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MOLECULAR CHARACTERIZATION OF ANTIBACTERIAL RESISTANCE GENES OF *SALMONELLA* IN DUCKS

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ABSTRACT

Due to financial losses associated with avian salmonellosis, high costs of prohibiting its spread and prevention and its multiple drug resistance, *Salmonella* infection, particularly in ducks, attracted interest of many researchers as duck is a main reservoir of *salmonella* transmitted to human. Therefore, this study aimed at identifying circulating *salmonella* of ducks in Assiut governorate and assessing their antimicrobial susceptibility profile. Five hundred and sixty samples (150 livers and 410 cloacal swabs) of infected, freshly dead and apparently healthy ducks, were obtained from different farms in Assiut governorate for bacteriological, serological and molecular examination. An overall 16.6% *Salmonella* detection rate was recorded, where 15isolates were identified serologically and molecularly as *S. typhimurium* (93.3%) and *S. infantis* (6.7%). Basing on antibiogram guidelines, detected *Salmonella* isolates were completely resistant (100%) to cephradine and amoxicillin, but had variable resistance degrees to colistin sulfate (80%), streptomycin (60%), chloramphenicol (33.3%), ampicillin and neomycin (26.7% of each). MIC test presented that all isolates were absolutely sensitive to colistin and doxycycline, but completely resistant to sulfaquinoxaline. High resistance rates occurred to cephradine, amoxicillin, streptomycin and florfenicol. *sul-1, strA-strB, bla TEM, aadA* and *floR* antibacterial resistance genes were assigned in variable frequencies (100%, 73.3%, 73.3%, 66.7% and 46.7%, respectively). In conclusion, *S. typhimurium* and *S. infantis* serovars are circulating among duck farms in Assiut. These serotypes exhibited genetic multiple drug resistance, that require special strict biosecurity and searching alternative effective control strategies.

Keywords: Ducks, *Salmonella,* PCR, MIC, Resistance gene.

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INTRODUCTION

Having unique characters over other poultry types, duck industry forms important part of poultry sector worldwide either rural or enterprises. For many years, *Salmonella* infection was concerned researchers, veterinarians, and public health authorities due to its associated losses in livestock and risks of food poisoning, rank number one among the potentially foodborne bacteria. In spite exerted efforts and advances in prevention and control measures *Salmonella* infection still induce great economic losses. Being the most important reservoir of *Salmonella*e transmissible to human, duck received great interest among researchers (Yang *et al.,* 2019). *Salmonella* infected birds show non-specific signs include depression, poor growth, weakness and diarrhea. The majority of mortalities usually occur in the first weeks of life. Rapidly developed septicemia is the origin of high mortality with limited or no clinical signs (Gast, 2008). Many *Salmonella* serotypes were detected in ducks, most of public health significance but some, including *S. gallinarum, S. pullorum, S. typhimurium, S. enteritidis and S. anatum* caused considerable losses in birds younger than few-weeks old (Buxton, 1957). *Salmonella* contains sequences unique invasion (*invA*) gene to this genus and confirmed to be a suitable target designed for PCR with a potential diagnostic application (Jamshidi *et al.,* 2009). Like other pathogens of clinical and economic significance, *Salmonellae* face the problem of drug resistance, principally multidrug resistance that must be periodically assessed and monitored. Antimicrobial resistance is a phenomenon of huge concern for *Salmonella* and other foodborne pathogens. The extensive and misuse of antimicrobials in veterinary medicine is regularly incriminated in transfer of antibiotic resistance to human pathogens and spreading of multiple antibiotic resistant. Moreover, World Health Organization emphasized increasing number of non-typhoid *Salmonella*e resistant to antibiotics (McEwen, 2012). Polymerase chain reaction (PCR) is a molecular technique took up an increasingly significant space in the field of laboratory diagnostics, allowing the detection of various pathogens and their genetic properties like antibacterial resistance genes (Santos *et al.*, 2001). So this study aimed at molecular identification of *Salmonella* isolates by conventional PCR, determining antibacterial susceptibility pattern and molecular characterization of antibacterial resistance genes among prevalent *Salmonella* serotypes.

MATERIALS AND METHODS

Sampling

Altogether, 560 samples (150 livers and 410 cloacal swabs) of infected, freshly dead and apparently healthy ducks, were obtained from different farms in Assiut and transported to laboratory of Faculty of Veterinary Medicine- Assiut University, Egypt.

Bacteriological and biochemical examination:

According to ISO 6579 (2002), samples were bacteriologically examined for *Salmonella* isolation. Briefly, swab from each sample was inoculated separately - under complete aseptic conditions- into 1:10 Buffered Peptone Water (pre-enrichment) and incubated aerobically at 37ºC for 18 hours. Then, 0.1ml from the incubated broth was transferred to 10ml Rappaport Vassilidis Soy (RVS) broth

and incubated at 41.5ºC for 24 hours. A loopful from the incubated RVS was streaked onto Xylose Lysine Deoxycholate (XLD) and incubated at 37ºC for 24 hours. The suspected *Salmonella* colonies were gram stained for cellular morphology and identified biochemically by using (urease, TSI, lysine decarboxylation, indole and citrate utilization tests).

