THE IRAQI JOURNAL OF
VETERINARY MEDICINE

eISSN:2410-7409
pISSN: 1609-5693

OPEN ACCESS

Application of RAPD-PCR and Phylogenetic Analysis for Accurate Characterization of *Salmonella* spp. Isolated from Chicken and Their Feed and Drinking Water

Thamer O Hasan[1], Inam J Lafta[2*], Emad A Ahmed[3], Samah A Jassam[4]

[1]Ministry of Iraqi Agriculture, Karbala, Iraq, [2]Department of Microbiology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq, [3]Department of Biological Science, College of Science, King Faisal University, Hofouf, Alhasa, 31982, Saudi Arabia, [4]Molecular Pathology, Diagnostic Laboratory, University Hospital Southampton NHS FT, England, United Kingdom

***Correspondence**:
Inam.j@covm.uobaghdad.edu.iq

Received: 6 January 2023
Revised: 23 January 2023
Accepted: 13 February 2023
Published: 28 Jun 2023

DOI:
https://doi.org/10.30539/ijvm.v47i1.1493

Cite:
Hasan TO, Lafta IJ, Ahmed EA, Jassam SA. Application of RAPD-PCR and phylogenetic analysis for accurate characterization of *Salmonella* spp. isolated from chicken and their feed and drinking water. Iraqi J. Vet. Med. 2023;47(1):11-20.

ABSTRACT

The aim of this study was the discrimination of *Salmonella* isolated from chicken and their feed and drinking water for the epidemiological control of salmonellosis. Totally, 289 samples, including 217 chicken cloaca swabs, 46 water, and 26 feed samples were collected from five different farms in Karbala governorate, Iraq. Conventional bacteriology tests, API 20E, Vitek 2, and serology were used for bacterial identification. Random amplified polymorphic DNA (RAPD)-polymerase chain reaction (PCR) was applied to analyze the genetic relationships among *Salmonella* isolates. The isolation rate of *Salmonella* spp. was 21.1% (61/289). While the water samples constituted the highest rate (30.4%), a rate of 21.7% was reported for the cloaca swabs, with no isolate at all from chicken feed. Vitek 2 was able to identify some isolates to the serotype level, such as *S.* Enteritidis, *S.* Paratyphi B, and *S.* Paratyphi C. However, the isolates were diagnosed as *S. enterica* by API 20E, and as *S. enterica* subsp. *arizonae* through serology. Analyzing the samples by the RAPD-PCR assay showed the presence of genetically different *Salmonella* spp. Dendrograms created by the GelJ software successfully delineated the genetic relationships. Therefore, RAPD-PCR can be used as a surrogate tool for the fast, reliable, and accurate detection of *Salmonella* in epidemiological surveys when compared with other biochemical-based identification methods.

Keywords: *Salmonella*, chicken, API 20E, Vitek 2, serology, RAPD-PCR

INTRODUCTION

Salmonella species, the important foodborne pathogens, have overwhelming distribution and often infect poultry herds (1). These infections still instigate important economic losses and cost huge budgets in many nations (2, 3). Annually, 93.8 million cases and 155,000 deaths due to foodborne illnesses caused by these bacteria have been estimated to occur throughout the world (4). Infected birds are considered reservoirs important for *Salmonella* transmission to humans via the food chain (2). Among the most important biological factors posing a hazardous threat to public health are zoonotic serotypes of *S. enterica* subsp. *enterica* (5).

Several routes of dissemination have been reported for *Salmonella* spp. affecting poultry flocks (6). For instance, vertical transmission can occur mostly through the ovary of infected birds or due to contaminating eggshells following

laying (7). In addition, horizontal transmission has been shown to take place through contaminated water, feed, feces, litter, dust, equipment, fluff insects, fomites, infected chicks, and diseased rodents (8). Furthermore, broiler chickens can be infected by other wild birds, domestic animals as well as personnel during the rearing period (7). *Salmonella* infections can result in many common clinical signs in chicken including diarrhea, appetite loss, dehydration, pale and shrunken combs, and ruffled feathers. While in mature birds, the main clinical findings involve ruffled feathers, decreased fertility, and egg production, as well as eggs of soft shell; however, infections without clear clinical signs have also been reported to occur in chicken farms owing to infections by numerous serotypes of these bacteria (9).

Importantly, the last status of under-reported infections is an aggravated problem, especially in developing countries. This makes it difficult to measure and control this disease (10). Therefore, cheap, accurate, and reliable diagnosis of *Salmonella* infections is necessary to be done periodically for chicken farms in order to control or at least reduce the economic losses of *Salmonella* epidemics. *Salmonella* detection methods can be classified based on their principles into several groups: conventional microbiological methods, miniaturized biochemical assays, immunology-based assays, nucleic acid-based assays, and biosensors (3). Drawbacks have been reported for most commercial diagnostic kits, particularly those based on biochemical tests (11). Generally, six criteria have been necessitated to be present in a typing method in order to be considered as an ideal technique, such as reproducibility, type-ability, discriminatory power, low cost, ease of use, and ease of interpretation (12). Most if not all these features can be present in RAPD-PCR. This method does not require prior knowledge of DNA sequences to design primers (13). It uses oligonucleotide primers that amplify arbitrary regions within the organism's genome by using PCR to generate identifiable banding patterns useful in strain differentiation (14). Therefore, this study aimed at identifying and discriminating *Salmonella* spp. from broiler and layer chicken and their feed and drinking water from five flocks in Karbala, Iraq. Serology, API 20E, Vitek 2, and RAPD-PCR were used for this purpose, with the last procedure also applied to analyze the genetic relationships among *Salmonella* isolates to locate the epidemiological source of infection in the five farms.

MATERIALS AND METHODS

Ethical Statement

The local Committee for Animal Care and Use at the College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq reviewed and approved all procedures involved in the current study.

Sample Collection

A total of 289 samples were collected from two different locations in the holy Karbala governorate, Iraq, including Al-Husseinia and Al-Zubeilia throughout the period from August to November 2020. *Salmonella* spp. were isolated from three different types of samples, including cloaca cotton swabs, water, and feed given to the birds under study (Table 1). All samples were treated aseptically, in which 1 gm of chicken feed sample was inoculated in a test tube containing peptone water (10 mL) and incubated at 37 °C for approximately 18 to 24 h. While cotton swabs were inoculated into peptone water (10 mL), water samples were centrifuged, and 1 mL of the sediment was moved to another test tube containing 9 mL of tetrathionate broth and incubated as mentioned above.

