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Serological and antibacterial characteristics of salmonella isolates from chickens in Assiut, Egypt

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ABSTRACT

Two hundred and twenty specimen from diseased and freshly dead chickens were gathered under complete aseptic conditions and transported to the laboratory of Faculty of Veterinary Medicine, Assiut University. Samples were processed for bacteriological and biochemical examinations. Suspected *Salmonella* isolates were serologically identified. Standard disc diffusion technique was used for antibacterial sensitivity testing of the isolated *Salmonella.* Minimum antibacterial inhibitory concentration (MIC) was also determined. 11.36% (25/220) out of the examined chicken samples were infected with *Salmonella*. The infection was more prevalent in two-weeks old chickens (20%) than four-weeks-old (5%). *Salmonella* was frequent in liver and spleen (13.33%, 11.26%, respectively) than caecum and yolk sac (9.3%, 6.25%, respectively). The isolated *Salmonellae* were serotyped as *S.* Kentucky (27.77%), *S.* Enteritidis (22.22%), *S.* Typhimurium (16.66%), *S.* Molade (11.11%), while *S.* Inganda, *S.* Papuana, *S.* Wingrove and *S.* Larochelle encountered in the same percent (5.55%). These obtained isolates exhibited complete antibacterial resistance to amoxicillin and ampicillin (100%), but (88.88%) resistance to cefotaxime and oxytetracycline, (83.33%) to erythromycin, (72.22%) to doxycycline, (66.66%) to neomycin and (61.11%) resistance to amikacin. Somewhat, antibacterial sensitivity was noticed to colistin (55.55%), spectinomycin (44.44%) and norfloxacin (33.33%). Based on MIC, colistin and florfenicol were the most sensitive antibacterial at a lower MIC value (<2µg/ml).

1. INTRODUCTION

Over 125 years, salmonellosis has been known to cause great losses in poultry, animals, and human. Poultry are a primary *Salmonella* reservoir, as avian gut provides a diverse polymicrobial environment that could potentially provide selective pressure to alter the genetic composition of *Salmonella* in such a manner to better adapt to the poultry environment (Foley *et al.*, 2013; CDC, 2017). Chicken salmonellosis either acute or chronic causes high financial losses in poultry industry due to high mortalities in young chicks and debilitating effect predisposing to other diseases (Crump *et al.*, 2015).

Salmonella belongs to the family *Enterobacteriaceae* and divided into 51 serogroups which collectively have 2600 serotypes (Gal-Mor, 2018). Chicken salmonellosis is manifested in pasty diarrhea, inappetence, dehydration, growth retardation, blindness, and lameness in one-week old broiler chicks. The main gross lesions are hepatomegaly with necrotic foci, splenomegaly, pericarditis, panophthalmitis, persistent yolk sac and arthritis (Swayne, 2020). Paratyphoid infection affects chickens at any age and of any type. In young birds, high mortality rates may reach 80% or higher while older ages, over 3 weeks old, paratyphoid infection rarely causes mortality, but the survivors become carriers and excrete the organisms in the environment (Gast and Beard, 1992). Flagella, capsule, plasmids, adhesion systems, toxins, and type 3 secretion systems (T3SS) play important role in *Salmonella* pathogenicity (Boko *et al.*, 2013).

Salmonella infection can be detected via bacterial isolation, identification, serological tests (Hendriksen, 2003) and polymerase chain reaction (PCR) which is more accurate especially in case of rough strain lacking O-antigen (Roy *et al*., 2002). Efforts to reduce *Salmonella* include guidelines adoption for antibiotic misuse, continuous antibacterial susceptibility surveillance to identify the changing pattern of *Salmonella* resistance (Kumar *et al*., 2012). Therefore, this study aimed at investigate the incidence of Salmonellae spp. in freshly dead and diseased broiler chickens, serological identification of Salmonella serotypes and studying the antimicrobial susceptibility of the prevalent Salmonella isolates to different antimicrobial drugs.

2. MATERIAL AND METHODS

2.1. Sampling:

220 specimens (liver, spleen, cecum, and yolk sac) from diseased and freshly dead chickens aging 1-40-days old were gathered under complete aseptic conditions from different diagnostic labs and farms in Assiut, Egypt, during December 2019 to May 2020, and transported to the laboratory of Faculty of Veterinary Medicine, Assiut University.