Molecular identification of *Salmonella* isolates:

Biochemically suspected *Salmonella* colonies were molecularly ascertained according to Dashti *et al*. (2009) and Oliveira *et al.* (2002). In brief, pure colonies were suspended in 5ml phosphate buffered saline (PBS) and centrifuged at 3000rpm (4°C) for 10minutes (repeated thrice till obtaining pellet). Pellets washed twice with PBS, re-suspended in 100 µl of degrade free water, heated for 10 min and chilled in ice for 30 min. and centrifuged at 3000×*g* for 5 min at 4°C. finally, supernatants were taken and used as template DNA it was amplified by Polymerase Chain Reaction assay using *Salmonella* specific *inv*A gene primer set:

Forward (F): (5´-GTGAAATTATCGC CACGTTCGGGCAA3´) and

Reverse(R) :(5´TCATCGCACCGTCA AAGGAACC-3´) and Go Taq® Green Master mix (Promega) in Veriti thermocycler (Applied biosystems, Germany) following Oliveira *et al.* (2002) cycling conditions. Accurately, an initial hot start at 94°C for 5minutes, followed by 35 cycles, each consisting of 94°C for 30s, 55°C for 30s, and 72°C for 30s and the step of final extension at 72°C for 7minutes. The amplified products (5µl) were identified on 1.5% agarose gel stained with ethidium bromide and visualizing them with UV light in comparison to molecular size of 100-1.500bp DNA ladder (RTU, Cat.No.DM001.R500, 11bands).

Molecular typing of *Salmonella* isolates:

Multiplex and conventional PCR assays were carried out using primer sets specific for *S. typhimurium* with sequences:

F1:(5'CAGCACCAGTTCCAACTTGA TAC-3').

R1:(5'GGCTTCCGGCTTTATTGGTA AGCA -3').

F2:(5'ATAGCCATCTTTACCAGTTCC CCC-3').

R2:(5'GCTGCAACTGTTACAGGATA TGCC-3') (Lim *et al.*, 2003) and *S. infantis* with sequence **F:** (5'-AACAACGACAGCTTATGCCG-3') and **R:** (5'-CGCAGCGTAAAGCAACT 3') (Kardos *et al*., 2007), producing amplicons with molecular weight of 663bp, 183bp and 413bp, respectively. The reaction conditions for *S. typhimurium* (*Rfbj* and *FliC* genes) consisted of a primary denaturation at 95ºC for 2 min, followed by thirty cycles of denaturation at 95ºC for 1min, annealing at 57ºC for 1min and extension at 72°C for 1min followed by final extension at 72°C for 10min. While, the PCR reaction conditions for *S. infantis* (*fljB* gene) consisted of an intial denaturation at 95ºC for 6minutes, followed by 35 cycles of final denaturation at 95ºC for 1minutes, annealing at 56ºC for 15seconds and extension at 72°C for 1minutes followed by final extension at 72°C for 4minutes. PCR products were screened as previously described for *invA* gene PCR.

Assessing Antibacterial Susceptibility Pattern of *Salmonella* isolates using Disc diffusion method:

Antibacterial sensitivity of *Salmonella* isolates was assessed using 15antibacterial agents. The sensitivity and the resistance were determined by criteria of Clinical and Laboratory Standard Institute (CLSI, 2018).

Determining antibacterial Minimum Inhibitory Concentration (MIC) to *Salmonella* isolates: according to **(Stanković *et al.*, 2017).**

Susceptibility of *Salmonella* to streptomycin, gentamycin, neomycin, florfenicol, sulphaquinoxalin, doxycycline, cephradine and amoxicillin was checked in microtiter plate 96 wells using double fold micro-dilution method against all isolated *Salmonella* in a density of 10^5 CFU (CLSI, 2018). Each antimicrobial had a concentration of 10µg/mL, and 2.56µl of each antimicrobial was added into two wells in the first row of the plate, followed by 50 µl tryptone soya broths with bacteria was added to all wells. Extra 50µl tryptone soya broth containing bacteria was put in to the 1st row of plate (antimicrobials wells) then two-fold serial dilution method was made and remove the last 50 µl. The broth containing bacterial inoculum was taken as a positive control while, broth without bacterial inoculum used as a negative control. after a 24-hours of incubation at 37ºC, the microtiter plates were examined for the lowest concentration showing no detectable growth (MIC).

Molecular identification of antibacterial resistance genes among *Salmonella* isolates using PCR:

Table 1: Primer's sequences and amplicon size (bp) for identification of Florfenicol, β-lactams and Sulfonamides resistance genes among *Salmonella* isolates.

