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Identification and Antimicrobial Susceptibility Profiles of *Salmonella* spp. Isolated from Chicken Flocks and their Feed and Water in Karbala, Iraq

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***Abstract*:** The objective of study was determining the most prevalent *Salmonella* spp. and their antimicrobial susceptibility in broilers and laying chickens and their feed and drinking water in five chicken farms in Karbala, Iraq over the period from August to October 2020. A total of 289 samples, including 217 cloaca swabs, 46 water and 26 feed samples were collected. *Salmonella* spp. was identified firstly by routine diagnostic methods, followed by applying the API 20E kit, the Vitek2 system, and serology. There was significant differences in *Salmonella* prevalence among different types of samples, mainly cloaca swabs reported a high isolation rate (21.7%). In contrast, feed samples were completely free of contamination. The highest rate of isolation was in September on the 4th to 6th weeks of age. The presence of different *Salmonella* types in the collected samples excludes the possibility of outbreak occurrence among these farms. However, many isolates were diagnosed as *S. paratyphi* B. The tested isolates were 100% resistant to Ampicillin, Amikacin, Gentamicin, and Ciprofloxacin. By contrast, they were susceptible to Ceftazidime, Cefepime, and Ertapenem. The study provides an insight into the distribution and antimicrobial resistance of *Salmonella* spp. circulating in several poultry farms in Karbala, Iraq.

***Keywords*:** *Salmonella* spp, Broiler chicken, Layer chicken, Identification, Serotyping

Salmonella spp. is a Gram-negative intracellular enteric bacteria that is important to public health (Li et al 2018) and is a major zoonotic pathogen that causes illness in both humans and animals, adding to being the most common bacterial foodborne illness in both developed and developing countries (Wibisono et al 2020). Typhoidal and non-typhoidal salmonellosis are caused by *Salmonella*, and millions of these cases occur annually, resulting in substantial economic losses and even human death (Luvsansharav et al 2020). The majority of cases of non-typhoidal *Salmonella* (NTS) illness are linked to infected animal-based foods, notably poultry meat and, in some circumstances, vegetables (Pandey and Goud 2021). *Salmonella* infections are important in the poultry production industry as well as in humans. Salmonellosis in poultry is an important disease that seriously hinders the development of the poultry industry (Sarker et al 2021). It causes reduced growth and fatality in chickens (Jazi 2019). Every year, *Salmonella* infection causes not only reduced performance of poultry production and even death, but also contaminate the human food chain, resulting in significant economic losses in the poultry sector as well as posing a threat to public health (Sylejmani et al 2016). Despite that chickens are typically considered as asymptomatic carriers who shed the germs in their feces and are significant reservoirs of bacteria. Infected birds can serve as a vehicle for disease transmission (Sylejmani et al 2016). Infection may be contracted through both direct and indirect contact with animals. Indirect transmission might result due to contact with contaminated things around poultry farms or with the environment around (Wibisono et al 2020). Chicks can become infected with *Salmonella* spp. by vertical transmission from infected parents or horizontal transmission from hatcheries, cloacal infection, or transfer through feed and equipment (Al Mamun et al 2017). Antimicrobial resistance to *Salmonella* has been a serious public health problem across the world (Sarker et al 2021). Resistance to antimicrobials by pathogenic bacteria is a global public health problem, particularly in developing countries (Akbar and Anal 2015, Rahman et al 2018). The primary determinants for the occurrence of multidrug-resistant pathogenic bacteria are the outcomes of unwise use of antimicrobial drugs to reduce bacterial infection or as a growth booster in poultry production (Akbar and Anal 2015, Rahman et al 2016). Therefore, the management of *Salmonella* infections with standard treatments is extremely challenging because of the occurrence of multidrug-resistant *Salmonella* isolates (Nair et al 2018).