Table 1. Information related to the samples from which *Salmonella* spp. were isolated

				Type and no. of samples		
Farm	Region	Type	Age (day)	Cloaca	Water	Feed
A	Al-Husseinia	Broiler	12-45	41	6	6
B	Al-Husseinia	Broiler	13-45	41	10	6
C	Al-Husseinia	Broiler	14-45	44	10	5
D	Al-Zubeilia	Layers	12-47	44	10	5
E	Al-Zubeilia	Layers	13-47	44	10	4

Bacterial Isolation

For bacterial isolation, a loopful of the cultured samples either in peptone water or tetrathionate broth was streaked onto selective and differential media, such as MacConkey's agar (Tm media, India), *Salmonella-Shigella* (SS) agar (Himedia, India), xylose lysine deoxycholate (XLD) agar (Oxoid, UK), Brilliant green agar (Tm media, India), and HiCrome *Salmonella* agar (Himedia, India), and incubated at 37 °C for 24 h. On the basis of colony features seen on the above media, staining properties by Gram's stain, and biochemical tests, including urease test (Himedia, India), triple sugar iron agar (Tulip diagnostic, India) and Simmon's Citrate (Oxoid, UK), the organisms were isolated and initially identified (15, 16).

Vitek 2 Diagnostic Method

Some isolates suspected to be *Salmonella* were identified by the automated Vitek 2 system with its identification card at Imam Al-Hijjah Hospital, Karbala, Iraq.

Identification with the API 20E System

Analytical profile index 20 for Enterobacteriaceae (API 20E) kit was used for the detection of some *Salmonella* spp. The identification of the bacterial isolates by this system was done according to the procedure stated by the manufacture (BioMerieux).

Serological Tests

The serological diagnosis was done at The Central Health Laboratories, Baghdad, Iraq, by the use of slip

stacking assay with a standard polyvalent antigen of the O and H antigen groups (phase I and II).

RAPD-PCR

A modification of PCR test, known as RAPD, was applied in this study for the genetic analysis and relatedness of *Salmonella* isolates. Using the protocol of G-spin™ Genomic DNA Extraction Kit (Intron), the genomic DNA was isolated from the bacterial culture. After optimizing the conditions, the RAPD-PCR reaction mixture involved: 5 µL of Master Mix (Pioneer, Korea), 3 µL of the DNA template (20-25 ng/µL), one of the primer formulas: P2, P3 or the mixture (containing P1 to P5) (each 1 µL; 20 pmoL), and up to 25 µL of double distilled water (ddH_2O) were used. The primer sequences are shown in Table 2.

Table 2. Primers used in the RAPD test

No.	Primer symbol	Primer name	Sequence 5′-3′
1	P1	AP-7	GTGGATGCGA
2	P2	P5	AACGCGCAAC
3	P3	OPP-16	CCAAGCTGCC
4	P4	OPE-20	AACGGTGACC
5	P5	OPE-4	GTGACATGCC

Concerning the PCR cycling conditions, they involved initial denaturation at 95 °C for 5 min and 35 cycles of: denaturation at 94 °C for 1 min, annealing at 40 °C for 1 min, and extension at 72 °C for 1 min. Then, final extension at 72 °C for 7 min and holding at 12 °C were also included. Afterwards, the PCR products were resolved by electrophoresis on 1% agarose gel dissolved in 1× Tris-borate EDTA (TBE) buffer and contained Ethidium Bromide (5 µg/mL) to stain the bands. Ultimately, the bands in the gel were visualized under UV light using the Gel imaging system, and the photographs were captured.

In silico Analysis

In silico analysis was done to analyze and compare the results of RAPD-PCR by using GelJ version 2.0 software. This program, a Java application, was designed for analyzing DNA fingerprint images, making dendrograms, and comparing the banding patterns from different experiments (17).

RESULTS

Incidence of *Salmonella*

The results of the isolation of *Salmonella* spp. on different culture and biochemical media are not shown here. Out of 289 collected samples, the isolation rate of *Salmonella* spp. was 21.1% (61 positive samples). A higher isolation rate (30.4%) occurred in water samples than in cloaca cotton swabs (21.7%). While no isolation was found in feed samples (Table 3).

Table 3. Isolation rates of *Salmonella* spp. from the collected samples

Source	No. samples	No. positive samples	Isolation %
Cloaca swabs	217	47	21.7
Water	46	14	30.4
Feed	26	0.0	0.00
Total	289	61	21.1

Diagnosis Using Vitek 2

Ten isolates of suspected *Salmonella* collected randomly from the five farms were confirmed by the Vitek 2 system as *Salmonella* spp. The diagnosis probabilities ranged from 97% to 99%. Most of the isolates were identified to the serotype level. The water and cloaca samples of farm A were diagnosed to the serotype level as *S.* Paratyphi B. However, samples of farm B were detected as *S. enterica* subsp. *enterica* for the water sample, and as *S.* Enteritidis/ *S.* Paratyphi B/ or *S.* Paratyphi C for the cloaca isolate. Different results were reported for farm C, while the water isolate was diagnosed as *S.* Enteritidis or *S.* Paratyphi C, the cloaca sample was *S.* Paratyphi B. Concerning farm D, both water and cloaca isolates were similar and identified as *S.* Paratyphi B. Finally, farm E showed isolation of *S.* Paratyphi B or *S.* Enteritidis from water sample and *S. enterica* subsp *enterica* from a cloaca swab.

Diagnosis by API 20E

The API 20E system identified 7 out of 10 suspected isolates as *S. enterica*. Figure 1 represents an example of a *Salmonella* isolate identified by the API 20E test strip**.**

Serotyping of *Salmonella* Isolates

Initially, serological tests were able to diagnose 7 out of 10 *Salmonella* spp. isolates to the serotype level as *S. enterica* subsp *arizonae*. While 2 of the isolates were incorrectly diagnosed as *E. coli*, another isolate was misdiagnosed as *Serratia liquefaciens* (Table 4).