Primers		Target	primer sequence	Amplicon size	References
Florfenicol	StCM-L	*floR*	CACGTTGAGCCTCTATATGG	888bp	Ahmed *et al.*, 2007
	StCM-R		ATGCAGAAGTAGAACGCGAC		
β-lactams	*bla TEM-F*	*bla* TEM	ATCAGCAATAAACCAGC	517bp	Colom *et al.*, 2003
	bla TEM-R		CCCCGAAGAACGTTTTC		
Sulfonamides	*Sul 1-F*	*Sul 1*	TCACCGAGGACTCCTTCTTC	316bp	Randall *et al.*, 2004
	Sul 1-R		AATATCGGGATAGAGCGCAG		

Table 2: Showing PCR conditions applied for the detection of Florfenicol, β-lactams and Sulfonamides resistance genes among *Salmonella* isolates.

Stage	Temperature/Time		
	***floR* gene**	***bla* TEM gene**	***Sul*-1gene**
Initial denaturation	95ºC/5min	94ºC/5min	94ºC/1min
Denaturation	95ºC/45s	94ºC/30s	94ºC/1min
Annealing	52ºC/45s	54ºC/30s	60ºC/1min
Extension	72ºC/1min	72ºC/1 min	72ºC/1min
Final extension	72ºC/10min	72ºC/10 min	72ºC/10min

Molecular identification of Streptomycin resistance gene by multiplex PCR:

Table 3: Presenting primers, its sequences and amplicon size (bp) for identification of streptomycin resistance gene among *salmonella* isolates.

	Primers	Target	Primer sequence	Amplicon size	References
streptomycin	*aadA*-F	aadA	GTGGATGGCGGCCTGAAGCC	525bp	Madsen *et al.*, 2000
	aadA-R		AATGCCCAGTCGGCAGCG		
	strA-strB–F	strA-strB	ATGGTGGACCCTAAAACTCT	891bp	Tamang *et al.*, 2007
	strA-strB–R		CGTCTAGGATCGAGACAAAG		

PCR reaction conditions consisted of a primary denaturation at 94°C for 4min, followed by 35 cycles of denaturation at 94°C for 45s, annealing at 60°C for 45s and primary extension at 72°C for 45s, followed by a last extension at 72°C for 10minutes.

RESULTS

The overall incidence of *Salmonella* infection in ducks was 16.6%. 36 suspected *Salmonella* isolates were undergoing serological identification according to Kauffman – White scheme gave 15 isolates were positive for *Salmonella spp.* with a percentage rate (41.6 %) and 21 gave negative for *Salmonella spp.* with a percentage rate (58.3%). 14 (93.3%) of isolates belonged to *S. typhimurium* and one (6.6) isolates belonged to *S. infantis*.

All fifteen *Salmonella* isolates were harbored *invA* gene and amplified at 284 bp fragments as shown in Fig. (1). Out of 15 *invA* gene positive *Salmonella* isolates, 1 (6.6%) isolate was positive for *fljB gene* indicating *Salmonella infantis.* Out of 15 *invA* gene positive *Salmonella* isolates, 14 (93.3%) isolates were positive for *Rfbj* and *fliC* genes indicating *Salmonella typhimurium* as shown in Fig. (2).

Antibacterial sensitivity test of *Salmonella* isolates using the disc diffusion technique:

All *Salmonella* isolates were completely sensitive (100%) to amikacin, ciprofloxacin, enrofloxacin and Sulfamethoxazole/trimethoprim and they had a variable sensitivity to tetracycline and oxytetracycline (86.7% of each), doxycycline and gentamicin (80% of each), chloramphenicol and ampicillin (60% of each) and colistin sulfate (20%). they had intermediate sensitivity to neomycin (73.3%), streptomycin (33.3%), Ampicillin (13.3%), and (6.7%) for doxycycline and chloramphenicol. All isolates were completely resistance (100%) to cephardine and amoxicillin, while they showed a variable degree of resistance to colistin sulfate (80%), streptomycin (60%), chloramphenicol (33.3%), ampicillin and neomycin (26.7% of each), gentamicin (20%) and (13.3%) for tetracycline, oxytetracycline and doxycycline as shown Fig. (3).

Antibacterial sensitivity test of *Salmonella* isolates using MIC technique:

All examined *salmonella* isolates were were absolutely sensitive to colistin and doxycycline, while they were completely resistant sulphaquinoxalin and highest

rate of resistance was against cephradine, amoxicillin, streptomycin and florfenicol, but the lowest degree of resistance for gentamicin and neomycin.

Detection of resistance genes in *Salmonella* isolates:

All *Salmonella* isolates were positive (100%) for *Sul-1* gene (Sulfonamide resistance gene) which giving amplification at 316 bp fragments as shown in Fig. (4). Eleven *Salmonella* isolates (11/15) (73.3%) were positive for *bla TEM* gene (β-lactams resistance gene) from the 15 examined *Salmonella* isolates which giving amplification at 517 bp fragments as shown in Fig. (4). Seven *Salmonella* isolates (7/15) (46.7%) were positive for *floR* gene (florfenicol resistance gene) from the 15 examined *Salmonella* isolates which giving amplification at 888 bp fragments as shown in Fig. (5). Eleven *Salmonella* isolates (11/15) (73.3%) were positive for *strA-strB* (streptomycin resistance gene) while, Ten *Salmonella* isolates (10/15) (66.7%) were positive for *aadA* gene (streptomycin resistance gene), from the 15 examined *Salmonella* isolates which giving amplification at 891bp and 525bp fragments as shown in Fig.(6).