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2.2. Bacteriological and biochemical examinations:
Under complete aseptic conditions, swabs from each organ were inoculated separately into Rappaport-Vassiliadis broth (*Salmonella* selective broth- HiMEDia, India) and incubated at 42°C for 24 hours. Then, a loopful from the incubated broth was streaked onto Xylose Lysine Deoxycholate (XLD) agar (*Salmonella* selective medium-HiMEDia/India) and incubated at 37 ºC for 24 hours. The *Salmonella* suspected colonies were identified morphologically and biochemically according to Quinn et al. (2002). colonies were sub-cultured on MacConkey's agar plates to exclude any lactose fermenter coliforms and stained with gram's stain for microscopical examination (Jawetz *et al.*, 2007; WHO, 2010). The purified suspected *Salmonella* colonies were biochemically identified (urea hydrolysis, triple sugar iron agar, lactose fermentation, indole, catalase, and oxidase test) according to Barrow and Feltham, (1993) and MacFaddin, (2000).

2.3. Serological examination:
Isolates preliminarily identified as *Salmonella* isolates were serologically identified according to Kauffman – White scheme (Kauffman, 1974) through determination of their Somatic (O) and flagellar (H) antigens using *Salmonella* antiserum (DENKA SEIKEN Co., Japan).

2.4. Determining antibacterial sensitivity pattern of the isolated Salmonella:
Standard disc diffusion technique was used for in-vitro antibacterial sensitivity testing of the isolated *Salmonella* to 15 antibacterial agents according to Bauer *et al.* (1966) following Clinical Laboratory Standards Institute (NCCLS, 1999; CLSI, 2010; CLSI, 2018) Table 1, as the following:
2.4.1. Bacterial inoculum preparation:
Three to five well isolated colonies were suspended in 5 ml of brain heart infusion broth and incubated at 37 °C for 24 hours.
2.4.2. Streaking of test plates:
1.About 2 ml of the broth culture were transferred into the plate and the plate rotated approximately 60ºC each time to ensure of well distribution of the inoculum.
2.All excess fluids were removed using sterile pipette and leave the plate to dry for 15-20 min. at room temperature before the application of the antimicrobial discs.
3.The antimicrobial discs were dispensed onto the surface of the inoculated agar plate and each disc has been pressed gently to ensure complete contact with the agar surface by using sterilized forceps.
4.The plates were inverted then placed in an incubator at 37 °C for 24 hours.
5.The diameter of growth inhibition zones was measured by using the transparent ruler.
6.The sensitivity and the resistance were determined by the criteria of the Clinical and Laboratory Standard Institute (CLSI, 2010; CLSI, 2018)

2.5. Determining minimum antibacterial inhibitory concentration (MIC) to Salmonella isolates:
The antibacterial effect of Neomycin, Spectinomycin, Streptomycin, Erythromycin, Florfenicol, Colistin, Sulphaquinoxaline, and Chlortetracycline (Table 2) was checked in 96 wells microtiter plate using double fold micro-dilution method against all obtained *Salmonella* serovars in a density of 10^5 CFU **(CLSI, 2018)**. The concentration of each antibacterial was 10µg/mL, 2.56µl of each antibacterial was added in 2 wells of the first row of plate then 100µl broth with bacteria was added to first row of plate (wells of antibacterial) and 50 µl broth with bacteria was added to the rest of wells. Two-fold serial dilution technique was made and discard the last 50µl. The bacterial inoculum broth was taken as a positive control and another broth without bacterial inoculum was considered as a negative control. The microtiter plates were incubated at 37ºC for 24 hours and examined for the lowest concentration showing no detectable growth (MIC).

Tables: Antimicrobials used in disc diffusion test

Antibiotic group	Antimicrobial agent	Symbol	Conc. (µg)
Aminoglycosides	Amikacin	AK	30
	Gentamicin	GN	10
	Streptomycin	S	10
	Spectinomycin	SH	100
	Neomycin	Neo	10
Cephalosporines	Cefotaxime	CTX	30
Fluroquinolones	Ciprofloxacin	CIP	5
	Norfloxacin	NOR	10
Macrolides	Erythromycin	E	15
Miscellaneous	Chloramphenicol	C	30
	Colistin	CL	10
Penicillines	Ampicillin	AMP	10
	Amoxicillin	AM	10
Tetracyclines	Doxycycline	DO	30
	Oxytetracycline	OT	30

Table 2 Antimicrobial used in MIC.