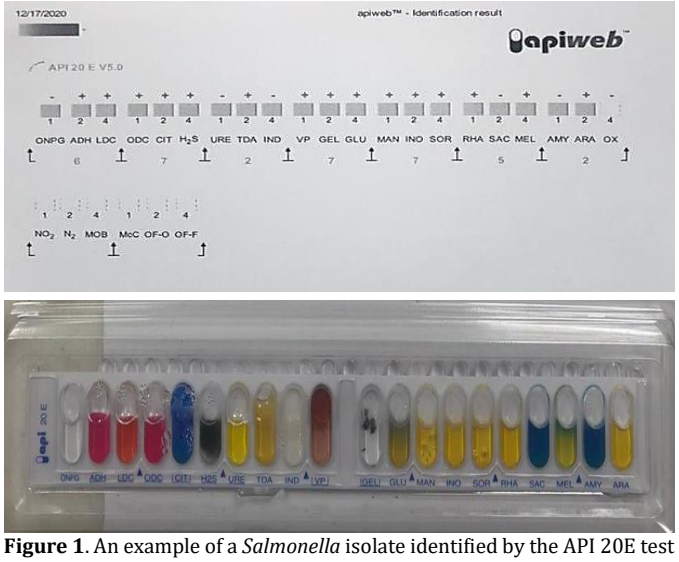

Figure 1. An example of a *Salmonella* isolate identified by the API 20E test strip

Table 4. Serotyping of *Salmonella* isolats

No.	Sample code	Breed	Serotype
1	AW	Broiler	*S. enterica* subsp. *arizonae*
2	AC	Broiler	*S. enterica* subsp. *arizonae*
3	BW	Broiler	*S. enterica* subsp. *arizonae*
4	BC	Broiler	*S. liquefaciens**1
5	CW	Broiler	*S. enterica* subsp. *arizonae*
6	CC	Broiler	*S. enterica* subsp. *arizonae*
7	DW	Layer	*E. coli**2
8	DC	Layer	*E. coli**3
9	EW	Layer	*S. enterica* subsp. *arizonae*
10	EC	Layer	*S. enterica* subsp. *arizonae*

A, B, C, D, E: represent the five farms where the isolates were detected; W: water sample; C: cloaca swab; *: misdiagnosis, [1, 2, 3] were diagnosed again by Vitek 2 as *S.* Paratyphi B or C, *S. enterica* subsp. *diarizonae*, and *S. enterica* subsp. *diarizonae*, respectively

RAPD-PCR and Vitek 2 Analysis of Samples

The RAPD-PCR test revealed amplification of similar *Salmonella* DNA bands for both water and cloaca isolates of farm A. Importantly, there were identical bands when either P3, P4 or the mixed primers were used to amplify the water sample and the cloaca swab obtained from farm A. This might indicate that the same genotype of *Salmonella* was found in these two samples, which were detected as *S.* Paratyphi B by the Vitek 2 system (Figure 2A). Similarly, water and cloaca isolates of farm B and amplified by primers P3, P4 or the mixture might be genetically identical, as shown in Figure 2B. These isolates were diagnosed by the Vitek 2 system as *S.* Enteritidis *S.* Paratyphi B or C for the cloaca isolate, and as *S. enterica* subsp. *enterica* for the water sample.

With respect to farm C, the water and cloaca isolates amplified with any of the primers were genetically different from each other (Figure 2C). This result could be consistent with that of the Vitek 2 system, in which the water isolate was diagnosed as *S.* Paratyphi C or *S.* Enteritidis, while the cloaca isolate was *S.* Paratyphi B. However, the cloaca and water samples collected from farm D showed similar band patterns resulted from each of the used primers (Figure 2D). This might also agree with the findings of the Vitek 2 system, where both isolates were diagnosed to be similar (i.e., *S.* Paratyphi B). Overall, the findings obtained from farm D were similar to those of farms A and B, according to RAPD findings.

Figure 2E demonstrates the presence of a high degree of genetic similarity between the water and cloaca samples collected from farm E. These isolates were already diagnosed as *S.* Paratyphi B or *S.* Enteritidis for the water sample, and as *S. enterica* subsp *enterica* for the cloaca sample, by the Vitek 2 system. Thus, the RAPD result might denote that both isolates belong to the same serotype, and the cloaca isolate could be *S.* Paratyphi B or *S.* Enteritidis as the water sample.

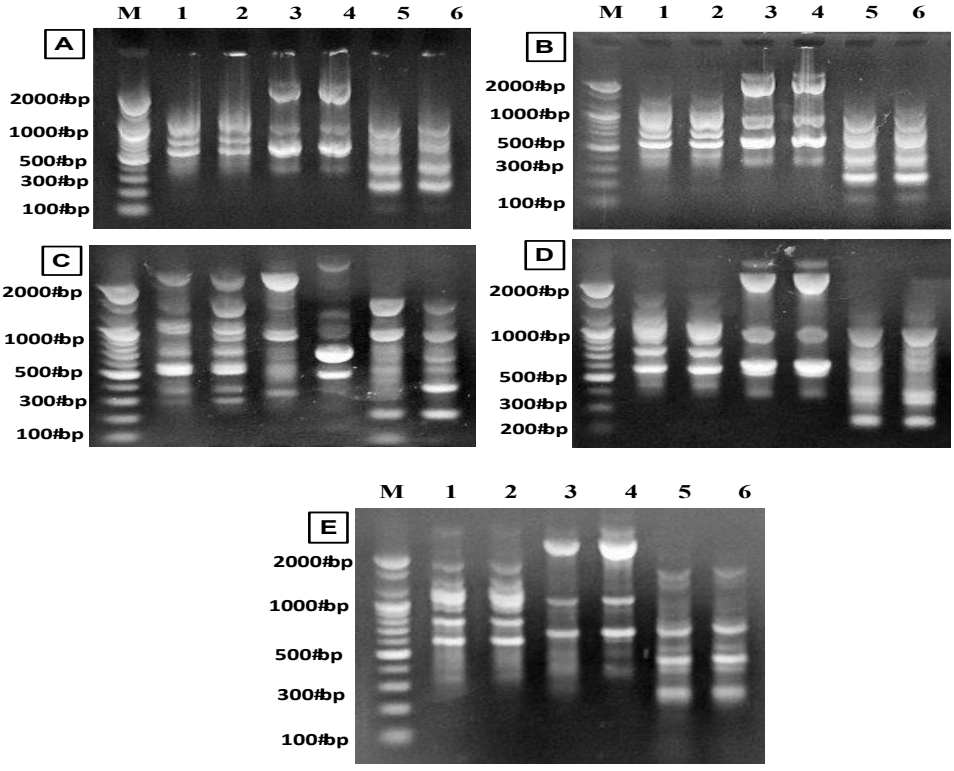

Figure 2. RAPD-PCR analysis for the *Salmonella* samples detected by the Vitek 2 system. The *Salmonella* genomic DNA was extracted from five farms A-E. M: 100 bp DNA size marker, lanes 1, 3, and 5 water samples, lanes 2, 4, and 6 cloaca samples. The P2 primer is in the lanes 1 and 2, the primer P3 is in the lanes 3 and 4, and the mixed primers are in the lanes 5 and 6

RAPD-PCR and API 20E Analysis of Samples

Random amplified polymorphic DNA was also performed for *Salmonella* isolates of water and cloaca samples diagnosed by the API 20E and confirmed by the serology. The findings of farms A and B revealed the existence of genetic resemblance between these isolates. The three primers were successful in amplifying the same polymorphic bands in these samples and in both farms. Although serology identified the presence of *S. enterica* subsp. *arizonae* in water and cloaca samples of farm A and a water sample only from farm B, the cloaca isolate of farm B was identified as *S.* Paratyphi B or C by the Vitek 2 system. Despite that technical error did occur that distorted the bands' shapes while running the samples of farm B (Figure 3A, B).