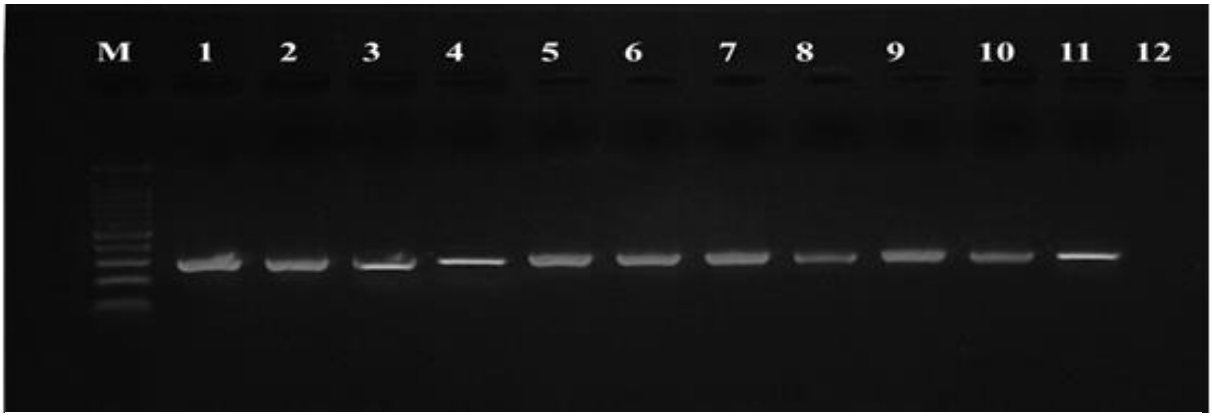

Fig. (1): Detection of *invA* gene in *Salmonella* isolates. Positive samples produce band (284 pb), Lane M: 1Kb DNA Ladder, Lane: 1 to 11 were positive samples produce band (284 bp)

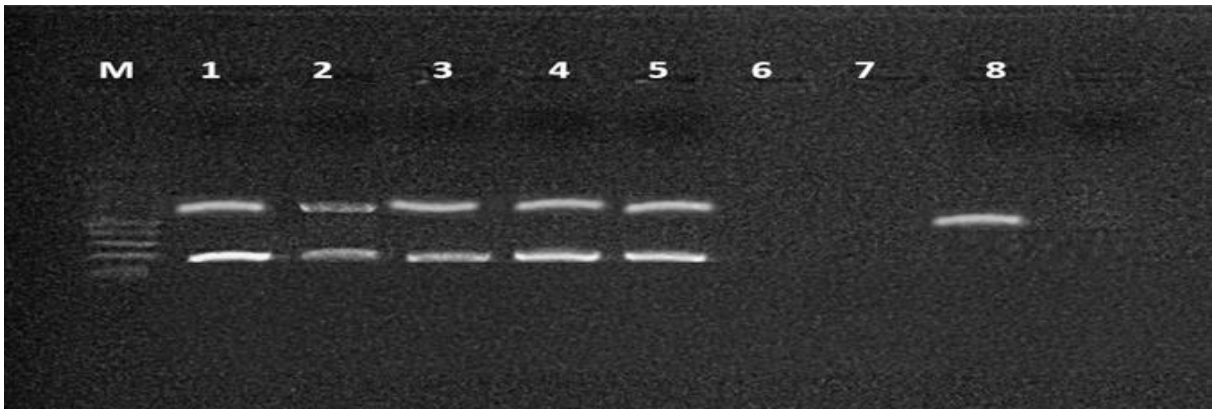

Fig. (2): Detection of ***Rfbj*** and ***fliC*** genes specific for *S. typhimurium* and ***fljB*** gene specific for *S. infantis* in samples. Positive samples produce bands (**663 and 183bp**) specific for *S. typhimurium* and (**413 bp**) specific for *S. infantis*. **Lane M**: 1 Kb DNA Ladder, **Lane: (1 to 5)** positive samples for *S. typhimurium* (produce bands 663 and 183bp) while, **Lane: (8)** positive samples for *S. infantis* produce band (413bp).

