
Prevalence of Different *Salmonella* serovars in Broiler Farms in Sharkia Governorate

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Abstract:

Bacteriological examination of 300 samples from broiler internal organs (liver, cecum, unabsorbed yolk sac) from one day old chicks suffering from omphalitis and respiratory disorders after hatching, revealed 37 *Salmonella* isolates 12.3%. Conventional methods for isolation and identification of *Salmonella* isolates from chicks showed that *Salmonella* appear as a colorless colony on Macconkeys agar medium. It gave the characteristics slightly transparent zone of reddish color with or without black center on XLD and colorless colonies with black center on SS agar medium. It appeared as Gram –negative straight rods , non-spore forming and arranged single in pairs and in groups .Moreover , *Salmonella* isolates were citrate test positive (blue color),urease test negative (yellow color)and they also gave acid but(yellow) and alkaline slant (red) with H₂S production (black correlation on TSI agar medium. Serotyping of 37 *Salmonella* isolates by slide agglutination test using specific monovalent and polyvalent O and H *Salmonella* sera revealed eleven different *Salmonella* serotypes, with *S. Enteritis* as the most prevalent serotype (27.1%) followed by *S. Tamale* (16.2%)., *S. Typhimurium* and *S. Kentucky* (13.5%) for each one, *S. Anatum* (10.8%), *S. Gallinarum* 5.4%, while *S. Infntis*, *S. Bargny* ,*S. Takorade* ,*S. Heldberg* and *S. Larochele* were the last detected serovars (2.7%) for each one. Anti-microbial sensitivity test revealed that most *Salmonella* isolates were sensitive to ciprofloxacin, sulfa methoxasole – trimethoprim , chlorumphenicol and gentamycin .while they were resistant to erythromycin,rifamycin ,amoxycillin /clavulnic acid ,colistin sulfate and cefotaxime .

Key words: *Salmonella*, serovars, Broiler.

Introduction

Salmonella serovars are one of the primary foodborne pathogens. Poultry consumption is responsible for the majority of disease cases worldwide. The prevalence of virulence determinants among Salmonella serovars appears to be lacking in Egypt. Therefore, this study investigated the occurrence, antibiotic resistance patterns, and virulence gene profiling of Salmonella serovars in broilers *Ammar et al., (2016)*.

Salmonella is an important pathogen for poultry production industry as well as for human due to zoonotic importance. It has more than 2600 identified serovars despite of this identification of Salmonella isolates into different serovars is critical for study of incidence and surveillance *Sedeik et al., (2019)*.

The aim of work prevalence of different Salmonella Serovars in broiler farms in Sharkia Governorate

Materials and Methods

1. sampling

A total of 300 samples (liver, cecum and unabsorbed yolk sac; 100 from each organ) were collected from diseased and freshly dead broiler chickens. Clinical tissue samples were collected aseptically to prevent cross contamination using

sterile sampling materials (swabs, bags and syringes) and wearing disposable gloves. The samples were collected and transported in ice boxes with ice packs as early as possible to the laboratory for bacteriological examination.

2 Isolation of Salmonella and serotyping

The procedure for isolation and identification of Salmonellae were conducted according to ISO 6579 (2002) procedure. Suspected Salmonella colonies were confirmed serologically by Kauffman – White scheme (Kauffman, 1974) for the determination of Somatic (O) and flagellar (H) antigens using Salmonella antiserum (DENKA SEIKEN Co., Japan). and biochemically by (TSI), Urea hydrolysis test, Lysine decarboxylation test, Indole production test and Citrate utilization test. The isolates were then serotyped by the Animal health research institute in Dokki -Giza. Only confirmed Salmonella were tested for their susceptibility to antimicrobial agents and the presence of the antimicrobial resistant genes.

3 Resistance to the antimicrobial agents

The antibiotic susceptibility was determined according to the recommendations set by the Clinical and Laboratory Standards Institute (Clinical and

Laboratory Standards Institute, CLSI, 2007) for the disk diffusion technique. The antimicrobials and concentrations tested were ampicillin (10 µg), gentamicin (10 µg), tetracycline (30 µg) and sulfamethoxazole (25 µg) (Oxoid, United Kingdom). The inhibition zones were measured and scored as sensitive, intermediate susceptibility or resistant according to the CLSI recommendations

Results and Discussion

Isolation and identification of *Salmonella* isolates:

Colonial appearance:

Salmonella grown onto MacConkey's agar medium gave pale colonies (non-lactose fermenter), subculturing from MacConkey's agar onto xylose lysine desoxy cholate agar medium yielding colonies with a slightly transparent zone of reddish colour with or without black center.

