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RESEARCH ARTICLE

Effect of Age and Season on Salmonella Infection in Broiler Chickens with Special Reference to Virulence and Antibiotic Resistance Genes

Heba Roshdy[1], Sahar R. Mohamed[1], Hossam S.H. Elsebaey[2] and Ghada O. El-Demerdash[3]
[1]Reference Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, Ministry of Agriculture, P.O. Box 246 – Dokki, 12618 – Giza, Egypt
[2]Animal Health Research Institute, Damanhur branch, Agriculture Researches, Egypt
[3]Animal Health Research Institute, Fayoum branch, Agriculture Researches Center, Egypt
* Corresponding author: roshdy2019 @yahoo.com

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Abstract

Salmonella species are causing high morbidity and mortality rates in broiler chickens and other birds particularly young ages resulting in great economic losses. The present study was performed to detect the effect of age and season on isolation rates of *Salmonella* species from 120 examined broiler chickens with different ages (1-7 days- old and more than 7days -old) in different seasons (summer, winter, autumn and spring). The highest isolation rates of *Salmonella* species were obtained from broilers of 1-7 days – old in summer (40%) followed by broilers of more than 7 days old in summer (33.3%). The increased isolation rates of *Salmonella* species was detected from liver of broilers of both ages (1-7 and more than 7 days old). Seventeen *Salmonella* isolates were obtained in this study from both ages. Serotyping of these *Salmonella* isolates revealed *S.* Enteritidis (5 isolates), *S.* Typhimurium (3 isolates), *S.* Infantis, *S.* Bargny, *S.* Newport, *S.* Magherafelt (2 isolates) and finally *S.* Apeyeme in (1 isolate). The most frequent resistance phenotypes were tetracycline (70%-71.4%), from 1 -7 days - old and more than 7 days- old chicks and finally ampicillin (60%, 57.1%). From the result obtained by using VITEK2 system, the highest resistance was recorded against Tetracycline in all *Salmonella* species. All *Salmonella* isolates showed -associated genes. The *inv*A gene was identified in all *Salmonella* serovars isolated from chickens of the two age groups, whereas, *avr*A gene was detected in all the isolates except *S.* Magherafelt recovered from the age group of more than 7 days-old birds . The tetracycline resistance gene (tetA) was identified in all the examined isolates.

Keywords: *Salmonella* species -virulence genes - VITEK2 system -Antimicrobial resistance genes.

Introduction

Salmonellosis is one of the most common infectious diseases for poultry and humans worldwide, the genus *Salmonella* is divided into, *Salmonella enterica* and *Salmonella bongori* species. *Salmonella enterica* is comprised of six subspecies [1]. Poultry is considered one of the main reservoirs that has a role in the spread of *Salmonellosis* [2]. International trade of poultry is considered as one of the factors affects spread of *Salmonellosis*, in this case it acts as a portable reservoir for infections such as *Salmonella* [3]. *Salmonellosis* is transmitted to humans through food and the significance of *Salmonella* species as causes of human and animal disease has increased in the recent years [4]. *Salmonella* is ubiquitous in the environment; birds can be infected and therefore contaminate poultry meat [5]. The incidence of *Salmonellae* in the closed system farms was higher than the open system farms [6]. The birds at the ages of the first week are exposed to infection, unlike birds at the age of 3-6 weeks, they are less susceptible to infection due to more rapid and higher antibody response [7]. The best overall organs to culture for *S.* Enteritidis are the spleen, liver and yolk sac, from 1-day-old infected chickens and fewer organs of 7-days-old infected chicks until 42 days of ages [8]. Factors that affect the