Group	Antimicrobial	Concentration (mcg/ml)
Aminoglycosides	Neomycin	10
	Spectinomycin	10
	Streptomycin	10
Macrolides	Erythromycin	10
Miscellaneous	Florphenicol	10
	Colistin	10
Sulphonamides	Sulphaquinaxalin	10
Tetracyclines	Chloratetracyclin	10

3. RESULTS

The birds were examined for clinical signs and postmortem lesions. The common encountered signs in the checked birds were retarded growth, depression, lameness, ruffled feathers, huddling, respiratory troubles, whitish watery diarrhea, and dirty vent. Septicemia, bronzy discolored enlarged liver with necrotic foci, splenomegaly with necrotic foci, congested kidneys, enteritis and typhlitis with chalky cecal core, and unabsorbed yolk sac in young chicks were the main postmortem lesions. (Fig. 1,2)

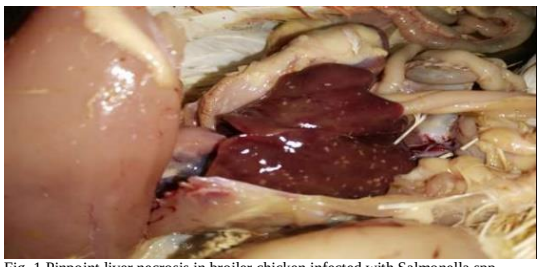
Fig. 1 Pinpoint liver necrosis in broiler chicken infected with Salmonella spp.


Fig. 2 Cheesy cecal core in chicken infected with Salmonella spp

Salmonella was identified in twenty-five samples (11.36 %, 25/220),
In relation to age, prevalence of Salmonella infection was 6.66% (2 / 30) in 1 -7 days old, 20% (10 / 50) in 8-15 days old chickens, 16% (8 / 50) in 16- 21days old, 6% (3 / 50) in

22-28 days old, and 5% (2 / 40) in 29 days old till slaughtering age (Fig.3).
Regarding internal organs liver, spleen, cecum, and yolk sac showed 13.33%, 11.26%, 9.3%, 6.25% *Salmonella* recovery rates, respectively (Fig. 4).

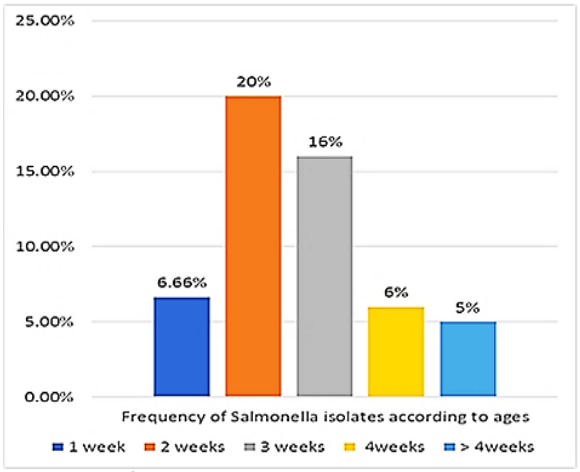

Fig. 3: Frequency of *Salmonella* isolates according to ages.

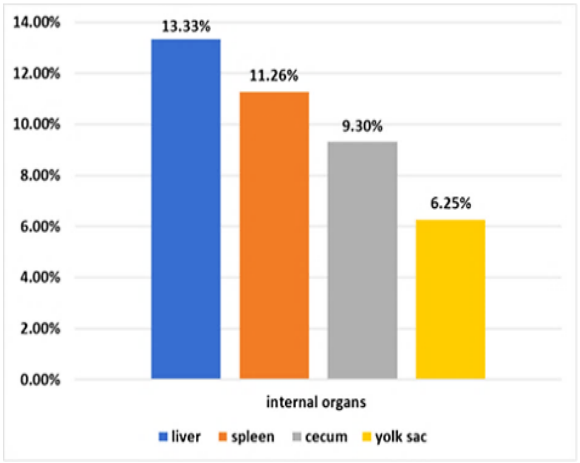

Fig. 4: The incidence of *Salmonellae* isolated from different organs.