Concerning farm C, where both the cloaca and water samples were detected by serotyping as *S. enterica* subsp. *arizonae*, both primers P2 and P3 showed the presence of similarity between the samples; conversely, the mixed primers revealed differences in banding patterns (Figure 3C). Thus, the mixed primers could be more powerful in showing discrepancy than individual ones. In farm D, on the other hand, the three primers revealed occurrence of compatibility between the cloaca and water samples (Figure 3D), and their banding patterns were different from those revealed in the other farms. This may agree with the result of Vitek 2, in which the repeated water and cloaca samples of farm D were only identified as *S*. *enterica* subsp. *diarizonae*.

Although *Salmonella* isolates of farm E were detected by serotyping as *S. enterica* subsp. *arizonae*, RAPD test showed completely different bands for water and cloacal samples (Figure 3E). This result might denote the higher accuracy of RAPD test than serotyping in differentiating epidemiological isolates.

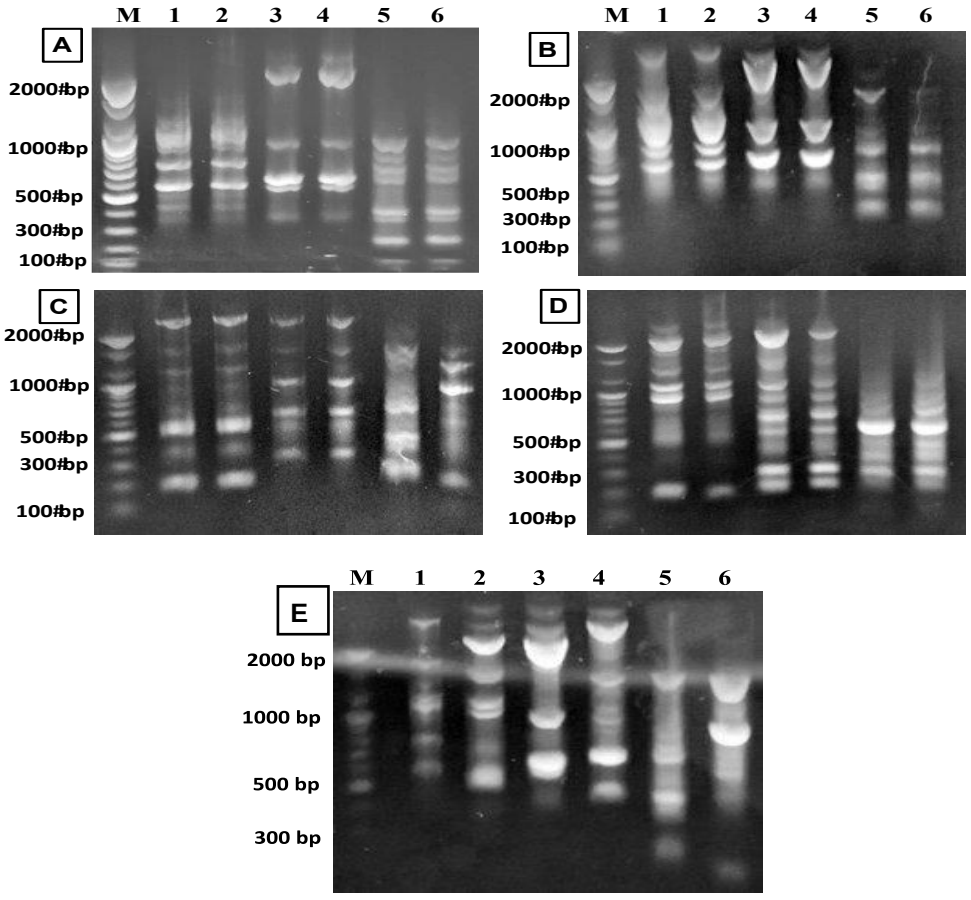

Figure 3. RAPD-PCR analysis for the *Salmonella* samples detected by the API 20E system and confirmed by serotyping. The *Salmonella* genomic DNA was extracted from five farms A-E. M: 100 bp DNA size marker, lanes 1, 3, and 5 water samples, lanes 2, 4, and 6 cloaca samples. The P2 primer is in the lanes 1 and 2, the primer P3 is in the lanes 3 and 4, and the mixed primers are in the lanes 5 and 6.

In silico Analysis for the Samples Detected by the Vitek 2 System

The visual analysis of electrophoresis banding patterns of the RAPD products was compatible with the *in silico* data of the same samples. Visually, farms A and B looked 100% similar to each other using the three different primers. The *in silico* analysis showed also similar result (Figure 4 above). Regarding farm B, 100% identity was reported between the water and cloaca isolates using the three primers (Figure 4 below).

In the comparison of the *in silico* data of farm C with that of the electrophoresis for RAPD products, obvious dissimilarity appeared between the isolates (Figure 2C; Figure 5 above). However, a similarity of 75% existed between samples number 2 and 3 amplified by the primer P2 (Figure 5 above). This result agreed with that of Vitek 2 where the water isolate was diagnosed as *S*. Enteritidis or *S.* Paratyphi C, while it was identified as *S.* Paratyphi B for the cloaca swab. Concerning farm D, complete matching appeared between cloacal and water samples using the various primers (Figure 5). Likewise, both isolates were identified as *S*. Paratyphi B by the Vitek 2 test. Upon examining the polymorphic bands of the cloaca swab (diagnosed as *S*. Paratyphi B) that were collected from farm C with the samples of farm D (also diagnosed as *S*. Paratyphi B), there was no matching, or at least partial similarity indicating an incorrect diagnosis of the Vitek 2 system.