***Corresponding author e-mail: (roshdy2019 @yahoo.com), Reference Laboratory for Veterinary Quality Control on Poultry Production, Animal Health.**

survival and growth of *Salmonella* in the environment include the warm temperatures and humidity [9]. The increased spread of multiple drug-resistant Salmonella spp. is due to random use of antibiotics, which in turn led to increased severity of the disease [10]. The widespread overuse of antimicrobial agents in food animal production as growth has led to the development of antimicrobial resistant pathogens such as *Salmonella* that has emerged as a major public health implication [11]. The Vitek2 automated system has been developed for the identification of Gram-negative strains in ordinary clinical microbiology because it is fast and rapid. The Vitek2 system has many advantages, first, it can avoid environmental contamination or cross-contamination as it is a closed system, second, Vitek2 system can detect specimen card if it is misplaced on its cartridge. So, during its operation it owns a dependable recheck system. Third, it is easy to prepare and load bacterial specimens by laboratory staff and the Vitek2 system can detect dozens of specimens automatically at the same time. Therefore, Vitek2 system gave reliable, rapid and highly reproducible results [12]. Virulence genes are important for *Salmonella* pathogenesis; such genes are located on different genome elements such as plasmids, chromosome and *Salmonella* pathogenicity islands [13]. The *inv*A gene is present only in *Salmonella* species and is used in genetic diagnosis; this explains the high prevalence used of *inv*A and *avr*A virulence genes in *Salmonella* serovars [14-15]. Tetracycline resistance gene has usually been related to resistance in human and animal *Salmonella* isolates [16]. This study was designed to detect the effect of age and season on the isolation rate of *Salmonella* species from broiler chickens. Moreover, the presence of virulence genes and tetracycline resistance gene in the identified isolates was also investigated.

Materials and Methods

Samples

A Total of 120 broiler chickens (live diseased and freshly dead) were sampled during the study. The ages of these chickens were 1-7 days old and more than 7days old. The samples were collected in different seasons (summer, winter, autumn and spring during 2019) from internal organs (heart, liver, lungs, yolk sac and intestine). The samples were collected under aseptic conditions and safety precautions to prevent cross contamination according to Middleton *et al.* [17].

Bacteriological examination

Isolation and identification of *Salmonella* species were performed according to ISO 6579-1 [18]. Samples were weighed and suspended in buffer peptone water (BPW, Oxoid®) and incubated at 37°C for 16-18 h, then 0.1 ml of the incubated pre-enrichment medium were transferred to modified semisolid Rappaport-Vassiliadis medium (MSRV, LABM®) and incubated at 41.5°C for 24 h, as well as 1 ml was transferred to Muller-Kauffmann tetrathionate-novobiocin broth (MKTTn, LabM®) and incubated aerobically then streaked onto Xylose Lysine Deoxycholate agar (XLD ,LabM®) and Hektoen Enteric (HE agar). The plates were incubated at 37°C for 24 h aerobically. The typical and selected colonies were identified by biochemical tests (urea agar, triple sugar iron, lysin iron, indole production, methyl red test, Voges-Proskauer and Simmons citrate agar (Oxoid®). The isolated *Salmonella* species were serotyped according to ISO 6579-3 Using Salmonella antiserum (Sifin Co., Japan®) [19]. Reading of *Salmonella* species by Kauffman – White scheme was according to Grimont and Weil [20].

In-Vitro antibiotic sensitivity test for Salmonella isolates

The antibiogram of *Salmonella* isolates was done by the Kirby–Bauer disc-diffusion method according to Koneman *et al.* [21] against (11) antimicrobials (Oxoid®), and the zones of inhibition were measured and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines **[**22]. The used antibiotics were Ampicillin (Amp, 10µg), Chloramphenicol (C^{30}, 30µg), Ciprofloxacin (CF^{5}, 5µg), Gentamicin (G^{10}, 10µg) , Nalidixic acid (NA^{30}, 30µg), Nitrofurantoin (F^{300}, 300µg), Norfloxacin (NX^{10}, 10µg),Trimethoprim-sulfamethoxazole (SXT, 1.25-23.75µg), Tetracycline (T^{30},30µg), levofloxacin(LEV,

5µg) and Ceftriaxone (CRO, 30 µg). Moreover, one representative isolate from each serotype (seven serotype) was tested against different antibiotics by the Vitek2 system, according to Chatzigeorgiou *et al.* [23].