The produced colonies on XLD agar were typical reddish with black center, white to white grey, small, smooth, rounded on Nutrient agar. Fig. 5a, b The gram-stained films from these colonies showed gram-negative bacilli.

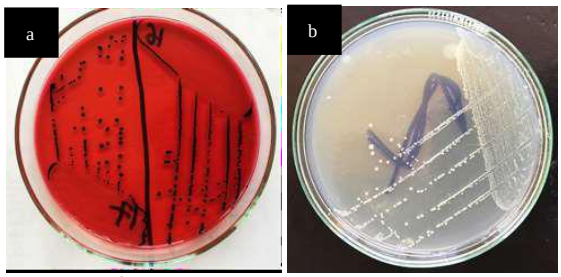

Fig. 5 Colonies of isolated serovars on the isolation media **a)** XLD agar b) nutrient agar

Biochemically the isolated suspected *Salmonella* were, catalase positive, urease, indole, and oxidase negative, and produced red butt /yellow slant and H_2S on TSI agar. (Table 3).
The biochemically identified *Salmonella* isolates were serotyped according to Kauffman – White scheme as *S.* Kentucky (27.77%), *S.* Enteritidis (22.22%), *S.* Typhimurium (16.66%), *S.* Molade (11.11%) while *S.* Inganda, *S.* Papuana, *S.* Wingrove and *S.* Larochelle encountered in the same percent (5.55%) as shown in Fig. 6 and Table 4.

Table 3 Biochemical properties of isolated Salmonella

Biochemical test	*Result*
TSI	Red slant /Yellow butt with H2S and gas production
Lactose fermentation	Non-lactose fermenter
Urease	Negative
Indole	Negative
Catalase	Positive
Oxidase	Negative

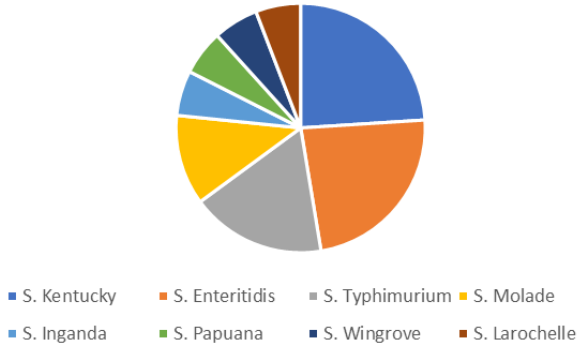

Fig. 6: Frequency of isolated Salmonella serovars

Table 4 Serogroups and serotypes of the obtained *Salmonella* isolates

Serial No.	Identified strains	Group	Antigenic structure	
			O	H
1	*Salmonella* Molade	C2	8,20	Z10 : Z6
2	*Salmonella* Enteritidis	D1	1,9,12	g,m : -
3	*Salmonella* Kentucky	C3	8,20	I : Z6
4	*Salmonella* Kentucky	C3	8,20	I : Z6
5	*Salmonella* Enteritidis	D1	1,9,12	g,m : -
6	*Salmonella* Kentucky	C3	8,20	I : Z6
7	*Salmonella* Typhimurium	B	1,4,5,12	I : 1,2
8	*Salmonella* Papuana	C1	6,7	r : e,n,Z15
9	*Salmonella* Enteritidis	D1	1,9,12	g,m : -
10	*Salmonella* Wingrove	C2	6,8	c : 1,2
11	*Salmonella* Molade	C2	8,20	Z10 : Z6
12	*Salmonella* Typhimurium	B	1,4,5,12	I : 1,2
13	*Salmonella* Inganda	C1	6,7	Z10 : 1,5
14	*Salmonella* Kentucky	C3	8,20	I : Z6
15	*Salmonella* Enteritidis	D1	1,9,12	g,m : -
16	*Salmonella* Typhimurium	B	1,4,5,12	I : 1,2
17	*Salmonella* Larochelle	C1	6,7	e,h : 1,2
18	*Salmonella* Kentucky	C3	8,20	I : Z6

In-vitro antibacterial susceptibility of the isolated Salmonellae:
All the obtained *Salmonella* serotypes were resistant to most tested antibacterial. Complete resistance (100%) to amoxicillin and ampicillin was observed. The resistance rates were (88.88%) to cefotaxime and oxytetracycline, (83.33%) to erythromycin, (72.22%) to doxycycline, (66.66%) to neomycin and (61.11%) to amikacin. Sensitivity rates (55.55%, 44.44%, and 33.33%) to colistin, spectinomycin, and norfloxacin, respectively. as presented in Fig. 7 and Table 5.
All the obtained isolates showed multidrug resistance (MDR) as they exhibited resistance to at least 5 antibacterial while 83.3% of isolates were resistant to 8-14 antibacterial. Table 6.