Finally, control measures, such as those contained in National Control Plans, have been undertaken in the EU to minimize the prevalence of salmonellosis and other

foodborne illnesses in poultry production due to the involvement of poultry in the spread of *Salmonella* spp. (Sibanda et al 2018). Despite numerous preventative and control methods, such as drug and vaccine use as well as eradication campaigns, *Salmonella* infection is still one of the most serious concerns globally. From what was mentioned above, *Salmonella* continues to cause major economic losses in many countries and consumes a significant amount of resources in other countries for testing and control efforts. In Iraq, this bacterium still poses a significant threat to poultry flocks. Therefore, the present study aimed at investigating the possible sources of *Salmonella* occurrence in five chicken farms in Karbala, Iraq, either in chicken themselves or their feed and water.

MATERIAL AND METHODS

Specimens' collection: A total of 289 samples were collected from two different locations in the holy Karbala governorate, Iraq, including: Al-Husseinia and Al-Zubeilia during the period from August till November 2020. *Salmonella* spp. was isolated from three different types of samples, including: cloaca cotton swabs, water and feed (Table 1). All samples were treated aseptically, in which 1 gm of chicken feed sample was individually inoculated in a test tube containing 9 ml of peptone water and incubated at 37°C for approximately 18-24 hr. Cotton swabs were inoculated into 10 ml of peptone water, water samples taken from the same fields were centrifuged and 1 ml of the sediment was moved to another test tube containing 10 ml of tetrathionate broth (TTB) and incubated at 37°C for 18-24 hr.

Bacterial isolation and identification: For bacterial isolation, 1 ml of peptone water medium already inoculated with a sample was transferred to 10 ml of tetrathionate broth (TTB), which inhibited the growth of all bacteria except for *Salmonella*, and then the medium was incubated at 37°C for 24 hr. Later, a loopful from the cultured enriched broth was streaked onto plates of MacConkey, SS, XLD, and Brilliant green agars, and incubated at 37ºC for 24 hr. Salmonella-Shigella (SS) agar, was used as a moderately selective and differential medium for the isolation, cultivation and differentiation of *Salmonella* spp. MacConkey's agar was used for the isolation of Gram-negative enteric bacteria and the differentiation of lactose fermenting from lactose non-fermenting bacteria (Jaffer 2013). Moreover, other *Salmonella* spp. were identified and differentiated by using HiCrome Salmonella agar.

On the basis of colony features, staining properties, and routine biochemical tests, organisms were isolated and initially identified. Afterwards, the bacterial growth was purified on Brian hart infusion agar to be used the next day for inoculating the commercial kits. Analytical profile index 20 for *Enterobacteriaceae* (API 20 E) kit was used for the detection of *Salmonella* spp and this was followed by serotype identification by 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). In addition, the Vitek2® system was used for confirmation of some isolates.

Identification with the API 20E system: The identification of the bacterial isolates by the API 20E system was done according to the procedure stated by the manufacture (BioMerieux). Twenty standard biochemical tests were determined by this system. To do this test, the bacterial suspension was prepared from the well-isolated colonies of the suspected isolates by using the API suspension medium, and the turbidity was adjusted to 0.5 McFarland tube ($1x10^8$ CFU/ml). By using a sterile Pasteur pipette, the bacterial

Table 1. Types, numbers and locations of samples from which *Salmonella* spp. were isolated

Location	Chicken age day^{-1}	No. samples	Type of samples	Specimen	Farm
Al-Husseinia	12-45	41 6 6	Cloaca cotton swabs Water Feed	Broiler chicken	A
Al-Husseinia	13-45	44 10 6	Cloacal cotton swabs Water Feed	Broiler chicken	B
Al-Husseinia	14-45	44 10 5	Cloacal cotton swabs Water Feed	Broiler chicken	C
Al-Zubeilia	12-47	44 10 5	Cloacal cotton swabs Water Feed	Layers chicken	D
Al-Zubeilia	13-47	44 10 4	Cloacal cotton swabs Water Feed	Layers chicken	E

suspension was transferred to the 20 micro tubes and inoculated according to the manufacturer's instructions. Then, after incubation at 37°C for 24 hr, the isolates were identified by matching the numerical coding of the API system.