Detection of virulence and antibiotic resistance genes

The DNA from 17 Salmonella isolates was performed by the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) according to the manufacturer's recommendations. Briefly, 200 µl of the sample suspension were incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56°C for 10 min. After incubation, 200 µl of 100% ethanol were added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. The nucleic acid was eluted with 100 µl of elution buffer provided in the kit. Oligonucleotide Primers were supplied from Metabion (Germany). The sequences of the primers used to amplify the *inv*A gene are F: (GTGAAATTATC GCCACG TTCGGG CAA), R: (TCATCGCA CCGTCAAAGG AACC) and they produced and amplicon of 284 bp [24], those for avrA gene are F: (CCTGTATTGTTGAGCGTCTGG), R: (AGAAGAG CTTCGTTGAATGTCC) and they amplified a product of 422 bp [25], while, the primers for the amplification of tetA gene are F: (GGTTCACTCGAACGACGTCA), R: (CTGTCCGACAAGTTGCATGA) and they produced 576 bp amplicon [26]. The reaction was performed in 25- µl volume containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentrations, 5.5 µl of water, and 5 µl of DNA template. The reaction was performed in an applied bio system 2720 thermal cycler.

Analysis of the PCR Products

The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of the conventional PCR products were loaded in each gel slot. Generuler 100 bp ladder (Fermentas, Thermo, Germany) and gel pilot 100 bp Ladder (Qiagen, Gmbh, Germany) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Results

Prevalence of Salmonella species recovered from examined chickens in different ages and seasons

Table (1) illustrates the prevalence of *Salmonella* spp. in the examined chickens at the different ages and seasons. The highest prevalence of *Salmonella* species was reported in summer and one day old chicks (40%), followed by in summer, more than 7days old chicks (33.3%). Meanwhile, the isolation rates in winter from one day and more than 7 days-old chickens were 20% and13.3%, respectively. On the other hand, dusting autumn, the isolation rate from one day - old chicks was 6.6%.

Tables 1: Incidence of *Salmonella* species recovered from examined broiler chickens in different ages and seasons.

Age	Season	No. of examined chickens	No positive (%)
1-7 days old	Summer	15	6 (40%)
	Winter	15	3 (20%)
	Autumn	15	1 (6.6%)
	Spring	15	0
More than 7 days old	Summer	15	5 (33.3%)
	Winter	15	2 (13.3%)
	Autumn	15	0
	Spring	15	0
Total	-	120	17 (14.1%)

* Percentage according to total number of the examined.

Distribution of Salmonella species isolated from the examined broiler chicken organs

The examination of liver, heart, yolk sac and intestine from 1-7 days old broiler revealed the isolation of 7, 0, 1 and 2 *Salmonella* isolates, respectively. While, 4, 1 and 2 *Salmonella* were isolated from liver, heart and intestine of more than 7 days-old chickens, respectively. The highest isolation rates were from liver of both 1-7 days-old and more than 7 days –old with an overall percentage of 64.7% (11 out of 17 isolates). The clinical examination of these chickens showed dehydration, diarrhea. The postmortem lesions of freshly euthanized chicks from which salmonella were isolated are signs of enlarged liver, unabsorbed yolk sac, turbid yellow color fluids in the peritoneal cavity and the lesion of old chicken, white necrotic foci in the liver, turbid yellow color fluids in the peritoneal cavity and enteritis.

Serotyping of Salmonella isolates from broiler chickens

A total of 17 *Salmonella* isolates were recovered from the examined birds. Ten and 7 isolates were isolated from 1 -7 days - old and more than 7 days- old chicks, respectively .The serotypes identified from 1-7 days old vs more than 7 days - old broilers were *S.* Enteritidis (3 vs 2), *S.* Typhimurium (2 vs 1), *S.* Infantis (2 vs 0), *S.* Apeyeme (1 vs 0), *S.* Bargny (2 vs 0), *S.* Newport (0 vs 2) and *S.* Magherafelt (0 vs 2).