Biochemical identification:

All *Salmonella* isolates were urea negative (yellow color), citrate positive (blue color), *Salmonella* isolates gave acid butt (yellow) and alkaline slant (red) with H₂S production (black coloration) on TSI agar medium.

Serotyping of *Salmonellae* isolates from chickens

Serotyping of 37 *Salmonella* isolates was applied by slide

agglutination test using specific polyvalent "O" I, II, III and "H" *Salmonella* sera. Three different serotypes were identified among selected *Salmonella* isolates. The different serogroups were identified and *Salmonella* Enteritidis was the most prevalent one with a percentage of (27.1%) followed by *S. Tamale* (16.2%), *S. Typhimurium* & *S. kentucky* (13.5%) for each one, *S. Gallinarum* (5.4%), *S. Apeyme* (10.8%), *S. Larochelle*, *S. Takorade*, *S. Bargny*, *S. Infantis* and *S. Heldberg* with percentage of 2.7% for each one.

Prevalence of *Salmonella* in different organs of chickens in El-Sharkia Governorate.

Thirty-seven *Salmonella* isolates were recovered from 300 examined samples collected from chickens (12.1%).

Salmonella was previously isolated from chicken by (Alshawabkeh and Yamani, 1996; Mohammed et al., 1999; Taha, 2002; Ahmed, 2003; Orji et al., 2005; Pieskus et al., 2006; Moawad, 2009; Maripandi and Ali 2010; Ahmed et al., 2016 and Ammar et al., 2019).

Nearly the same rates were obtained by Moawad (2009) who isolated *Salmonella* from chicken in Dakahlia Governorate with percentage 13.3% while higher rates were recorded by Al-Shawabkeh and Yamani (1996)

who recovered *Salmonella* with percentage 20.5% ,19% and 17.9%, in layers, followed by broilers and breeders respectively.

Lower percentage rates than the previously mentioned were reported by *Sadoma (1997)* who isolated *Salmonella* from six out of 300(2%) cloacal swabs collected from 30 chicken farms at different localities in Garbia.

Moreover, *Mohamed et al. (1999)* isolated *S. Typhimurium*, *S. Anatum*, and *S. Pullorum* from 2.5% of chickens and 4% of duck samples from fattening and laying farms in Kafr-Elshikh Governorate and *Ahmed (2003)* isolated *Salmonella* from chickens reared in rural village in Sharkia province with a percentage of 1.7%.

This differences in prevalence rate may reflect considerable disparity in the sampling scheme, sample type, *Salmonella* detection protocol and geographic location.

Beside, eleven different serotypes were identified among selected isolates by slide agglutination test using polyvalent "O" 1,11,111 and H antisera.

The results of serological identification in present study detected eleven different serovars from 37 *Salmonella* isolates, *S. Enteritidis* predominated with higher

percentage (27.1%), while the remained isolates were serotyped as *S. Tamale*, *S. Typhimurium*, *S. Kentucky*, *S. Apeyme*, *S. Gallinarum*, *S. Infantis*, *S. Takorade*, *S. Heldberg*, *S. Bargany* and *S. Larochele* with percentages (16.2%), (13.5%), (13.5%), (10.8%), (5.4%), (2.7%), (2.7%), (2.7%), (2.7%), (2.7%) respectively as in table (7).

In current study *S. Enteritidis* is the predominant serotype that go hand in hand with *Sedeik et al. (2019)*.

Several authors isolate *Salmonella Typhimurium* from chickens at a higher rate than *S. Enteritidis* in India with a percentage of 18.10% and 9.87% respectively (*verma and Gupta, 1995*) and in Singapore with a percentage of 23.8% and 3.3% respectively (*Toh et al., 1996*). On the other hand, *S. Enteritidis* was the predominated serotype in Saudi Arabia with percentage of (55.6%) followed by *S. Typhimurium* (22.2%) *Moussa et al. (2010)*.

Poultry farms are *Salmonella enterica* serovar *Typhimurium* and *Salmonella enterica* serovar *Enteritidis* *Abd El-Gany et al. (2012)*.

Indicating the health hazard of poultry as a major source of *S. Typhimurium* and *S. Enteritidis* as a food borne pathogens and the commonest serotype causing

disease in human (*Baggesen et al., 2000 and Aktas et al., 2007*).

Antimicrobial resistance in *Salmonella* has received consider attention as the emergence of MDR *Salmonella* may result in treatment failure *Yan et al. (2003)*.

With the emergence of antibiotics resistance bacteria presenting a serious challenge in human and veterinary medicine globally, there is an abundant of evidence showing that the antimicrobial resistance of *Salmonella* in the chicken supply chain is more possibly attributed to the use of antibiotics in animal husbandry (*Cui et al., 2016*).