Vitek2 diagnostic method (BCL identification card): Some isolates suspected to be *Salmonella* were identified by the automated Vitek2 system with its identification card at Imam Al-Hijjah Hospital, located in Karbala, Iraq. The 64-well card contained 43 colorimetric substrates for the phenotypic identification of bacterial species. For detecting of the bacterial identity using Vitek2, the isolate was plated onto XLD agar and incubated overnight at 37°C. The next day, a suspension of the organism was prepared in saline (0.45-0.50% NaCl) inside a polystyrene tube to a density equivalent to a McFarland tube number 0.5. The density was determined using a Vitek2 DensiChek spectrophotometer. Subsequently, the tube and the card were inserted into the Vitek2 cassette, and the card was auto-inoculated within the Vitek2 instrument via a vacuum-release method. The wells of the card were optically scanned and read each 15 min, with a total incubation time of approximately 8 hours.

Antimicrobial susceptibility test: Using the Vitek2 system, the antimicrobial resistance of 11 of the *Salmonella* isolates was examined against 21 antimicrobial agents according to the manufacturer's instructions. To determine the microbial resistance, the purified isolates were streaked onto nutrient broth and incubated overnight at 36°C. The antimicrobial agents included: Ampicillin, Cofotaxime, Ceftazidime, Cefepime, Ertapenem, Amikacin, Gentamicin, Ceftriaxone, Amoxicillin/Clavulanic acid, Piperacillin/Tazobactam, Imipenem, Meropenem, Ciprofloxacin, Cefazolin, Cefoxitin, Levofloxacin, Tigecycline, Fostomycin, Norfloxacin, Nitrofurantoin and Trimethoprim/Sulfamethoxazole.

Statistical analysis: A paleontological statistics software package for education and data analysis (PAST3) version 3.09 was used to analyze the data of this study. The findings were evaluated by using chi-square analysis, in addition, the probability P-value was estimated, in which values equal to or less than 0.05 were indicated as significant differences.

RESULTS AND DISCUSSION

Detection of *Salmonella* spp.

Routine bacteriological diagnosis: Figure 1 and 2 show detection of suspected *Salmonella* spp. on many different bacteriological media. In addition, biochemical tests were also indicative of this bacterium (Fig. 3).

Diagnosis by Api 20E: The Api 20E system identified the suspected isolates as *S. enterica* (Fig. 4). By comparing the API 20E system with the conventional biochemical tests, the first one was able to identify *Salmonella* isolates at rates of 84%, while the traditional tests showed identification rate of 76% (Ahmed and Khudor 2019).

Diagnosis using the Vitek2 system: Thirteen isolates of suspected *Salmonella* collected randomly from the five farms were confirmed by the Vitek2 system as *S. enterica* subsp. *enterica*. The diagnosis probabilities ranged from 97% to 99% (Table 1).

Serotyping of *Salmonella* isolates: Serology was able to confirm some of the isolates to the serotype level as *S. enterica* sub sp. *enterica* serotype Arizonae. *Salmonella* spp. may be present in 65% of individuals in a flock. Serotypes colonizing the gastrointestinal tract of poultry are variable, depending on the geographic location and the time of the year (Nidaullah et al 2017). *Salmonella* serovars are distributed differently in poultry across countries and areas. In Jordan, for example, *S. enteritidis* and *S. typhimurium* caused poultry infection rates of approximately 8% and 13%, respectively (Nisafi and Abdelaziz 2006).

Similarly, in Egypt, the most frequent serotypes isolated from retail stores and broiler chickens were *S. enteritidis* and *S. typhimurium* (Elkenany et al 2019). By contrast, Singh et al (2013) isolated *S. typhimurium* at a prevalence rate of 4.4% from cloaca samples of layer chickens. Moreover, a Japanese study found only *S. infantis*, *S. manhattan*, and *S. schwarzengrund* in broiler chicken cecal samples (Duc et al 2019). In comparison with a study performed in China, both *S. typhimurium* and *S. enteritidis* were the most common serotypes that constituted 15.3% and 69.8%, respectively (Zhu et al 2017). Despite serotyping is most widely used phenotypic method, it fails to provide correct information because of the complex serotyping scheme and lacking of comparison among various laboratories, thereby limiting its application to the reference laboratories only (Parmley et al 2013).

