Isolation and characterization of

enterobacteria associated with health and disease in Sonali chickens. Bangladesh J Vet Med. 2004;2(1):15-21.
2. Yoshida C, Gurnik S, Ahmad A, Blimkie T, Murphy SA, Kropinski AM, et al. Evaluation of molecular methods for identification of Salmonella serovars. J Clin Microbiol. 2016;54(8):1992-8.
3. Rodriguez J, Rondón I, Verjan N. Serotypes of Salmonella in Broiler Carcasses Marketed at Ibague, Colombia. Braz J Poult Sci. 2015;17:545-52.
4. Ibrahim WA, Abd El-Ghany WA, Nasef SA, Hatem M. A comparative study on the use of real time polymerase chain reaction (RT-PCR) and standard isolation techniques for the detection of Salmonellae in broiler chicks. Int J Vet Sci. 2014;2(1):67-71.
5. Saif Y, Fadly A, Glisson J, McDougald L, Nolan L, Swayne D. Diseases of poultry. Ames. Iowa, USA: Blackwell Publisng; 2008.
6. Control CfD, Prevention. Reports of Salmonella outbreak investigations from 2018. 2018.
7. Taddele MH, Rathore R, Dhama K, Agarwal R. Epidemiological characterization of Salmonella gallinarum isolates of poultry origin in India, employing two PCR based typing methods of RAPD-PCR and PCR-RFLP. Asian J Anim Vet Adv. 2011;6(11):1037-51.
8. Altwegg M, Hickman-Brenner F, Farmer III J. Ribosomal RNA gene restriction patterns provide increased sensitivity for typing Salmonella typhi strains. J Infect Dis. 1989;160(1):145-9.
9. Navarro F, Llovet T, Echeita M, Coll P, Aladuena A, Usera M, et al. Molecular typing of Salmonella enterica serovar Typhi. J Clin Microbiol. 1996;34(11):2831-4.
10. Thong K-L, Puthucheary S, Yassin RM, Sudarmono P, Padmidewi M, Soewandojo E, et al. Analysis of Salmonella typhi isolates from Southeast Asia by pulsed-field gel electrophoresis. J Clin Microbiol. 1995;33(7):1938-41.
11. Thangaraj M, Vishruth P, Ramesh T, Lipton A. RAPD fingerprinting and demonstration of genetic variation in three pathogens isolated from Mangrove environment. Asian J Biotechnol. 2011;3(3):269-74.
12. Cheah Y-K, Salleh NA, Lee L-H, Radu S, Sukardi S, Sim J-H. Comparison of PCR fingerprinting techniques for the discrimination of Salmonella enterica subsp. enterica serovar Weltevreden isolated from indigenous vegetables in Malaysia. World J Microbiol Biotechnol. 2008;24(3):327-35.
13. Gomes AR, Muniyappa L, Krishnappa G, Suryanarayana V, Isloor S, Prakash B, et al. Genotypic characterization of avian Escherichia coli by random amplification of polymorphic DNA. Int J Poult Sci. 2005.
14. Hassanien H. Use of randomly amplified polymorphic DNA (RAPD) analysis to detect genetic variation in Seabass Dicentrarchus labrax. J Fish Aquat Sci. 2008;3:39-46.
15. Maripandi A, Suresh S, Ponmurugan P, Gurusubramanian G. Random amplification of polymorphic DNA (RAPD) of Salmonella enteritidis isolated from chicken samples. Biotechnology. 2007;6(2):278-82.
16. Idil N, Bilkay IS. Application of RAPD-PCR for determining the clonality of methicillin resistant Staphylococcus aureus isolated from different hospitals. Braz Arch Biol Technol. 2014;57:548-53.
17. R Jaffer M, K Nazal K. Contamination of local laying hen's egg shell with salmonella serotypes. Iraq J Vet Med. 2013;37(1):13-6.
18. Heras J, Domínguez C, Mata E, Pascual V, Lozano C, Torres C, et al. GelJ–a tool for analyzing DNA fingerprint gel images. BMC Bioinform. 2015;16(1):1-8.
19. Al-Abadi I, Al-Mayah A. Isolation and identification of Salmonella spp. from chicken and chicken environment in Basrah province. Afr J Biol Sci. 2011;7:33-43.
20. Meteab BK, Abed AAA. Isolation and identification of Salmonella serotypes in poultry. Al-Qadisiyah J Vet Med Sci. 2018;17(1):75-80.
21. Taha ZM, Ahmed MS, Abdo JM. Occurrence and antimicrobial resistance of Salmonella serotypes isolated from chicken carcasses in Duhok, Kurdistan Region/Iraq. 2015;17: 119-128.
22. Betancor L, Schelotto F, Martinez A, Pereira M, Algorta G, Rodríguez MA, et al. Random amplified polymorphic DNA and phenotyping analysis of Salmonella enterica serovar Enteritidis isolates collected from humans and poultry in Uruguay from 1995 to 2002. J Clin Microbiol. 2004;42(3):1155-62.
23. Quintaes BR, Leal NC, Reis EMF, Hofer E. Optimization of randomly amplified polymorphic DNA-polymerase chain reaction for molecular typing of Salmonella enterica serovar Typhi. Rev Soc Bras Med. 2004;37:143-7.