Antimicrobial sensitivity test

Variable degrees of resistance of the isolates are shown in Table 2. The most frequent resistance phenotypes were against tetracycline (70%-71.4%) for the strains isolated from birds of the age group from 1 -7 days - old and more than 7 days- old chicks, ampicillin (60%, 57.1%) and finally, trimethoprim-sulfamethoxazole and ampicillin (57.1%) in the birds more than 7 days- old broiler chickens. Detection of antibiotic sensitivity of *Salmonella* serotypes by VITEK2 system revealed that all the examined serovars were resistant to tetracycline (Table 3).

Table 2: Results of antimicrobial resistance of salmonella isolates from 1- 7days-old (N=10) and more than 7 days-old (N=.7) broiler chickens:

Antibiotic	Resistance		Intermediate		Sensitivity	
	No. of isolates		No. of isolates		No. of isolates	
	1-7 days old	More than 7 days old	1-7 days old	More than 7 days old	1-7 days old	More than 7 days old
Ampicilin	6(60%)	4(57.1%)	2(20%)	3(42.8%)	2(20%)	-
Chloramphenico	2(20%)	1(14.3%)	2(20%)	1(14.3%)	6(60%)	5(71.4%)
Ciprofloxacin	3(30%)	1(14.3%)	1(10%)	2(28.6%)	6(60%)	4(57.1%)
Gentamicin	4(40%)	3(42.8%)	2(20%)	2(28.6%)	4(40%)	2(28.6%)
Nalidixic acid	3(30%)	3(42.8%)	4(40%)	2(28.6%)	3(30%)	2(28.6%)
Nitrofurantoin	3(30%)	3(42.8%)	3(30%)	1(14.3%)	4(40%)	3(42.8%)
Norfloxacin	3(30%)	3(42.8%)	2(20%)	3(42.8%)	5(50%)	1(14.3%)
Ceftriaxone	2(20%)	2(28.6%)	4(40%)	2(28.6%)	4(40%)	3(42.8%)
Trimethoprim-sulfamethoxazole	4(40%)	4(57.1%)	2(20%)	-	4(40%)	3(42.8%)
Tetracycline	7(70%)	5(71.4%)	2(20%)	1(14.3%)	1(10%)	1(14.3%)
Levofloxacin	2(20%)	1(14.3%)	2(20%)	1(14.3%)	6(60%)	5(71.4%)

*Percentage calculated according to total number of the examined samples.

Table 3: Detection of antibiotic sensitivity of salmonella serotypes using VITEK2 system

AB	S.E		S.T		S.I		S.A		S.B		S.N		S.M	
	MIC	I.P	MIC	I.p	MIC	I.p	MIC	I.p	MIC	I.p	MIC	I.p	MIC	I.p
Gentimycin	≥16	I	≤1	R	≤1	R	≤1	R	≤4	S	≤4	S	≤1	R
Ciprofloxacin	≥32	R	≤2	S	≤2	S	≥32	R	≤2	S	≤2	S	≤2	S
Levofloxacine	≤0.12	S	≤0.12	S	≤0.12	S	≤0.12	S	≤0.12	S	≤0.12	S	≤0.12	S
Meropenem	≤o.25	S	≤o.25	S	≤o.25	S	≤o.25	S	≤o.25	S	≤o.25	S	≤o.25	S
Nitrofurantoin	≤8	S	≤8	S	16	I	16	I	≤8	S	≥32	R	≥32	R
Ampicillin	≤2	S	≤2	S	≥o.5	R	≤2	S	≥o.5	R	≥o.5	R	≤2	S
Trimethoprim-sulfamethoxazole	≤20	S	≤20	S	≤20	S	≥80	R	≤20	S	≥80	R	≥80	R
Norfloxacin	≤1	S	≥4	R	≥4	R	≥4	R	2	I	2	I	≤1	S
Tetracycline	≥128	R	≥128	R	≥128	R	≥128	R	≥128	R	≥128	R	≥128	R
Ceftriaxone	≤1	S	≤1	S	≤1	S	≥8	R	4	I	≤1	S	4	I
Cefeprime	≤1	S	≤1	S	≤1	S	≤1	S	≤1	S	≥16	R	≤1	S