Incidence of *Salmonella* in different sources of samples: In this study, out of 289 collected samples, *Salmonella* spp. were isolated from 61 (21.1%) of the samples. In comparison with another study performed in Basra, Iraq Al-Abadi and Al-Mayah (2011) obtained 34 *Salmonella* spp. isolates from 370 samples with a prevalence rate of 9.2%. These isolation rates were higher than the rate of 5% assumed by the National *Salmonella* Control Program in 2004. Many other studies were performed in other countries, for example, the study of Choi et al (2014) Korea, in which *Salmonella* spp. were isolated from 195 out of 1214 (16.1%) samples collected from various stages of the integrated broiler production firm, such as broiler mother farms, broiler farms, broiler trucks, slaughterhouses, and retail chicken meat. There were significant differences in *Salmonella* spp. isolation rates

among different types of samples. In the present study, water was highly contaminated with *Salmonella* spp. with an isolation rate of 30.4%, followed by cloaca swabs (21.7%), compared with the feed samples where no contamination was reported at all. Concerning water contamination with *Salmonella* spp., these microorganisms have an important characteristic, which is their ability to grow and multiply outside the bodies of living host organisms, this increases the chance of their survival in comparison with other organisms (Winfield and Groisman 2003). These microorganisms have been identified in approximately 29% of water samples collected from South India (Patel et al 2020). Poultry drinking water was found to be contaminated with *Salmonella* at an isolation rate of roughly 17.2% (Islam et al 2014), while the rate of contamination was 28.6% in the study of Al Mamun et al (2017). By contrast, other studies performed in Argentina and Algeria, for instance, found that the drinking water was either negative for *Salmonella* or carry very low rate of 2.18%, respectively (Soria et al 2017, Djeffal et al 2018).

In contrast to this study, cloaca swabs collected from chickens in South Africa showed very low *Salmonella* incidence rate of 3.2% (Mathole et al 2017). However, Karim et al (2017) isolated *Salmonella* spp. from 46% of cloaca samples. This result was also consistent with other studies (Akond et al 2012, Rahman et al 2018). Interestingly, Paul et al (2017) obtained extremely high prevalence (80%) in

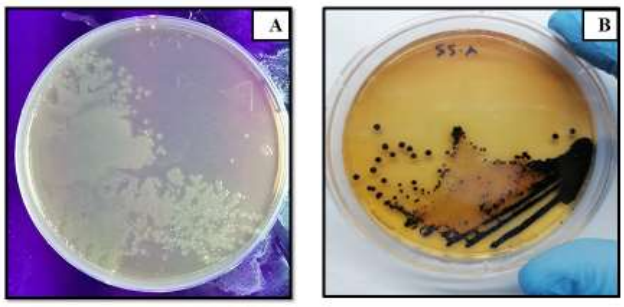

Fig. 1. Lactose non-fermenter bacteria suspected to be *Salmonella* spp. grown on MacConkey's agar (A), and SS agar with H_2S production (B)

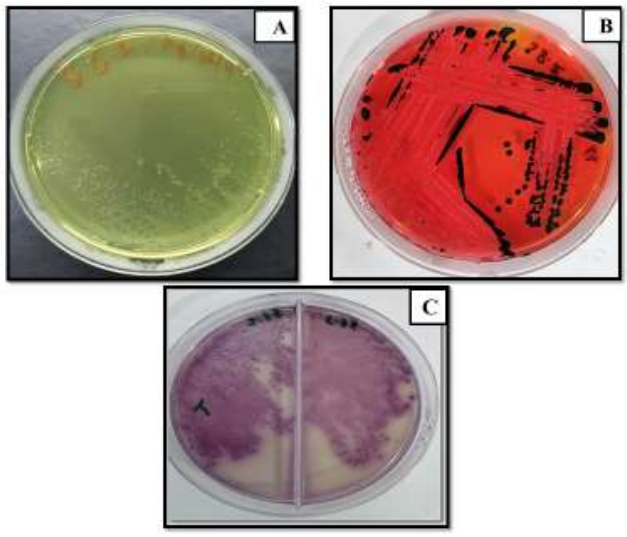

Fig. 2. Bacteria suspected to be *Salmonella* spp. grown on Brilliant green agar (A), XLD agar (B), and HiCrome Salmonella agar (C)