Minimum antibacterial inhibitory concentration:
Basing on MIC test for the tested antibacterial, 14 Sulphaquinoxaline and 7 neomycin resistant isolates showed very high (MIC) value (>256µg/ml), 12 spectinomycin, 10 florfenicol, and 7 colistin resistant isolates showed high MIC values (> 128 µg/ml), 16 streptomycin resistant isolates showed 4–8fold increase in MIC compared to the CLSI resistance breakpoint of 16 µg/ml. All erythromycin resistant isolates showed 4–10fold increase in MIC compared to CLSI resistance breakpoint (>

8µg/ml). 15 chlortetracycline resistant isolates showed 2–8 fold increase in MIC compared to CLSI resistance breakpoint (> 16µg/ml). 9 and 3 isolates showed sensitivity to colistin and florfenicol respectively at a lower MIC value (< 2µg/ml) as illustrated in Table 7.

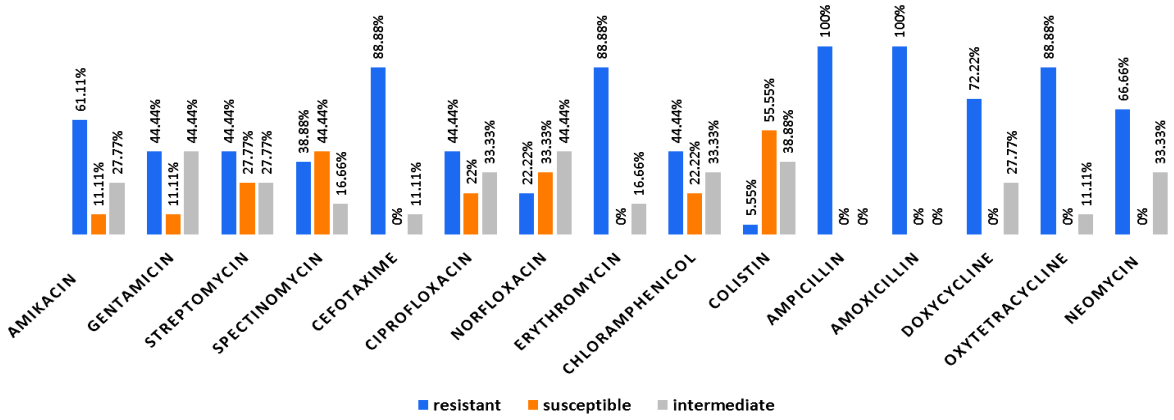

Fig. 7 Antibacterial resistance profiles of isolated *Salmonella* serovars

Table 5: Antimicrobial resistance profiles of isolated Salmonella serovars

antibiotic	isolated Salmonella serovars															
	S.Kentucky		S.Enteritidis		S.Typhimurium		S.Molade		S.Inganda		S.Larochelle		S.Papuana		S.Wingrove	
	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S
Amikacin	*	-	*	-	*	-	*	-	-	*	-	*	*	-	-	-
Gentamicin	*	-	*	-	*	-	-	*	*	*	*	-	*	-	-	-
Neomycin	*	-	*	-	*	-	*	-	*	-	-	*	*	-	*	-
Streptomycin	*	-	*	-	*	-	*	-	-	*	*	-	*	-	-	-
Spectinomycin	*	-	*	-	*	-	*	-	-	*	*	-	*	-	-	-
Cefotaxime	*	-	*	-	*	-	*	-	*	-	*	-	*	-	*	-
Ciprofloxacin	*	-	*	-	*	-	-	*	*	-	*	-	*	-	-	-
Norfloxacin	-	*	*	-	-	*	*	-	-	*	*	-	*	-	-	-
Erythromycin	*	-	*	-	*	-	*	-	*	-	*	-	*	-	*	-
Chloramphenicol	*	-	-	*	-	*	-	*	-	*	-	*	-	*	*	-
Colistin	*	-	*	-	*	-	*	-	-	*	*	-	*	-	-	-
Ampicillin	*	-	*	-	*	-	*	-	*	-	*	-	*	-	*	-
Amoxicillin	*	-	*	-	*	-	*	-	*	-	*	-	*	-	*	-
Doxycycline	*	-	*	-	*	-	*	-	-	*	*	-	*	-	*	-
Oxytetracycline	*	-	*	-	*											