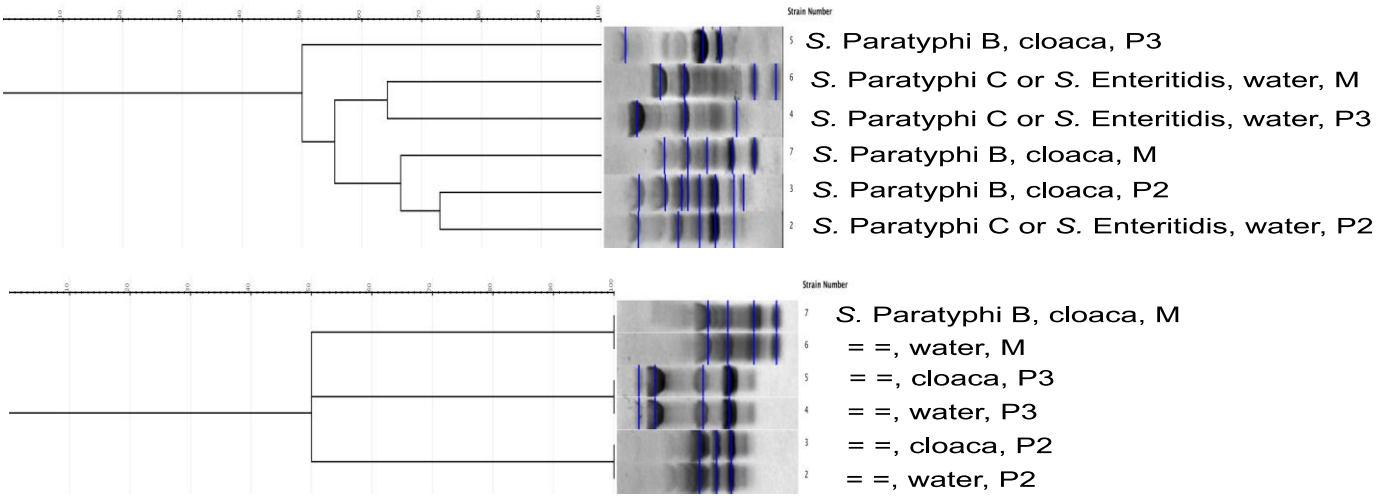

Figure 4. Dendrograms show analysis of the electrophoresed PAPD-PCR products of *Salmonella* spp. already detected by Vitek2. Above: farm A and below: farm B.

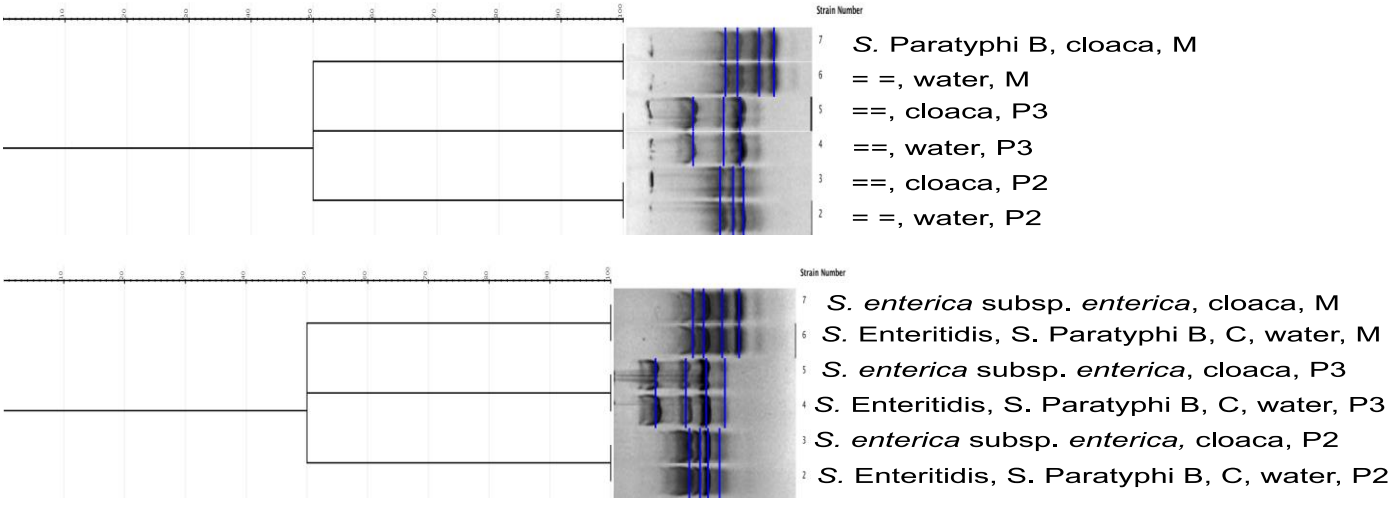

Figure 5. Dendrograms show analysis of the electrophoresed PAPD-PCR products of *Salmonella* spp. already detected by Vitek 2. Above: farm C and below: farm D.

Figure 6 shows the *in silico* analysis of farm E isolates where the water sample was identified as *S.* Paratyphi B or *S*. Enteritidis, and the cloaca sample as *S*. *enterica* subsp. *enterica*. Apparently, the samples look genetically identical as they showed similar banding profiles. Consistently, the *in silico* analysis revealed existence of 100% identity between the cloaca and water isolates amplified by primers P3, P2, and the mixture.

In silico Analysis for Samples Detected by the Serotyping

The isolates of the water and cloaca samples of farms A and detected by serotyping look apparently similar, on the first glace, on the electrophoresed RAPD products (Figure 3). In comparison, the *in silico* analysis revealed presence of 100% matching when each of P2, P3 or M primer was used in amplification (Figure 7 above). With respect to farm B, there was complete matching between water and cloaca isolates when amplified by P2 or P3 primers. However, the primer M was able to show little discrepancy between the cloaca and water isolates (Figure 7 below).

Analyzing the results of farm C isolates confirmed by serotyping as *S. enterica* subsp. *arizonae* showed clear differences between these samples, particularly when the mixed primers were used (Figure 8 above). Concerning farm D, although the isolates were incorrectly diagnosed as *E. coli*, and identified by Vitek 2 as *S. enterica* subsp. *diarizonae*, their *in silico* analysis revealed presence of 100% matching upon using each of the primers (Figure 8 below). Finally, the *in silico* analysis for farm E isolates was inconsistent with the results of serology (*S. enterica* subsp. *arizonae*); the samples appeared different from each other (Figure 9).

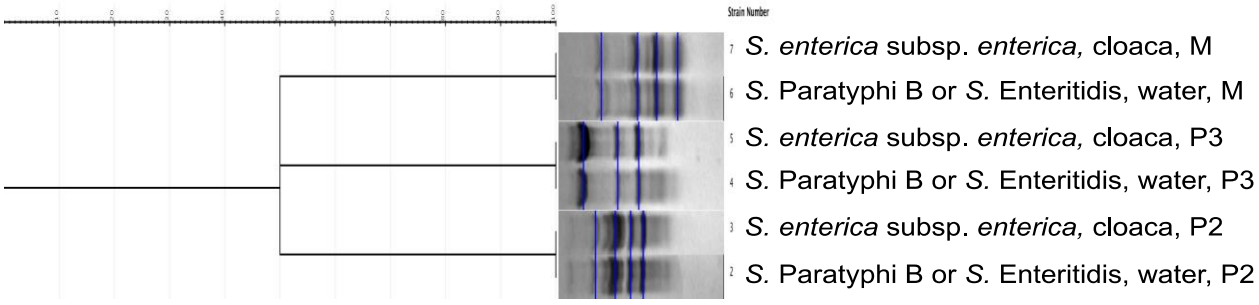

Figure 6. Dendrogram shows analysis of the electrophoresed PAPD-PCR products of *Salmonella* spp. of farm E already detected by Vitek2