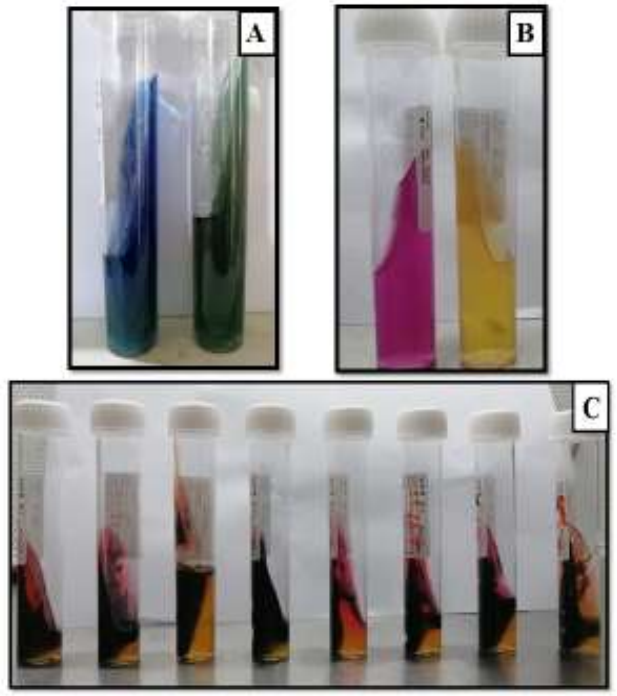

Fig. 3. Examples of biochemical tests inoculated with suspected *Salmonella* spp. A- Simmon's citrate agar: positive reaction (left tube). B- Urease test: negative result (right tube). C- Series of samples suspected to be *Salmonella* spp. inoculated into TSI agar

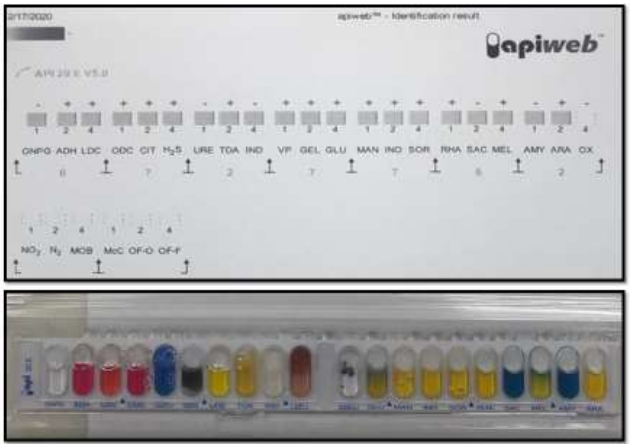

Fig. 4. Api 20E strip shows an example of a sample diagnosed as *S. enterica*

cloaca swabs. Sodagari et al (2020) reported *Salmonella* prevalence of 35% in the environment of 26 commercial layer farming flocks in Western Australia, and the greatest *Salmonella* recovery rates were seen in pooled fecal samples (54.5%). Similarly, another study conducted in China reported an isolation rate of 30% from different samples, approximately 48% of which were isolated from broiler cecal contents (Zhu et al 2017).

With respect to *Salmonella* spp. isolation from poultry feed, while Djeffal et al (2018) in this study found that all of the tested samples (n= 160) were Salmonella free, Kingsbury et al (2019) in New Zealand, found only one (3%) of 33 examined feed samples was positive for *Salmonella*. Despite the essential use of protein sources and feed additives in chicken ration for improving growth and performance, they have been implicated as reservoirs for a variety of *Salmonella* serovars (Almrsomi et al 2021). Contamination of feed components, such as animal protein sources, or the use of contaminated trucks for delivering feed, might have a big role in *Salmonella* outbreaks on farms (Hulaj et al 2016). The current study conflicts with that of Maqsood (2012), who stated that the major source of *Salmonella* infection in chicken was contaminated feed. Despite the fact that feed is generally recognized as a possible source of contamination, disease outbreaks linked to feed are extremely rare, and serotypes recovered from feed do not match those isolated from sick chicken flocks (Eguale 2018). Poultry feed pellets have been demonstrated to be effective to decrease the occurrence of *Salmonella* contamination (Boltz 2019).