AB: antibiotic, S.E: *Salmonella* Enteritidis, S.T: *Salmonella* Typhimurium, S.I: *Salmonella* Infantis, S.: A: *Salmonella* Apeyeme, S.B: *Salmonella* Bargny, S.N: *Salmonella* Newport, S.M: *Salmonella*. Magherafelt, M: minimum inhibitory concentration (MIC); I.P interpretation. R: Resistance, I: Intermediate, S: Sensitivity.

Molecular identification of virulence and resistance genes

The *inv*A gene was identified in all *Salmonella* serovars isolated from chickens of the two age groups, whereas, *avr*A gene was detected in all the isolates except *S.* Magherafelt recovered from the age group of more than 7 days-old birds (Table 4 and Figure 1A,B). The tetracycline resistance gene (*tet*A) was identified in all the examined isolates (Table 4 and Figure1 C).

Table 4: Detection of virulence and antibiotic resistance genes of salmonella isolates by PCR

Serotype	No. of isolate	Virulence genes						Antibiotic resistance genes			
		1-7 days old			More than 7 days old			1-7 days old		More than 7 days old	
		No	*inv*A	*avr*A	No	*inv*A	*avr*A	No	*tet*A	No	*tet*A
***Salmonella* Enteritidis**	5	**3**	3/3 100%	3/3 100%	**2**	2/2 100%	2/2 100%	**3**	3/3 100%	**2**	2/2 100%
***Salmonella* Typhimurium**	3	**2**	2/2 100%	2/2 100%	**1**	1/1 100%	1/1 100%	**2**	2/2 100%	**1**	1/1 100%
***Salmonella* Infantis**	2	**2**	2/2 100%	2/2 100%	-	-	-	**2**	2/2 100%	-	-
***Salmonella* Apeyeme**	1	**1**	1/1 100%	1/1 100%	-	-	-	**1**	1/1 100%	-	-
***Salmonella* Bargny**	2	**2**	2/2 100%	2/2 100%	-	-	-	**2**	2/2 100%	-	-
***Salmonella* Newport**	2	-	-	-	**2**	2/2 100%	2/2 100%	-	-	**2**	2/2 100%
***Salmonella.* Magherafelt**	2	-	-	-	**2**	2/2 100%	0/2 0	-	-	**2**	2/2 100%
Total	17	**10**	-	-	**7**	-	-	**10**	-	**7**	-

*Number of examined isolates from each age group.