R= resistance S= sensetive

Table 6: Multidrug resistance of isolated serovar

Serotype	*NO.*	*(%)*	*Resistance to NO. Of antibiotics*									
			5	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*
S.K	5	(27.77%)	1	-	1	-	-	3	-	-	-	-
S.E	4	(22.22%)	-	-	-	1	1	1	1	-	-	-
S.T	3	(16.66%)	-	-	-	1	-	2	-	-	-	-
S.M	2	(11.11%)	-	-	1	-	-	1	-	-	-	-
S.I	1	(5.55%)	-	-	-	-	1	-	-	-	-	-
S.P	1	(5.55%)	-	-	-	-	-	-	-	-	-	1
S.W	1	(5.55%)	-	-	-	1	-	-	-	-	-	-
S.L	1	(5.55%)	-	-	-	-	-	-	-	1	-	-
		Total no	1	-	2	3	2	7	1	1	-	1

Table 7: MIC breakpoint

Anti-biotic	*Minimum inhibitory concentration of 18 salmonella isolates*								*Resistance breakpoint*	*Resistance %*
	<2	*4*	*8*	*16*	*32*	*64*	*>128*	*>256*		
NEO	-	-	-	-	4	7	-	7	-	18 (100%)
S	-	-	-	-	2	2	3	11	>16	18 (100%)
SH	-	-	-	3	1	2	2	10	-	18 (100%)
SUL	-	-	-	-	-	-	4	14	-	18 (100%)
E	-	-	-	-	-	5	8	5	8	18 (100%)
F	3	5	-	-	-	-	7	3	8	10 (55.55%)
CL	9	2	-	-	-	-	5	2	4	9 (50%)
CHL	-	-	-	3	2	3	6	4	16	18 (100%)

4. DISCUSSION

Despite being old problem, salmonellosis still very interesting and worth pursuing. *Salmonella* particularly paratyphoid causes worldwide significant economic and public health losses particularly in developing countries due to its relevant implications in poultry industry and human. The extensive irrational use of antibiotics and chemotherapeutics to prevent and control bacterial diseases led to development of drug-resistant bacteria that became increasingly difficult to be controlled and eradicated. So, this study investigated chickens *Salmonella* infection in Assiut, Egypt, and determining the serological and antibacterial characteristics of isolated *Salmonellae*.

Depending on conventional bacteriological examination and biochemical identification, from 220 broilers chickens' samples, 25 (11.36%) were infected with *Salmonella*. These results indicate existence of *Salmonella* in commercial chickens although the extensive use of antibiotics particularly at the first days of chicken's life and need to be ascertained in large scale studies. Similarly, Kaoud *et al.* (2018) observed average salmonellosis prevalence 11.33 % in open broiler houses in Egypt.

Conversely, lower infection rates were obtained by Ibrahim *et al.* (2018) 6.8% in broilers in Beheira Provence, Egypt and Sedeik *et al.* (2019) 7.5% in newly hatched chicks in five different governorates in Egypt (El-Gharbia, Kafr-Elshiekh, El- Beheira, Alexandria and Matrouh).

Higher infection rates were documented by Abdeen *et al.* (2018) 14.3% in morbid Fayoumi chickens in Menoufia Governorate, Elkenany *et al.* (2019) 28.6% in live and dead broiler chickens in Sharkia Governorate, Egypt and EL-Sheikh (2018) 16% in Baladi and broiler chickens. The variation in the percentage of *Salmonella* detection among chicken species could be explained by different factors including management, biosecurity and prophylactic antibacterial used in each circumstance, different samples type, and different diagnostic techniques. Low biosecurity measures inside farms allow possibility of disease transmission via different reservoirs and workers in farms (El-Sharkawy *et al*., 2017).