Prevalence of *Salmonella* in broiler and layer chicken farms: *Salmonella* spp. is one of the diseases that affect broiler and layer hens at various ages and seasons. Among

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
Total	289	61	21.1
Statistics		Chi square (X^2) = 6.96 Degree of freedom = 2 P < 0.05	

Table 4. Prevalence of *Salmonella* spp. in broiler and layer farms

Loction	Breed	No. samples	No. positive samples	Incidence (%)
AL-Husseinia	Broiler	172	46	26.7
Al-Zubailia	Layer	117	15	12.8
Total	-	289	61	21.1
		Chi square (X^2) = 5.417		

Table 2. Identification of *Salmonella* spp. by the Vitek2 system

Farm code	Type of sample	Bacterial species	Probability	ID message confidence level
A	W	*S. enterica* subsp *enterica* *S. paratyphi* B	99%	Excellent identification
	C	*S. enterica* subsp *enterica* *S. paratyphi* B	99%	Excellent identification
B	W	*S. enterica* subsp *enterica*	97%	Excellent identification
	C	*S. enterica* subsp *enterica* *S. enteritidis* *S. paratyphi* B *S. paratyphi* C	99%	Excellent identification
C	W	*S. paratyphi* C *S. enteritidis*	99%	Excellent identification
	C	*S . enterica* subsp *enterica* *S. paratyphi* B	99%	Excellent identification
D	W	*S. enterica* subsp *enterica* *S. paratyphi* B	99%	Excellent identification
	C	*S. enterica* subsp *enterica* *S. paratyphi* B	99%	Excellent identification
E	W	*S. enteritidis* *S. paratyphi* B	99%	Excellent identification
	C	*S. enterica* subsp *enterica*	99%	Excellent identification

A, B and C: broiler farm; D and E: layer farm; W: water sample; C: cloaca swab

172 samples taken from broiler farms located in Al-Husseinia, 46 (26.7%) *Salmonella* spp. was isolated. However, lower than this rate (15 isolates, 12.8%) of *Salmonella* contamination was identified out of 117 samples collected from layer farms of Al-Zubeilia region (Table 3). Thus, this study shows that the infection rate is higher in broiler than laying chicken.

Effects of age and month on *Salmonella* infections in broiler and layer birds: Chicks in age of 30-37 days were more susceptible to infection (32.8%) followed by the age group of 21-27 days (30.8%). However, birds at the age of 45 days were less susceptible (10.5%). In case of layer birds, the age of 47 days witnessed the highest infection rate of 30.8% (Table 4). Similarly, a significant increment in infection rates occurred on day 35 (Marin and Lainez 2009). The last authors also observed that during the first 3 weeks of rearing, detection of these bacteria in feces increased. Furthermore, Djeffal et al (2018) found more *Salmonella* contamination occurred in the samples collected at the age of 3 weeks than those taken at the end of the production period. High infection rates of *Salmonella* at that age was due to the elimination of antimicrobial use as growth stimulants in poultry diets especially antimicrobials are known to alter bacterial flora in the chicken intestine (Al-Taie 2009). Conversely, other studies stated that the highest excretion of *Salmonella* occurred nearly 14 days of rearing due to immature immune system. As a result, detection of *Salmonella* decreased and persisted uncommon until the day of slaughter (Van Immerseel et al 2004). The least isolation rate occurred on day 45 in broiler chicken. Marin and Lainez (2009) mentioned that slight decrease in the infection rates was at the end of rearing. However, layer birds showed the opposite situation in the current study, in which high isolation rate was observed on day 47 (Table 4). Van Immerseel et al (2004) observed that the young chicks infected with *Salmonella* continue excretion of these bacteria for at least 18 weeks of rearing. Additionally, young birds, regardless of their age at *Salmonella* exposure, would persist in infection till 10 or 12 weeks, after the age of slaughtering broilers (Beal et al 2004).