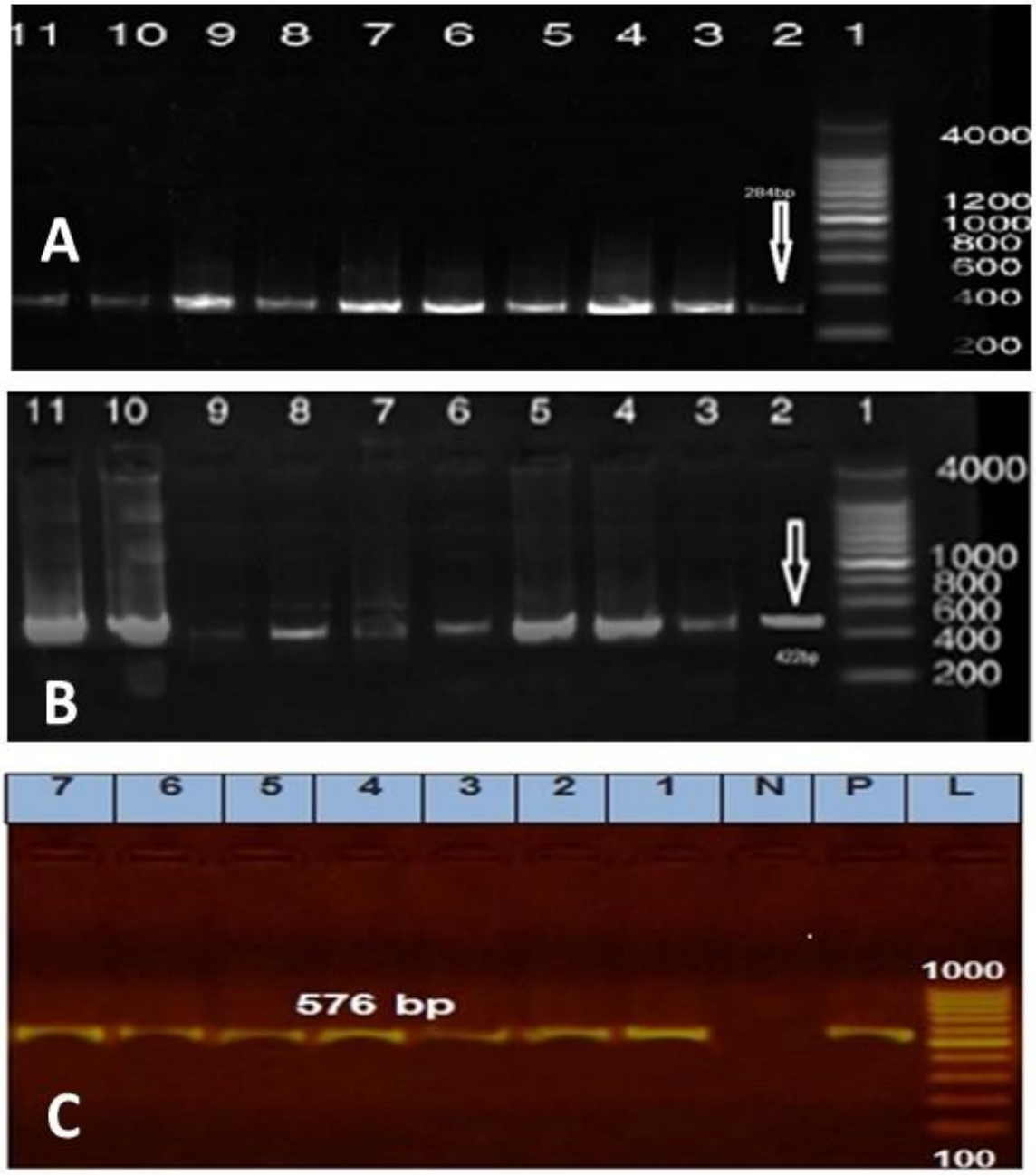

Figure 1: A: Agarose gel electrophoresis showing positive amplification of (284bp) fragments using PCR was performed with primer specific for *inv*A gene. Lane 1: 100 bp ladder, Lane 2: positive control and lanes 3-11: Positive isolates. B: Agarose gel electrophoresis showing positive amplification of 422 bp fragments using PCR was performed with primer specific for *avr*A gene. Lane 1: 100 bp ladder, Lane 2: positive control and lanes 3-11: Positive isolates. C: Agarose gel electrophoresis showing positive amplification of 576 bp fragments using PCR was performed with primer specific for *tet*A gene. L: 100 bp ladder, P: positive control, N: negative control, lanes 1-7: Positive isolates.

Discussion

Salmonellosis is an important cause of food borne diseases in humans. This study highlights the prevalence rate of *Salmonella* serotypes in broilers as well as demonstrates the presence of virulence and antibiotic resistance genes [27]. The findings of the present study demonstrated the persistence of different *Salmonella* serotypes in an integrated broiler supply that alarms for a potential public health hazard. The frequency reported in the present study was recently reported in Egypt [28].