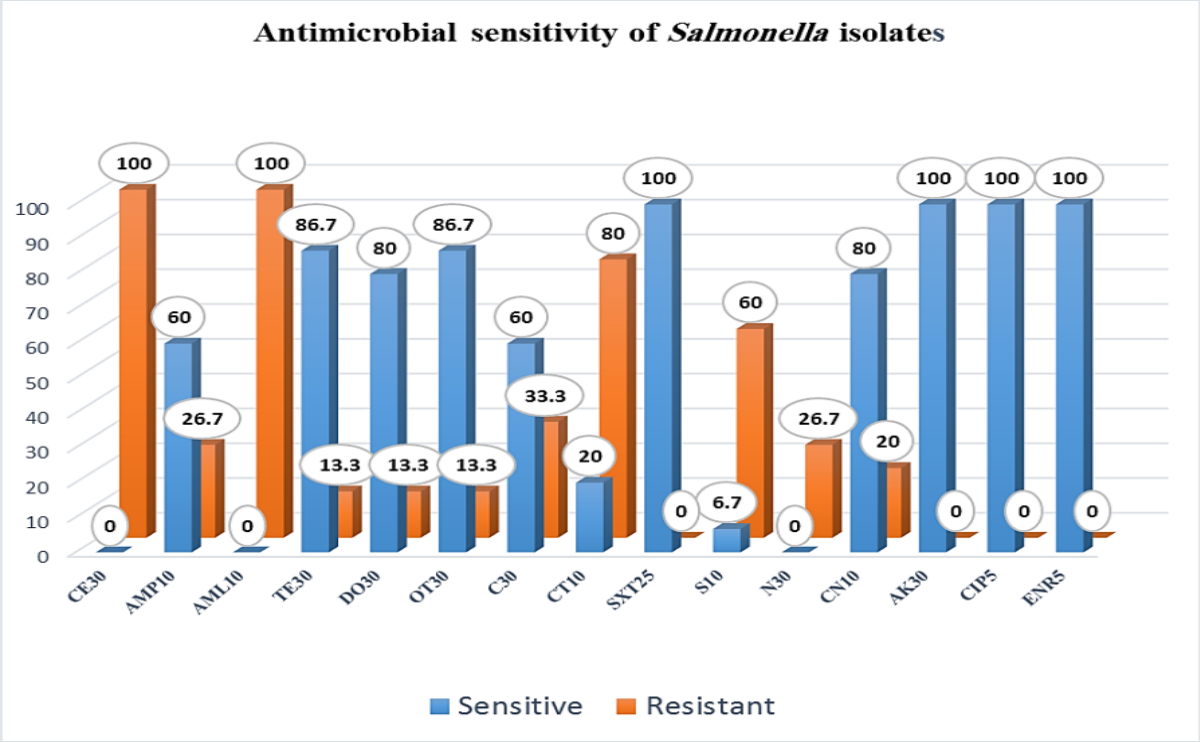

Fig. (3): antibacterial sensitivity and resistance percentages of isolated *Salmonellae*

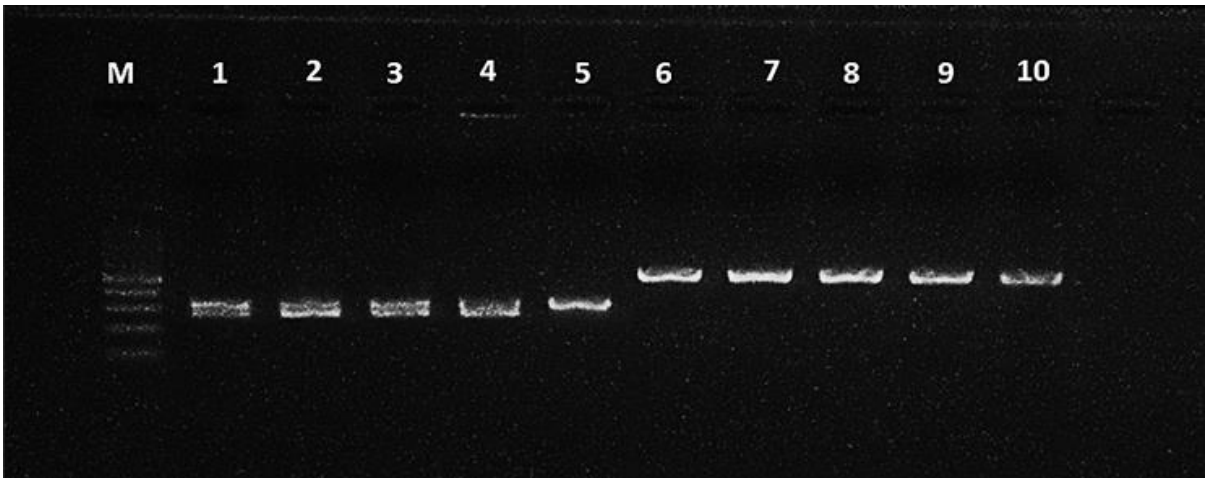

Fig. (4): Detection of *Sul-1* and *bla* TEM gene in *Salmonella* isolates. Positive samples produce band (316 and 517bp), respectively. **Lane M:** 1Kb DNA Ladder, **Lane:** 1, 2, 3, 4 and 5 were positive samples produce band (316bp) for *Sul*-1gene and **Lane**: 6,7,8,9 and 10 were positive samples produce band (517bp) for *bla* TEM gene.