The higher infection rates occurred in September than August or October in broiler flocks (Table 4). Generally, *Salmonella* infection is more common in the summer. The optimum conditions for *Salmonella* growth are warmer weather and unrefrigerated foods (CDC 2020). The salmonellae in chicken samples collected from China were more common during spring and summer than in autumn and winter (Li et al 2020). Regalado-Pineda et al (2020) in Mexico reported a significant high prevalence of these microorganisms during the spring, summer, autumn and winter. These findings emphasize the importance of health threats of *Salmonella* that need to be tackled urgently.

Antimicrobial susceptibility test: Multi-drug resistance (MDR) in *Salmonella* spp. is a growing worry across the world, especially in the developing nations where numerous antimicrobials are used indiscriminately at chicken farms to increase the production (Seo et al 2019). Antibiotic resistance can be developed as a result of the prophylactic use of several antimicrobials in chicken feed (Rajagopal and Mini 2013). The improper use of chemotherapeutic agents and growth promoters in poultry farms resulted in the emergence of MDR in *Salmonellae* (Magdy et al 2020). Against 21 antimicrobials tested in this study, *Salmonella* isolates collected from different farms showed MDR. Importantly, all the 11 isolates were 100% resistant to 4 antimicrobials, including: Ampicillin, Amikacin, Gentamicin, and Ciprofloxacin (Table 5).

In case of Ampicillin, other studies also documented *Salmonella* resistance to this antimicrobial agent, and the resistance increased significantly (98.4%) in 2019 compared with the 2017 report (87.8%) indicating that the farms have

Table 5. Effects of age and month on *Salmonella* infections in broiler and layer chicken

Breed	Age/days	No. examined samples	No. positive (%)	August	September	October
Broiler	12-14	18	4 (22.2)	1	3	0
	21-27	26	8 (30.8)	0	8	0
	30-37	67	22 (32.8)	0	15	7
	45	19	2 (10.5)	0	0	2
Layer	12-13	16	-	0	0	0
	20	20	5 (25)	0	5	0
	35	20	-	0	0	0
	43	18	2 (11.1)	0	0	2
	47	13	4 (30.8)	0	0	4
Total		217	47 (21.7)	1	31	15

Table 6. Susceptibility tests for *Salmonella* isolates to different antimicrobials

Antimicrobial	1 AW	2 AC	3 BW	4 BC	5 CW	6 CC	7 DW	8 DC	9 EW	10 EC	11 CC
Ampicillin	R	R	R	R	R	R	R	R	R	R	R
/Amoxicillin Clavulanic acid	I	I	I	I	R	I	R	I	I	I	S
/Piperacillin Tazobactam	S	S	S	S	I	S	S	I	S	S	S
Cofotaxime	S	S	S	S	S	S	S	S	I	I	S
Ceftazidime	S	S	S	S	S	S	S	S	S	S	S
Cefepime	S	S	S	S	S	S	S	S	S	S	S
Ertapenem	S	S	S	S	S	S	S	S	S	S	S
Imipenem	S	S	S	S	S	S	S	S	R*	S	S
Meropenem	S	S	S	S	S	S	S	S	I	I	S
Amikacin	R	R	R*	R*	R	R	R	R	R*	R*	R*
Gentamicin	R	R	R	R	R	R	R	R	R*	R*	R*
Ciprofloxacin	R	R	R	R	R	R	R	R	R	R	R
Cefazolin	/	/	/	/	/	/	/	/	R*	R*	/
Cefoxitin	/	/	/	/	/	/	/	/	R*	R*	/
Ceftriaxone	/	/	/	/	/	/	/	/	S	S	/
Levofloxacine	/	/	/	/	/	/	/	/	R	R	/
Tigecycline	/	/	/	/	/	/	/	/	S	S	/
Norfloxacin	R	R	R	R	R	R	R	R	S	I	R
Fostomycin	S	S	S	S	S	S	S	S	I	I	S
Nitrofurantoin	I	S	I	I	S	S	S	I	I	S	S
Trimethoprim/Sulfamethoxazole	R	R	R	R	R	R	R	R	S	S	S