Examination of samples collected from diseased and died saso chicks from different hatcheries in Sharkia using SMT, 37 *Salmonella* isolates was isolated in an over prevalence of 12.3% (37/300), 21% was from liver, while 11% was from 2ceciand 5% from yolk sac. The results of serological identification in present study detected eleven different serovars from 37 *Salmonella* isolates, *S. Entertides* predominated with higher percentage (27.1%), while the remained isolates were serotyped as.

S. Tamale, S. Typhymurium, S. Kentucky, S. Apeyme, S. Gallinarum S. infantis, S.

Takorade, S. Heldberg, S. Bargany and S. Larochele with percentages (16.2%), (13.5%), (13.5%), (10.8%), (5.4%), (2.7%), (2.7%), (2.7%), (2.7%), (2.7%) respectively.

Results of antimicrobial susceptibility testing (antibiogram of *Salmonella* isolates):

All *Salmonella* isolates were tested for their susceptibility to the following antimicrobial agents: gentamicin (CN), ciprofloxacin (CIP), amoxicillin-clavulanic acid (AMC), doxycycline (DO), chloramphenicol (C), erythromycin (E), sulfamethoxazole trimethoprim (SXT), cefotaxime (CTX), colistin sulfate (C.T), streptomycin (S), naidixic acid (N.A) and rifamycin (R). High rate of susceptibility was the most common finding obtained against sulfamethoxazole trimethoprim (97.3%), streptomycin (83.8%) chloramphenicol and ciprofloxacin (75.7%) as shown in table (8). Also, absolute resistance was obtained among *Salmonella* isolates against erythromycin, cefotaxime, colistin sulfate and rifamycin (100%) for each one followed by amoxicillin clavulanic acid (75.7%).

In particular, there are many reports of increasing prevalence of fluoroquinolone-resistant

Salmonella **Piddock, (2002) and Wasyl et al., (2013)** which might be a potential risk for human health.

The results of this study revealed the potential problem of widespread of multidrug resistant *Salmonella* species especially in chickens because of extensor use of antimicrobial agent, in human and veterinary medicine that was agree with **Briggs and Fratamsco (1999)** as we detect resistance to CIP was 24% that is nearly the same as **Ren et al. (2016) and Nhung et al. (2018) but Cui et al. (2019)** detect resistance to CIP with a higher percentage 37%.

In present study, it is noticed that there is a huge increase in quinolone resistance in *Salmonella* that is go hand in hand with **Mobalk et al. (2002)**. Controversially, no resistance of

Salmonella to ciprofloxacin in Egypt from broiler carcass **Mona (2014)** and in Brazil (**Cordoso et al. (2006)**). The burden of food born disease is increasing due to antimicrobial resistance which represents a great risk of treatment failure, however, very little is known about the resistance profile of food born pathogen **Bantawak et al. (2019)**

Therefore, efforts are needed to reduce the prevalence of resistant *Salmonella* in broiler chickens, including the adoption of guide lines for the prudent use antimicrobial agents in animals used for food. There is a need for continued surveillance to determine a regular antimicrobial susceptibility data to detect any changing of resistance pattern **Kumar et al., (2012)**.

Table (1): Prevalence of Salmonella serotypes among different organs of chicks (each organ represent one chick)

Organ (No)	No of <i>Salmonella</i> isolates	<i>Salmonella</i> serotypes(37)										
		S. Enteritides	S. Tamale	S. Typhimurium	S. kentucky	S. Apeyme	S. Gallinarum	S. Infantis	S. Takorade	S. Heldberg	S Bargny	S. Larochele
Liver(100)	21%	6	4	3	2	3	-	1	1	-	1	-
2ceci(100)	11%	4	2	1	2	1	-	-	-	1	-	-
Yalk sac(100)	5%	-	-	1	-	-	2	-	-	-	-	1
Total (300)	37 12.3%	10 27.1%	6 16.2%	5 13.5%	5 13.5%	4 10.8%	2 5.4%	1 2.7%	1 2.7%	1 2.7%	1 2.7%	1 2.7%