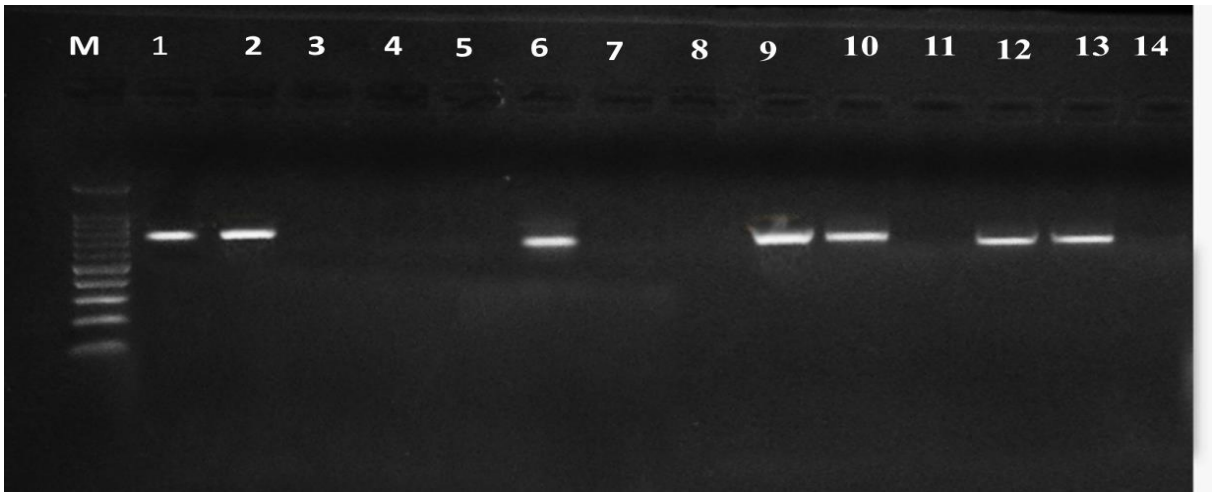

Fig. (5): Detection of floR gene in Salmonella isolates. Positive samples produce band (888pb), Lane M: 1Kb DNA Ladder, Lane: 1, 2, 6,9,10,12 and 13 were positive samples produce band (888bp) while, Lane: 3,4,5,7,8,11 and 14 were negative samples**.**

Fig. (6): Detection of *strA-strB* and *aadA* genes in *Salmonella* isolates. positive samples produce bands (891 and 525bp) respectively, **Lane M:** 1Kb DNA Ladder, **Lane**: 1,2,3,4,6,7,8,9,11 were positive samples produce band of 525bp for *aadA* gene. while, **Lane**: 3,4,5,7,8,9,10,11 were positive samples produce band (891bp) for *strA-strB* gene and **lane**: 3,4,7, 8,9,11were positive samples produce 2 bands (891 and 525bp) for *strA-strB and aadA* genes, respectively.

Table 4: Distribution of resistance genes among *Salmonella* isolates with different serotypes.

Serotype (No.tested)	***No. of resistance genes positive isolates (%)***				
	Sul-1	***blaTEM***	***StrA-StrB***	***aadA***	***floR***
S.typhimurium(14)	14(100)	10 (71.4)	10(71.4)	9(64.3)	7(50%)
S.infantis (1)	1(100)	1(100)	1(100)	1(100)	-
Total examined (15)	15(100)	11(73.3)	11(73.3)	10(66.7)	11(46.7)

Sul-1= resistant gene for sulfonamide.
***Bla* TEM**= resistant genes for β-lactams
FloR= resistant gene for florfenicol.
aad*A** and ***strA-strB= resistant gene for streptomycin.

DISCUSSION

Pathogenic *Salmonella* isolates in ducklings and duck farms can be identified using Polymerase Chain Reaction (PCR) (Yang *et al*., 2019). The invasion gene (*invA*) encodes a protein found in bacteria's inner membrane that is required for invasion of the host's intestinal mucosa (Singh *et al*., 2013) and a common unique marker gene in all isolates of *Salmonella* species (Liu *et al*., 2012). In this study, The PCR confirmed the conventional tests performed and all 15 examined isolates were positive for *invA* gene with 100% specificity, the size of amplified product was 284bp as shown in Fig. (1). similar findings have been described by (Elgohary *et al*., 2017) who detected *invA* genes in all *Salmonella* serovars isolated from duck farms.

Fourteen *salmonella* isolates (93.3%) were positive for *Rfbj* and *fliC* genes indicating *S. typhimurium* and one (6.7%) was positive for *fljB* gene indicating *S. infantis* as shown in Fig. (2). The predominant serotype in this study was *S. typhimurium* in duck farms. These results went in parallel with these reported by (Niu *et al.,* 2020) who found that *S. typhimurium* was the common serotype recovered from ducks in China, (Abel-Tawab *et al.,* 2020 and El shabrawy *et al.,* 2021) who reported that *S. typhimurium* was the predominant isolated from ducks in Egypt these results in contrast to (Enany *et al.,* 2018) who found that *S. ruzizi, S. give and S. entertidis* isolated from local duckling with 0.5% for each. (Han *et al.,* 2020) who found that S. Indiana (26.3%) as one of the prevalent serovars in duck carcasses from China. The 2nd common serotype in this study was *S. infantis* (6.7%) and detected in high percentage (14.6%) in retail duck meat in China by (Chen *et al.,* 2020). This was due to the fact that the distribution of the most common *Salmonella* serotypes is largely determined by geographical factors that change over time (Huehn *et al.,* 2010), and may be related to sampling methods and isolation techniques (Vanantwerpen *et al.,* 2016), despite the fact that several serotypes are consistently detected at a high rate around the world (Gast, 2007).