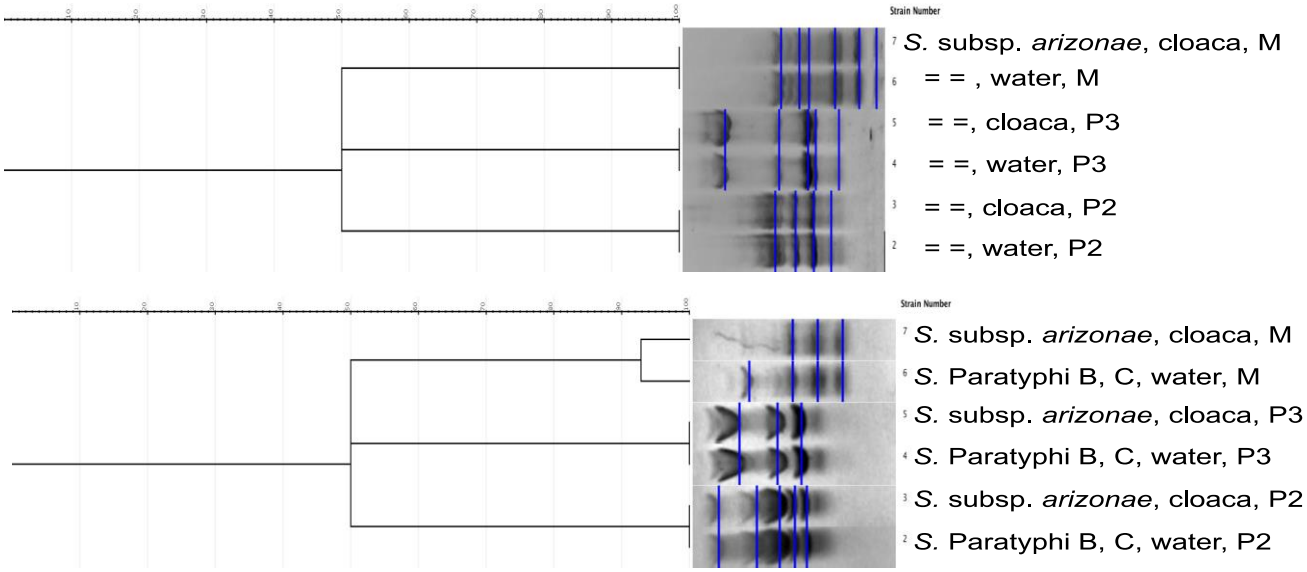

Figure 7. Dendrograms show analysis of the electrophoresed PAPD-PCR products of *Salmonella* spp. already detected by serology. Farm A above, Farm B below.

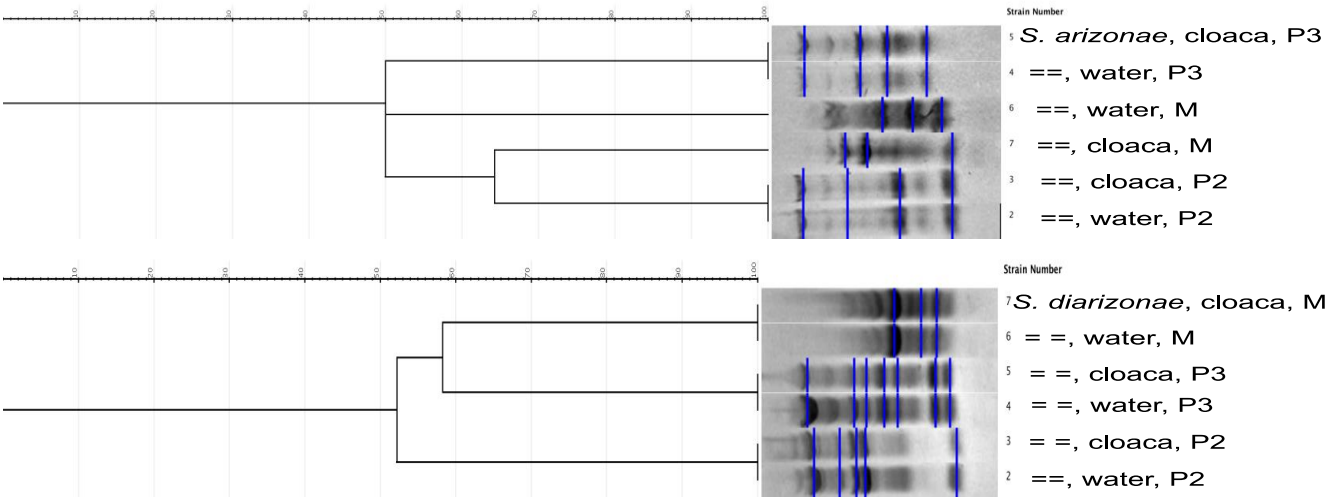

Figure 8. Dendrograms show analysis of the electrophoresed PAPD-PCR products of *Salmonella* spp. already detected by serology. Above: farm C and below: farm D

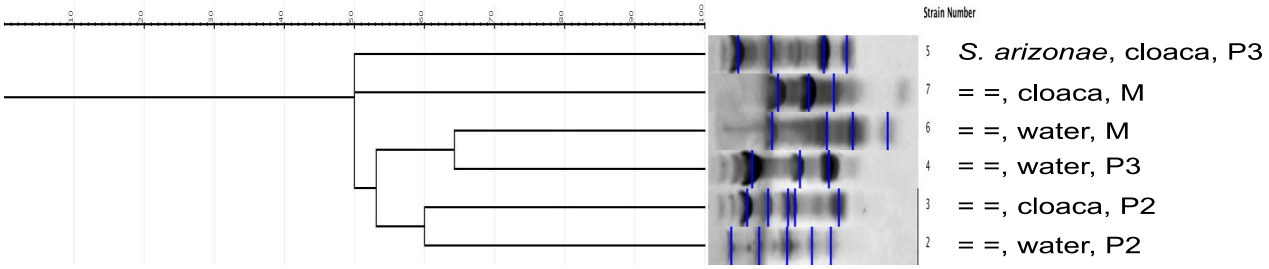

Figure 9. Dendrogram shows analysis of the electrophoresed PAPD-PCR products of *Salmonella* spp. of farm E already detected by serology

DISCUSSION

In this study, 289 samples of chicken cloaca swabs, chicken feed, and drinking water were collected from five farms in Karbala province suspected to have salmonellosis. The preliminary diagnosis based on Gram's staining, different bacteriological culture media, and the biochemical diagnostic test revealed the presence of 61 samples positive to *Salmonella* spp. with an incidence rate of 21.1%. While water samples had the highest rate (30.4%), a rate of 21.7% was reported for the cloaca swabs, with no isolate from chicken feed. This result might indicate water as the main source of infection in these flocks. The presence of these bacteria in cloaca samples of infected chicks could be another source to transmit the infection to other birds through contaminating drinking water, especially the fecal-oral route is well-known for foodborne diseases including salmonellosis. Concerning the absence of *Salmonella* contamination in food samples of the current study, a similar finding was also observed by Djeffal and co-workers (2018) (18) who found that all of the 160 food samples tested against *Salmonella* were negative. However, this bacterium was isolated from 3% only of the feed samples examined in New Zealand by Kingsbury et al. (2019) (19).