Table (2): Species susceptibility testing to different antimicrobial discs

Code no		AMC	S	CN	NA	CIP	CTX	SXT	DG	E	C	CT	RF
22	S.Enteritides	-	I	I	-	-	-	S	I	-	I	-	-
23		-	I	I	-	-	-	S	I	-	I	-	-
27		-	S	I	-	I	-	I	I	-	I	-	-
36		-	S	I	I	S	-	S	-	-	I	-	-
38		-	S	I	I	S	-	I	-	-	-	-	-
42		-	I	I	-	-	-	S	I	-	I	-	-
44		-	I	I	-	-	-	S	I	-	I	-	-
92		-	-	-	I	S	-	I	-	-	S	-	-
105		-	-	I	-	I	-	-	I	-	-	-	-
108		-	i	-	-	I	-	I	-	-	-	-	-
13	S. Tamale	I	I	s	I	S	-	I	I	-	I	-	-
18		-	I	I	-	-	-	S	I	-	I	-	-
46		-	I	S	I	S	-	S	-	-	S	-	-
87		I	S	I	-	I	-	I	-	-	S	-	-
91		I	S	S	I	S	-	I	I	-	I	-	-
93		-	S	S	I	S	-	I	-	-	I	-	-
17	S.Typhymurium	I	-	-	-	I	-	I	-	-	-	-	-
20		-	I	I	-	-	-	S	I	-	I	-	-
21		-	I	I	-	-	-	S	I	-	I	-	-
26		-	-	-	-	I	-	I	-	-	-	-	-
31		-	I	-	-	S	-	I	I	-	-	-	-
5	S.Kentucky	-	I	-	-	I	-	I	-	-	-	-	-
10		-	I	I	-	-	-	S	I	-	I	-	-
11		-	I	I	-	S	-	I	-	-	S	-	-
33		-	I	I	-	I	-	S	-	-	-	-	-
34		-	I	-	I	I	-	I	-	-	-	-	-
51	S.Apeyme	I	S	I	I	S	-	I	I	-	-	-	-
52		-	I	I	-	-	-	S	I	-	S	-	-
55		-	S	I	I	I	-	I	I	-	I	-	-
57		-	S	-	I	S	-	I	I	-	S	-	-
77	S.Gallinarum	I	S	I	I	S	-	S	-	-	S	-	-
79		I	S	I	I	S	-	s	I	-	S	-	-
74	S.Infantis	I	S	-	I	S	-	S	I	-	S	-	-
86	S.Takorade	I	I	I	I	S	-	I	I	-	I	-	-
97	S.Heldberg	-	I	-	-	I	-	I	I	-	I	-	-
101	S.Bargny	-	I	I	I	S	-	I	I	-	I	-	-
106	S.Larochelle	-	I	i	I	S	-	I	I	-	I	-	-

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الملخص العربي

تم تجميع 300 عينة من دجاج تسمين عمر يوم يعاني من مشاكل تنفسية والتهابات في الصرته بعد الفقس وتم فحصها بكتريولوجيا واسفرت نتائج الفحص عن وجود 73 معزوله من السالمونيلا بنسبه 12.3%.

أظهرت الطرق التقليدية لعزل وتحديد معزولات السالمونيلا من الكتاكيت أن السالمونيلا مظهر مستعمرات عديمة اللون على وسط أجار Macconkeys أعطت خصائص منطقة شفافة قليلاً من اللون المحمر مع أو بدون مركز أسود على XLD ومستعمرات عديمة اللون مع وسط أجار أسود المركز. ظهرت كقضبان مستقيمة سالبة الجرام، ومرتبطة مفردة في أزواج وفي مجموعات. عزلات السالمونيلا كانت نتيجة اختبار السترات إيجابية (اللون الأزرق)، واختبار البورياز سلبي (اللون الأصفر)، كما أعطت الحمض ولكن (أصفر) وميل قلوي (أحمر) مع إنتاج H₂S الارتباط الأسود على وسط أجار TSI. عن طريق اختبار تراض الشرائح باستخدام مصل السالمونيلا أحادي التكافؤ ومتعدد التكافؤ، أظهر 11 نمطاً مصلياً مختلفاً من السالمونيلا، مع *S. Enteritidis* باعتباره النمط المصلي الأكثر انتشاراً (27.1%) يليه *S. Tamali* (16.2%)، *S. Salmonella* كنتاكي (13.5%) لكل منها، 10.8% *S. Anatum*، 5.4% *S. Gallinarum*، بينما *S. Infantis*، *S. Bargny*، *S. Takorade*، *S. Heldberg* و *S. Larochelle* كانت آخر مصل تم اكتشافها (2.7%) لكل واحد مضاد للميكروبات أظهر الاختبار أن معظم عزلات السالمونيلا كانت حساسة للسيبروفلوكساسين، السلفا ميثوكساسول - تريميثوبريم، الكلوروفينيكول والجنتاميسين، بينما كانت مقاومة للاربيثروميسين، الريفاميسين، الأموكسيسيلين / حمض الكلافولينيك، كبريتات الكوليستين والسيوفوتاكسيم.