In this study the *Salmonella* recovery rate was relatively higher in 2-weeks old chickens (20%) than those >4-weeks old (5%). This can be explained by that *Salmonella* intestinal colonization, invasion to internal organs, and persistence in colonized tissues are all higher in newly hatched chicks than in older birds due to their acquisition of protective microflora that either competes with *Salmonella* for intestinal receptors or produces antagonistic factors (Al-baqir *et al.,* 2019; Swayne, 2020), These findings are in accordance with those of Al-baqir *et al.* (2019).

Focusing on body organs, the present study depended on investigation of Salmonella in more than one organ (liver, spleen, cecum, and yolk sacs). The liver showed the highest Salmonella recovery rate (13.33%), followed by spleen (11.26%) and cecum (9.3%) while the yolk sac revealed the lowest infection rate (6.25%). This denotes *Salmonella* ability to survive and multiply in internal organs with various frequency, especially liver and spleen as these areas provide sites where bacterial multiplication can occur without exposure to host defense mechanisms (Gast, 2003).

Coming along with our results Al-baqir *et al.* (2019) recorded the highest *Salmonella* recovery percent in liver (30.66%) followed by spleen (25.33%) caecum (20%) and yolk sac (15.7%).

The isolated *Salmonellae* were serotyped into 8 serotypes. *S.* Kentucky and *S.* Enteritidis were the most encountered serotypes, followed by *S.* Typhimurium and *S.* Molade. *S.* Inganda, *S.* Papuana, *S.* Wingrove and *S*. Larochelle were the least frequent and encountered in the same percent (Figure 6). This high variation in the prevalent *salmonella* serotypes can denote substantial burden on poultry industry particularly the high frequency of the *S.* Kentucky, *S.* Enteritidis, and *S.* Typhimurium that represent high public health significance.

These results were closely agreed with Awad *et al.* (2020) who reported the predominance of *S.* Kentucky (22.6%), *S.* Enteritidis (22.6%) and *S*. Typhimurium (19.4%) serovars in Egypt. Other previous studies recorded different results, Im *et al.,* (2015) documented *S.* Bareilly as the most prevalent *Salmonella* serovars in the flocks (41.2%), followed by *S.* Mbandaka (32.4%) and *S.* Rissen (17.6%). Abd El-Tawab *et al.* (2015) demonstrated low *S.* Papuana, *S.* Takoradi, *S.* Labadi, and *S*. Angers incidence (2.3% each) in different Governorates in Egypt. Sedeik *et al.* (2019) found the most prevalent serovar detected in Egypt was *S.* Enteritidis 2.4% followed by *S.* Virchow 1.4%, *S.* Typhimurium 1.2%, and *S.* Kentucky 0.8%.

Generally, the frequencies of serovar isolation varied from location to another due to different management and hygienic regimes as well as environmental and individual differences as mentioned by Kirn *et al.* (1991).

It is evident from this work that all isolated *Salmonella* serotypes belong to serogroup (C3) and (D1) with large percent then (B), (C1) and (C2) of Kauffman white scheme. These results are like Al-Nakhli *et al.* (1999) who mentioned that 90% of *Salmonella* serotypes infecting both poultry and human are belonged to serogroup B,C,D and E.

To antibacterial susceptibility, the dissemination of multidrug resistance MDR in *Salmonella* spp. became a major concern in poultry production. Certain *Salmonella* serovars were found to have a greater ability to acquire MDR than others.

The antibacterial susceptibility pattern of the isolated *Salmonella* serovars in this study was determined against the commonly used antibacterial in Egyptian poultry farms. All isolates (100%) exhibited resistance to at least 5 antibacterial, while 83.3 % of them showed resistance to 8-14 antibacterial. This is not surprising because these antibacterial are commonly used in humans and poultry. Moreover, the antibacterial misuse by poultry producers including subtherapeutic doses, unauthorized use without prescription and usage as preventive tool in poultry leading to development of enteric flora resistance, from which pathogenic *Salmonella* may acquire and transfer resistance to humans strains through food chain leading to emergence of multidrug resistance *Salmonella* (Cox *et al.*, 2003).

The present results closely coincided with Abdeen *et al.* (2018) who revealed that all tested *Salmonella* isolates (100%) were MDR to five classes of antibacterial and higher than Elkenany *et al.* (2019) who revealed that MDR against three or more antibacterial in 76.7% of isolates. Sharma *et al.* (2019) showed that all isolates were MDR and 92.86% of theses isolates showing resistance to 5 or more classes of antibacterial on disk diffusion.