According to the results concerning antimicrobial susceptibility test presented in Fig. (3). Fifteen *salmonella* isolates showed the highest percentage of resistance (100%) to cephradine and amoxicillin followed by colistin sulfate (80%), streptomycin (60%) and chloramphenicol (33.3%). These findings were higher than those reported by (Abouzeid *et al.,* 2020) who documented that amoxicillin/clavulanic resistance was 70% in Salmonella isolated from diarrheic ducklings. In contrary to these results (Abd El-Tawab *et al.,* 2018) who found that *salmonella* isolated from laying ducks were sensitive to amoxicillin and streptomycin. This was associated to excessive use of these antibacterial agents in duck farms as result of the increased rates of duck diseases due to the development of intensive animal husbandry and high stocking density (Guo *et al.,* 2020).

In this work, all *Salmonella* isolates were 100% sensitive to amikacin, ciprofloxacin, enrofloxacin and trimethoprim/ sulphamethoxazole, followed by tetracycline (86.7%), gentamicin (80%), ampicillin and chloramphenicol (60% for each). These results to some extent agree with (Abouzeid *et al.,* 2020) who reported that *salmonella* isolates were amikacin

sensitive by 100%, followed by gentamicin and sulphamethoxazole/ trimethoprim (50% for each). In contrary to these results (El-shabrawy *et al.,* 2021) who found that salmonella isolates displayed high resistance rate to tetracycline (85%), amikacin and sulphamethoxazole/ trimethoprim (62.8% for each) and ampicillin (51.4%).

In this study, antibacterial sensitivity test by using MIC showed that all examined *salmonella* isolates were absolutely sensitive to colistin and doxycycline, while the highest rate of resistance was against sulfaquinoxalin, cephradine, amoxicillin and florfenicol, while variable degree of resistance for streptomycin, gentamicin and neomycin. these findings nearly in agreement with (Chen *et al.,* 2020) who reported that the highest levels of resistance were observed for sulfadiazine, followed by florfenicol, streptomycin, and gentamicin. These findings differed with (Zhao *et al*., 2017) stated that most 56 isolates recovered from ducks were resistant to tetracycline, ampicillin and ciprofloxacin.

In this study, all fifteen *Salmonella* isolates (100%) were resistant to at least two antibacterial agents, while 93.3% (14/15) of the examined isolates exhibited Multidrug resistant (MDR) were resistant to 3or more antibacterial agents and Resistance to 3-8 antibacterial agents was detected in 12 isolates (80%), 2 isolates (14.3%) were resistant to 9-11 antibacterial agents. These results were higher than (Chen *et al.,* 2020) investigated that 133 (88.1%) of *salmonella* isolates exhibited MDR, (Han *et al.,* 2020) who found that 63.5% of *Salmonella* isolates were classified as MDR which were resistant to 3 or more antimicrobial agents.

PCR was a perfect tool for perfect detection of *Salmonella* resistant genes and the results that the *sul-1*gene, a gene encoded for sulfonamide resistance was reported in the present study with a percentage of 100% among *Salmonella* isolates as shown in Fig. (4). these results consistently, (Niu *et al.,* 2020) who detected *sul-1* gene in 92 *salmonella* isolates isolated from duck farms in south China was 97.8%, these results were higher than results obtained by (Chen *et al.,* 2020) who found that *sul-1* gene with percentage of 63% and (Abd El-Tawab *et al.,* 2015) who reported that *Sul*-1with percentage of 87%.

The *bla* TEM gene, a gene encoded for β-lactamases resistance was reported in the present study with a percentage of 73.3% (11 out of 15 isolates) which giving amplification at 517bp fragments as shown in Fig. (4). These results were higher than (Abdallah *et al.,* 2015) who reported that *bla* TEM gene with percentage of 41.2% and (Zhao *et al.,* 2017) who reported that *bla* TEM gene with percentage of 35.7% among 56 isolates recovered from ducks.

The *floR* gene, a gene encoded for florphenicol resistance was reported in the present study with a percentage of 46.7 % (7 out of 15 isolates) which giving amplification at 888 bp fragments as shown in Fig. (5). These results were higher than results obtained by (Zhao *et al.,* 2017) who reported that *floR* gene with percentage of 23.2% among 56 isolates recovered from ducks. These results were lower than results obtained by (Abd El-Tawab *et*

al., 2015) who reported that *floR* gene with percentage of 77.8% and (Niu *et al.,* 2020) who detected *flo*R in 92 *salmonella* isolates obtained from duck farms in south China was 97.8%.