A, B, C, D & E refer to the five farms; the 2nd C refers to cloaca sample; W refers to water sample. The numbers refer to: 1- *S. paratyphi* B, 2- *S.* paratyphi B, 3- *S. enterica* subsp *enterica*, 4- *S. enteritidis* or *S. paratyphi* B or C, 5- *S. paratyphi* C or *S. enteritidis*, 6- *S. paratyphi* B, 7- *S. paratyphi* B, 8- *S. paratyphi* B, 9- *S. enteritidis* or *S. paratyphi* B, 10- *S. enterica* subsp *enterica*, 11- *S. paratyphi* B; *: AES (Advanced Expert System) modified; R: Resistant, S: Susceptible; I: Intermediate with MIC within ±1 doubling dilution

had more applications of Ampicillin (Zhu et al 2017). A study conducted in Egypt reported that 95% of the isolates were resistant to Penicillin, 85% to Norfloxacin, and 75% to Gentamicin (Magdy et al 2020). In comparison with a previous study, all *Salmonella* isolates were resistant to Ampicillin, Kanamycin, Cefotaxime, Erythromycin, Streptomycin, Neomycin, Novobiocin and Spectinomycin (Shah and Korejo 2012). The isolates of the present study were 100% resistant to each of Gentamicin and Ciprofloxacin. *Salmonella* strains were highly sensitive to these antimicrobials in the research performed by Bahnass et al (2015) and Baran et al (2019)..

In comparison with the study of Yu et al (2021), partial simililarity was noticed, particularly, the sensitivity of the isolates to Imipenem; however, that study reported susceptibility of the isolates to each of Amikacin and Amoxicillin/Clavulanic acid, which contradict the data of this study. Here, Amikacin showed 100% resistance as mentioned above, whereas intermediate susceptibility was exerted by the combination Amoxicillin/Clavulanic acid towards 8 isolates, with 2 isolates were resistant to the same combination. Moreover, in the current study, 9 isolates were resistant to Norfloxacin (approximately 82% resistance, Table 5). This result is lower than that reported in China by Zhu et al (2017) where *Salmonella* isolates were found to be 99.5% resistant to Norfloxacin, respectively. This resistance could be due to the overuse of Norfloxacin in chicken farms in China compared to Iraq. Regarding Trimethoprim/Sulfamethoxazole used in the present study, this mixture did not inhibit the growth of 8 isolates (72.7% resistance). On the other hand, all of the isolates were susceptible to Ceftazidime, Cefepime, and Ertapenem. Furthermore, 10, 9, 9, and 6 isolates were inhibited by Imipenem, Meropenem, Fostomycin, and Nitrofurantoin (Table 5). Thus, controlling the use of growth promoters and antimicrobial drugs in animals is critical to prevent the development of resistant strains.

CONCLUSIONS

Different *Salmonella* spp. were isolated from cloaca and water samples, while all chicken feed samples were completely free of *Salmonella* contamination in five farms in Karbala province. The most prevalent *Salmonella* spp. isolated was *S. paratyphi* B according to Vitek2. *S. enteritidis* and *S. diarizonae* were identified in some samples. Identification of *Salmonella* spp. by Api 20E, serotyping, or by Vitek2 showed inconsistent and rather inaccurate results, particularly, at the serotype level. Therefore, these tests may be inefficient and inappropriate for *Salmonella* detection in general. The emergence of different types of *Salmonella* contaminating water samples collected from the five chicken farms excludes the possibility of the epidemic spread among these farms, especially they are located in different regions within the same governorate. A significant degree of antimicrobial profile similarity was exhibited by *Salmonella enterica* serotypes. All *Salmonella* spp. isolated were 100% resistant to four antimicrobials, including: Ampicillin, Amikacin, Gentamicin, and Ciprofloxacin. By contrast, all of the isolates were susceptible to Ceftazidime, Cefepime, and Ertapenem. Therefore, extensive study need to be done to explore the prevalent *Salmonella* serotypes circulating in different chicken farms throughout the Iraqi governorates.

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