All the examined *Salmonella* serovars in the current study were completely resistant (100%) to ampicillin and amoxicillin indicating the limited therapeutic value of these antibacterial to poultry, this may be due to their wrong and frequent use in the field. Higher rates of resistance were observed to cefotaxime, erythromycin, oxytetracycline, doxycycline, amikacin, gentamycin, chloramphenicol, neomycin, ciprofloxacin and streptomycin. While rates of intermediate sensitivity were observed to colistin, spectinomycin and norfloxacin.

Interestingly *S.* Papuana was resistant to (14) antibacterial, *S.* Larochelle was resistant to (12) antibacterial. All isolates showed sensitivity to colistin except one *S.* Kentucky isolate was resistant.

These results were in a partial accordance with Tarabees *et al.* (2017) who stated that *S.* Typhimurium isolates were resistant to ampicillin, amoxicillin, penicillin, neomycin, ofloxacin, doxycycline, and chloramphenicol and higher than Moawad *et al.* (2017) who showed that most of the *S.enterica* isolates showed resistance to ampicillin (87.0%) and cefotaxime (80.0%) and Awad *et al.* (2020) who reported that the isolates collected from chicken carcasses in Mansoura, Dakahlia Province show resistance to Erythromycin 96.78%, Doxycycline 93.55%, Streptomycin 80.65% and Amoxicillin 67.8%.

On the other hand, our data showed contrast to the data obtained by Helal *et al.* (2019) who revealed that the examined *Salmonella* serovars were highly sensitive to Doxycycline, Chloramphenicol, followed by Amoxicillin, Ampicillin, Gentamycin and Sulphamethoxazole+ Trimethoprim. Elkenany *et al.* (2019) Who reported that *Salmonella* serovars were highly susceptible to Ciprofloxacin, Cefotaxime, Chloramphenicol and Ezzat *et al.* (2019) who mentioned that all strains were sensitive to amikacin and norfloxacin (100%) which was the most effective chemotherapeutic agent against *Salmonella* infection.

Moreover, 14 sulphaquinoxaline and 7 neomycin resistant isolates showed very high MIC values (> 256µg/ml). 12 spectinomycin, 10 florfenicol and 7 colistin resistant isolates showed high MIC values (>128µg/ml). All erythromycin resistant isolates showed 6–10 folds increase in MICs compared to CLSI resistance breakpoint (> 8µg/ml). 16 streptomycin resistant isolates showed 4–8 folds increase in MIC compared to the CLSI resistance breakpoint of 16µg/ml. 15 chlortetracycline resistant isolates showed 2–8 folds increase in MICs compared to CLSI resistance breakpoint (> 16µg/ml). While 9 and 3 isolates showed sensitivity to colistin and florfenicol, respectively at a lower MIC value (<2µg/ml). This may be due to colistin competitively displaces divalent cations

(Ca2+ and Mg2+) from the phosphate groups of membrane lipids, which leads to disruption of the outer cell membrane, leakage of intracellular contents and bacterial death.

5. CONCLUSION

In conclusion, the data of the present study revealed the variation of *Salmonella* serovars recovered from chicken. These serovars expressed variable degrees of resistance to the available antibacterial used for the treatment of invasive Salmonellosis alarming the importance of the situation. This requires regular monitoring of the isolated *Salmonella* for their antibacterial susceptibility especially that of zoonotic importance and finding strict national plan for complete elimination of this disease. Therapeutic agents must not be given before microbial sensitivity testing and it's advisable not to use the therapeutic antibacterial as a preventive feed supplement. Further investigations are important to track the emergence of new *Salmonella* serovars and the frequencies and patterns of its antibacterial resistance.

The higher MDR value observed in this study might be attributed to the widespread use of antibacterial in the locality of our study in Egypt. So, regular testing of the prevalent *Salmonella* isolates in poultry for their antibacterial susceptibility is required to avoid transmission of these serovars to human food chains and possibility of increasing in their pathologic potential to poultry. The current findings alert for seeking alternative safe ways like herbal extract, probiotic, and prebiotic to combat Salmonellosis instead of the traditional insensitive therapeutics.

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