The *StrA-strB* and *aadA* genes, genes encoded for streptomycin resistance were reported with a percentage of (11 out of 15 isolates) 73.3 % and (10 out of 15 isolates) 66.7 %, respectively as shown in Fig. (6) these results were lower than results obtained by (Niu *et al.,* 2020) who detected *aadA1* in 92 *salmonella* isolates obtained from duck farms in south China was 100%, (Chen *et al.,* 2020) who documented that *StrA* gene and *aadA1* with percentage of 94.1% and 83.8%, respectively while these results were higher than (Abd El-Tawab *et al.,* 2015) who reported that *aadA2* gene with percentage of 53.1%, (Abdallah *et al.,* 2015) who reported that *aadA2* gene with percentage of 47%. The detection rates of resistant genes greatly differ among diverse studies due to the various circumstances and dosages of antibiotics used in the farms (Niu *et al.,* 2020) and as result of the excessive number of related genes that mediate resistance to these antibacterial agents or alternatively, due to changes in the *Salmonella* resistance mechanism result from geographical or other factors (Chen *et al.,* 2020).

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التوصيف الجزيئى للجينات المقاومة للمضادات البكتيرية للسالمونيلا فى البط

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نتيجة للخسائر الإقتصادية الناجمة عن محاولة التغلب على الإصابة بالسالمونيلا فى الدواجن والتكاليف الباهظة لمنع إنتشارها ومقاومتها المتعددة للأدوية. فقد حظيت الإصابة بهذه البكتيريا لا سيما فى البط باهتمام الكثيرين لكونه أهم مستودعات السالمونيلا التي تنتقل إلى الإنسان. ولذلك هدفت هذه الدراسة الى التحرى عن انتشار السالمونيلا في البط بمحافظة أسيوط وتقييم مدى حساسيتها لمضادات البكتيرية المتاحة فقد تم تجميع ٥٦٠ عينة (١٥٠ كبدًا و٤١٠ مسحة مجمع) من بط سليم ظاهريا أومصاب أو نافق حديثا. وخضعت العينات للاختبارات البكترولوجيه والسيرولوجية (وفقا لمخطط Kauffman-White) والبيولوجية الجزيئية ثم دراسة حساسيتها لمضادات الحيوية المختلفة باستخدام اختبار الحساسية وتاثير اقل جرعه مثبطه من هذه الادوية، والتحقق من الجينات المسئولة عن مقاومة المضادات الحيوية جينيا باستخدام تفاعل البلمرة المتسلسل.

وكشفت النتائج عن ١٦,٦٪ نسبة الإصابة الكلية بالسالمونيلا في البط، حيث تم تعريف ١٥ معزولة من السالمونيلا (سيرولوجيا وجينيا) كسالمونيلا تيفيميوريم (٩٣,٣٪) وسالمونيلا انفانتيس (٦,٧٪). وتم اجراء اختبار الحساسية معمليا لهذه المعزولات ضد المضادات البكتيرية المختلفة بإستخدام disc diffusion technique و MIC test والتى بينت أن معظم المعزولات مقاومة بنسبة ١٠٠% لكل من الاموكسيللين وسيفرادين وحساسة بنسبة ١٠٠% لكل من للاجيال الجديدة من المضادات البكتيرية وهى السيبروفلوكساسين وإنروفلوكساسين والسلفاميثوكسازول وترای ميثوبريم والأميكسين , وكانت المقاومة متفاوتة لباقى المضادات البكتيرية. عند دراسه مدى تاثير اقل جرعه مثبطه من المضادات البكتيرية علي معزولات السالمونيلا وجد أن جميع معزولات المختبرة كانت حساسة تماما للكوليستين والدوكسي سيكلين بينما كان أعلى نسبة مقاومة ضد سلفاكينوكسالين وسيفرادين وأموكسيسيلين وفلورفينيكول والستربتومايسين. وبعد ذلك تم تطبيق (PCR) للكشف عن الجينات المقاومة لمضادات مختلفة. كانت جينات المقاومة التي تم فحصها هي *bla TEM* لمعزولات مقاومة للبيتالاكتام ، و *floR* لمعزولات مقاومة للفلورفينيكول ، و*Sul1* لمعزولات مقاومة للسلفوناميد ، وال *aadA* و *StrA- StraB* لمعزولات مقاومة للستربتومايسين. ونسبة حدوث هذه الجينات المقاومة في المعزولات التى فحصت (١٠٠٪) للجين المقاومة الخاص بالسلفوناميد (*Sul*-1) ، (٧٣,٣٪) للجين المقاومة الخاص بالستربتومايسين والبيتالاكتام (*strA-strB* و *bla TEM*)، (٦٦,٧٪) للجين الخاص بالستربتومايسين (*aadA*) و (٤٦,٧٪) للجين المقاومة الخاص بالفلورفينيكول (*FloR*).