The API 20E system used in the present study was able to diagnose the suspected isolates to the species level only as *S. enterica*. It has been demonstrated that API 20E was an accurate technique for identifying *S. enterica* (20-22). In another study performed to assess the accuracy of identifying different bacteria by using API 20E, it was found that 87.6% of isolates had the exact identity, 12% nearest identity, and 0.4% no identity (11). Although it is a robust bacterial identification tool, the API 20E performance differed among different species (11). Nevertheless, this method is not useful for the definite diagnosis of this organism that has approximately 2600 serotypes, and it should not be used for this purpose, especially since three isolates of this study were incorrectly diagnosed as other enteric bacteria.

Concerning serotyping, it has been useful for *Salmonella* typing over the world throughout decades. Nevertheless, serotyping analysis has frequently been demoted to

reference laboratories causing difficulty to private laboratories in making fast analyses (12). In the current study, while serology identified all of the suspected isolates as *S. enterica* subsp. *arizonae*, the Vitek 2 system was capable of identifying different serotypes of *Salmonella*. The serotypes identified by this tool included: *S.* Enteritidis, *S.* Paratyphi B, *S.* Paratyphi C, *S. enterica* subsp. *diarizonae*, and others diagnosed as *S. enterica* subsp. *enterica*. However, in a previous study, out of 845 isolates including members of the Enterobacteriaceae family and other non-enteric bacteria, only 84.7% were correctly diagnosed to the species level, 0.8% strains were misidentified, and 1.2% were not identified by using the Vitek 2 system. Identification mistakes did occur randomly throughout various bacterial taxa (23).

Darbandi (2010) (24) compared the accuracy of API 20E and that of Vitek 2 in identifying microorganisms, the data showed that the acceptable findings increased from 80% in case of API 20E to 90% in Vitek 2 system. Thus, the Vitek 2 system can replace API 20E because of the economic and trustable values of the first, despite that some limitations still exist when using Vitek 2 exemplified mainly by the long time required for bacterial sample preparation (24). However, using Vitek 2 alone for *Salmonella* serotypes identification might have some inconsistencies. Therefore, surveillance and monitoring of *Salmonella* infections must depend on effective and reliable detection methods (25). In the current study, a molecular test known as RAPD- PCR was applied for analyzing the samples of different farms. This technique showed the occurrence of genetically different *Salmonella* spp., and it had a powerful discriminatory value of showing relatedness or discrepancy among the isolates of the same or different farms. Importantly, the uniplex primers P3 or P4 or the multiplex primers were useful in showing the genetic relatedness among the isolates. Dendrograms constructed by the GelJ software successfully described the phylogenetic relationships. This is consistent with the findings of Hasan and Lafta (2021b) (26) who used RAPD-PCR for studying the molecular diversity among *Salmonella* spp. obtained from broiler and chicken flocks on various farms.

The compiled-in silico analysis performed for the RAPD-PCR profiles of water and cloaca *Salmonella* isolates collected from the five farms shows that heterogeneous *Salmonella* spp. contaminated water samples and also different genotypes existed in birds' cloaca. This finding rule out the possibility of the occurrence of an outbreak among the studied farms, especially these farms are located in different areas within the same province. To conclude, drinking water but not feed can be considered as a main source of *Salmonella* infections in the studied farms. RAPD-PCR can be used as a surrogate tool for the fast, reliable, and accurate typing of *Salmonella* spp. in epidemiological surveys, and it can replace other biochemical-based identification methods.

ACKNOWLEDGEMENTS

N/A

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Al-Khatib GM, Al-Qutbey SH, Al-Mashhadany MS, Al-Adad BM. Isolation and identification of *Salmonella* spp. which contaminated poultry slaughter houses. Iraqi J. Vet. Med. 2005;29(1):92-97.
2. Crespo PS, Hernandez G, Echeita A, Torres A, Ordonez P et al. Surveillance of foodborne disease outbreaks associated with consumption of eggs and egg products: Spain 2002–2003. Europ Surveill. 2005;10(6):E050616.2.
3. Lee KM, Runyon M, Herrman TJ, Phillips R, Hsieh J. Review of *Salmonella* detection and identification methods: Aspects of rapid emergency response and food safety. Food Cont. 2015;47:264-276.
4. Heredia N, Garcia *S.* Animals as sources of food-borne pathogens: A review. Ani Nutr. 2018;4(3):250-255.
5. Trawinska B, Saba L, Wdowiak L, Ondrasovicova O, NowakowiczDebek B. Evaluation of *Salmonella* rod incidence in poultry in the Lublin Province over the years 2001–2005. Annu Agricult Environ Med. 2008;15(1):131–134.
6. Nayak R, Stewart T, Wand RF, Lin J, Cerninglia CE. Genetic diversity and virulence gene determinants of antibiotic-resistant *Salmonella* isolated from preharvest Turkey production sources. Inter J Food Microbiol. 2004;91:51–62.
7. Pande VV, Devon RL, Sharma P, McWhorter AR, Chousalkar KK. Study of *Salmonella* Typhimurium infection in laying hens. Front in Microbiol. 2016;7:203.
8. Tabo DA, Diguuimbaye CD, Granier SA, Moury F, Brisabois A et al. Prevalence and antimicrobial resistance of non-typhoidal *Salmonella* farms in N'Djamena, Chad. Vet Microbiol. 2013;166(1-2):293–298.
9. McWhorter AR, Chousalkar KK. A long-term efficacy trial of a live, attenuated *Salmonella* typhimurium vaccine in layer hens. Front in Microbiol. 2018;9:1380.
10. Barbour EK, Ayyash DB, Alturkistni W, Alyahiby A, Yaghmoor S et al. Impact of sporadic reporting of poultry *Salmonella* serovars from selected developing countries. J Infect in Develop Countr. 2015;9(1):1–7.
11. Maina D, Okinda N, Mulwa E, and Revathi G. A five year review of API20E bacteria identification system's performance at a teaching hospital. East Afr Med J. 2014;91(3):73-76.
12. Salem B, Ridha M, Mahjoub A. Laboratory Typing Methods for Diagnostic of *Salmonella* Strains, the "Old" Organism That Continued Challenges. In: *Salmonella*-A Dangerous Foodborne Pathogen. InTech; 2012.
13. Ellsworth DL, Rittenhousen D and Honeycutt RL. Artifactual variation in randomly amplified polymorphic DNA banding patterns. Biotechn. 1993; 14: 214–217.
14. Maurer JJ, Lee MD, Lobsinger C, Brown T, Maier M et al. Molecular typing of avian Escherichia coli isolates by random amplification of polymorphic DNA. Avi Dis. 1998;42:431-451.
15. Jaffer RM, KK Nazal. Contamination of local laying hen's egg shell with *Salmonella* serotypes. Iraqi J. Vet. Med. 2013;37(1):13-16.
16. Hasan TO, Lafta IJ. Identification and antimicrobial susceptibility profiles of *Salmonella* spp. isolated from chicken flocks and their feed and water in Karbala, Iraq. Ind J Ecol. 2021a;48(5):1542-1550.
17. Heras J, Dominguez C, Mata E, Pascual V, Lozano C et al. GelJ -a tool for analyzing DNA fingerprint gel images. BMC Bioinform. 2015;16:270.
18. Djeffal S, Mamache B, Elgroud R, Hireche S, Bouaziz O. Prevalence and risk factors for *Salmonella* spp. contamination in broiler chicken farms and slaughterhouses in the northeast of Algeria. Vet World. 2018;11(8):1102.
19. Kingsbury JM, Thom K, Erskine H, Olsen L, Soboleva T. Prevalence and genetic analysis of *Salmonella enterica* from a cross-sectional survey