The prevalence of *Salmonella* spp. in different seasons in the present study was higher during summer as opposed to rainy

season. Temperature may be a major factor for the survival and proliferations of *Salmonella*, warm temperatures provide suitable environment for the growth of *Salmonella* [29,30]. The highest prevalence of *Salmonella* in broiler chickens recorded in the current study might be due to overcrowding and improper sanitary measures of the farms. The results of this study indicated that broilers could be an important reservoir of *Salmonella* spp., this is in accordance with Li *et al*. [31].

The higher percentage of isolation from the internal organs from liver of both 1-7 days-old and more than 7 days –old birds was with an overall percentage of 64.7% in our study, followed by intestine (23.5 %) in both 1-7 days old and more than 7days old birds. These results reveal that the birds were infected at more than one week of ages, due to the production of more rapid and higher antibody response than those infected at less than one week of age. These results are in contrary to other studies [32,33]. However, other studies reported higher percentage of isolation from from yolk sacs (10%) then from livers (9%) and from intestines (9%) [34,35].

Different Salmonella serotypes were identified from 1-7 days old chicken (*S*. Enteritidis, *S*. Typhimurium, *S*. infantis, *S*. apeyeme and *S*. Bargny) and from birds of more than 7 days old (*S*. Enteritidis, *S*. Typhimurium, *S***.** Newport and *S*. Magherafelt). These results are in agreement with other studies [36-37]. These findings support the assumption that there is a difference in the occurrence of some salmonella types at young ages versus older ages [38-40]. The most commonly isolated serotype from different organs was *S*. Enteritidis which is consistent with results recorded in Egypt [41].

During recent years some bacteria have shown full or partial resistance to different antibiotics. This increasingly global phenomenon, called antimicrobial resistance, is a rising concern in both public and animals health, Tetracyclines were discovered in the 1940s and exhibited activity against a wide range of microorganisms including gram-positive and gram-negative bacteria, chlamydiae, mycoplasmas, rickettsiae, and protozoan parasites. They are inexpensive antibiotics, which have been used extensively in the prophylaxis and therapy of human and animal infections and also at subtherapeutic levels in animal feed as growth promoters. [42], in the present study chicken showing high resistance to be recorded against Tetracycline, [43,44]. Higher rates of resistance were observed to extend spectrum penicillin, these antimicrobial resistances of *Salmonella* spp. indicated that all isolated *Salmonella* strains exhibited absolute resistance (100%) against Trimethoprim-Sulfamethoxazole, indicating the limited therapeutic value of this antibiotic to poultry [45], In the current study chicks showed high resistance to Clindamycin and Lincomycin 100% and variable resistance to Trimethoprim-sulfamethoxazole 50% then Tetracycline, Ampicillin and Ciprofloxacin 33%. [46-48].

The VITEK2 system was approved by many laboratories worldwide for the identification of Gram negative or positive strains in ordinary clinical microbiology and for the determination of antibiotic sensitivity because it is fast and rapid [49]. *Salmonella* spp. isolates were confirmed resistant by the VITEK2 System. In accordance, high resistance of *Salmonella* isolates against nalidixic acid and tetracycline was reported [50].

Our study examined *Salmonella* isolates for the detection of *inv*A and *avr*A genes by conventional PCR. The *inv*A gene was detected in 100% of *Salmonella* isolates in both age groups. The gene *avr*A was also detected in all the isolates expect *S*. Maherafelt. The *inv*A gene encodes a protein structure in the bacterial membrane which is essential to invade the epithelial cells of intestine. Several studies detected *inv*A gene in 100% of *Salmonella* serovars [51- 53]. These findings suggested that any changes in the proteins arrangement, such as avrA, may cause changes in the capability of this serovar to adapt to new hosts and, consequently, the emergence of novel virulent strains [54,55]. However, their absence in some isolates, suggests that they are not essential for infectivity of *Salmonella* spp. in the human host.