of the New Zealand egg production environment. J Food Prot. 2019;82(12):2201–2214.
20. Nucera DM, Maddox CW, Hoien-Dalen P, Weigel RM. Comparison of API 20E and invA PCR for identification of *Salmonella enterica* isolates from swine production units. J Clinic Microbiol. 2006;44(9):3388–3390.
21. Saeed AA, Hasoon MF, Mohammed MH. Isolation and molecular identification of *Salmonella* typhimurium from chicken meat in Iraq. J World's Poult Res. 2013;3(2):63-67.
22. AL-mossawei MT, Kadhim AA, Hadi BH. A comparative study between conventional methods and Vidas UP *Salmonella* (SPT) to investigate *Salmonella* species from local and imported meat. Baghdad Sci J. 2015;2(12):242-248.
23. Funke G, Monnet D, Debernardis C, Graevenitz AV, Freney J. Evaluation of the VITEK 2 system for rapid identification of medically relevant Gram-negative rods. J Cli Microbiol. 1998;36(7):1948–1952.
24. Darbandi F. Parallel comparison of accuracy in Vitek 2 auto-analyzer and API 20 E/API20 NE microsystems. MSc, University College of Boras, School of Engineering. 2010.
25. Rodriguez-Lazaro D, Lombardc B, Smith H, Rzezutka A, D'Agostino M et al. Trends in analytical methodology in food safety and quality: monitoring microorganisms and genetically modified organisms. Trends in Food Sci Technol. 2007;18(6):306-319.
26. Hasan TO, Lafta IJ. RAPD Fingerprinting and Genetic Diversity of *Salmonella* spp. Isolated from Broiler and Layer Flocks in Karbala, Iraq. Arch Razi Inst. 2021b;76(5):1183-1190.

استخدام فحص RAPD-PCR وتحليل النشوء والتطور للتوصيف الدقيق لأنواع السالمونيلا المعزولة من الدجاج وأعلافها ومياه الشرب

ثامر عبيد حسن[١]، إنعام جاسم لفته[٢]،عماد عبد الجليل احمد[٣]، سماح علي جسام[٤]

[١]وزارة الزراعة العراقية، كربلاء، العراق، [٢]فرع الاحياء المجهرية، كلية الطب البيطري، جامعة بغداد، بغداد، العراق، [٣]قسم العلوم البيولوجية، كلية العلوم، جامعة الملك فيصل، الأحساء، المملكة العربية السعودية، [٤]علم الأمراض الجزيئي، مختبر التشخيص، مستشفى جامعة ساوثهامبتون، المملكة المتحدة

الخلاصة

الهدف من هذه الدراسة هو التمييز بين السالمونيلا المعزولة من الدجاج وعلفها ومياه الشرب للسيطرة الوبائية على السالمونيلا. جرى جمع ٢٨٩ عينة، منها ٢١٧ مسحة من مجمع cloaca الدجاج و ٤٦ عينة مياه و ٢٦ عينات علف من خمس حقول مختلفة في محافظة كربلاء، العراق. استخدمت الاختبارات التقليدية لعزل وتشخيص الجراثيم، فحص API 20E وفحص Vitek 2 فضلا عن الفحوصات المصلية لتحديد انواع السالمونيلا المعزولة بالدراسة. استخدم تفاعل البوليميراز المتضخم العشوائي (RAPD) لتحليل العلاقات الوراثية بين عزلات السالمونيلا. أوضحت النتائج عزل *Salmonella* spp بمعدل ٢١,١ ٪ (٢٨٩/٦١). شكلت عينات المياه أعلى نسبة عزل (٣٠,٤٪)، وسجلت نسبة ٢١,٧٪ لمسحات المجمع cloaca مع عدم وجود عزل على الإطلاق من علف الدجاج. تمكن Vitek 2 من تحديد بعض العزلات على مستوى النمط المصلي مثل *S.* Enteritidis و *S.* Paratyphi B و *S.* Paratyphi C. ومع ذلك، جرى تشخيص العزلات على أنها *S. enterica* بواسطة API 20E ، و *S. enterica* subsp. *arizonae* من خلال الاختبارات المناعية. أظهر تحليل العينات بواسطة RAPD-PCR وجود أنواع مختلفة جينيا من السالمونيلا. نجح Dendrograms الذي تم إنشاؤه بواسطة برنامج GelJ في تحديد العلاقات الجينية. لذلك، يمكن استخدام RAPD-PCR كأداة بديلة للكشف السريع والموثوق والدقيق عن السالمونيلا في المسوحات الوبائية عند مقارنتها بالطرائق البيوكيميائية الأخرى.

الكلمات المفتاحية: السالمونيلا، الدجاج، فحص API 20E ، فحص الفايتك Vitek 2، الفحص المصلي، فحص تفاعل البوليميراز المتضخم العشوائي