Antibiotic resistance transmitted between bacteria through mobile genetic elements such

as plasmids, transposons and integrons resulting in healthy animals become carriers for these antibiotic resistant bacteria, animal fecal matter acts as vehicle for transmission of antibiotic resistance to human [56]. The results of this study showed high antimicrobial resistance in broiler chicken to *tet*A gene 100%, in all salmonella strains [57-59], recorded that *tet* class genes are considered the most common types in gram negative bacteria, also *tetA* and *tet*B genes are located inside non-conjugative transposons which are important way for the horizontal transfer of antibiotic resistance.

Conclusion

The result presented in this study indicated the high prevalence of *Salmonella* spp. in most organs from 1-day-old infected chickens and fewer organs of 7-days-old. Most birds are affected in the summer, increasing resistance to several antibiotics, such as the resistance to tetracycline, which may be transferred to humans. Monitoring antibiotic resistance and associated genes is essential to study the epidemiology link between poultry and humans. Biosecurity in the poultry farms should be the first line of defense against infectious diseases.

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الملخص العربى

تاثير العمر والموسم علي عدوي السالمونيلا في دجاج التسمين مع اشارة خاصه الي جينات الضراوة وجينات المقاومة للمضادات الحيوية

هبة رشدي[1] -سحر رشدي محمد[1] – حسام السباعي[2] –غادة عمر الدمرداش[3]

[1] المعمل المرجعى للرقابة البيطرية على الإنتاج الداجنى. معهد بحوث صحة الحيوان. شارع نادى الصيد ص.ب ٢٤٦- الدقى-١٢٦١٨- الجيزة- مصر

[2] معهد بحوث صحة الحيوان ، فرع دمنهور ، مركز البحوث الزراعية ، م

[3] معهد بحوث صحة الحيوان ، فرع الفيوم ، مركز البحوث الزراعية ، مصر

السالمونيلا هي بكتيريا تسبب ارتفاع معدلات المراضة والوفيات في دجاج التسمين والطيور الأخرى بشكل خاص ، مما يؤدي إلى خسائر اقتصادية كبيرة. أجريت هذه الدراسة للكشف عن تأثير العمر والموسم على معدل عزل السالمونيلا من 120 جاجه من مراحل عمريه مختلفة (1-7 أيام وأكثر من 7 أيام) في الصيف والشتاء والخريف والربيع. تم الحصول على أعلى معدل عزل للسالمونيلا من دجاج التسمين عمر 1-7 أيام في الصيف (40٪) يليه دجاج التسمين أكثر من 7 أيام في الصيف (33.3٪). ان اعلي نسبة من عزلات السالمونيلا تم تسجيلها في كبدفراخ التسمين عند كلا العمرين (من 1-7 أيام و الاعمار الاكثر من7 ايام). ولقد تم الحصول علي 17 عزلة من السالمونيلا في هذه الدراسة في كلا العمرين. التنميط المصلي للسالمونيلا المعزولة ذات الصلة *S.* Enteritidis (5 عزلات) ، تليها *S.* Typhimurium (3 عزلات) ، *S.* Infantis، *S.* Bargny، *S.* Newport, S. Magherafelt في (عزلين) وأخيراً *S.* A peyeme في (1 عزلة). من الواضح أنه تم تسجيل أعلى مقاومة ضد التتراسيكلين (70%-71.4%) من اعمار 1-7 أيام و الاعمار الاكثر من7 ايام والأمبيسلين (57.1%-60%). من النتيجة التي تم الحصول عليها باستخدام نظام VITEK2 ، واتضح تسجيل أعلى مقاومة ضد التتراسيكلين في جميع السالمونيلا.وأظهرت جميع معزولات السالمونيلا جينات مرتبطة.حيث تم التعرف على جين invA بالسالمونيلا المعزولة من الدجاج بالمجموعتين العمريتين ، بينما تم الكشف عن جين avrA في جميع العترات باستثناء S. Magherafelt الذي تم عزله من المجموعة العمرية لأكثر من 7 أيام من الطيور. اضافة الى تحديد جين مقاومة التتراسيكلين (tetA) في جميع العزلات السالمونيلا .