Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

Since 1990

Original Paper

Bacteriological and molecular studies on Salmonella isolated from duckling farms at Kaliobia, Egypt

Ashraf A. Abd El Tawab[1], Ahmed A. A. Maarouf[2], Fatma I. El Hofy[1] and Ibtehal M. S. Abd El- Ghaffar[3]

[1]*Bacteriology, Immunology and Mycology Dep., Fac. Vet. Med., Benha Univ.*

[2]*Animal Health Research Institute "Benha branch" ARC*

[3] *Veterinarian*

ARTICLE INFO

Keywords

Antibiotic resistant
Duckling
Salmonella
Virulence genes

Received 11/08/2020
Accepted 04/09/2020
Available On-Line
01/10/2020

ABSTRACT

This study was conducted on 21 commercial duckling farms (1- 20 days old) inspected to show Salmonella infection in different localities at Kaliobia Governorate. Samples were taken from diseased ducklings and freshly dead for bacteriological examination which resulted in, 94 samples were positive from 630 isolates, where 28 isolates from 33 diseased ducklings and 66 isolates from 72 freshly dead ducklings. Three serogroups of Salmonella were obtained by serological identification (*Salmonella* Typhimurium, *Salmonella* Enteritidis and *Salmonella* Blegdam). The antibiotic sensitivity tests for the isolated strains showed multiple antibiotic resistances (oxytetracycline; amoxicillin; ampicillin; streptomycin; erythromycin and trimethoprim/ sulphamethoxazol) but gentamycin, norfloxacin and ciprofloxacin are the most effective antibiotic on the isolated Salmonella and can be used for treatment of Salmonellosis in duck farms. PCR results appeared that, *inv*A and *stn* genes were detected in all studied Salmonella isolates; *pef*A gene was detected in four out of five studied isolates but *sef*C gene was detected in two isolates only. Finally, isolated Salmonellae are virulant pathogens responsible for disease in ducklings resulting in high mortality and morbidity, gentamycin, norfloxacin and ciprofloxacin are the most proper antibiotics used for treatment of Salmonellosis in duck farms.

1. INTRODUCTION

Salmonella infection is one of the most important duck diseases with a significant economic losses and public health importance as infected duck flocks are considered the most important reservoir of Salmonellae which can transmit it to human (Yang *et al*., 2019).They are Gram-negative, short plump shaped rods, non- spore forming, non- capsulated, aerobic and facultatively anaerobic organisms and classified under the family Enterobacteriaceae (Mondal *et al*., 2008a). One of the antigens classify Salmonella into serotypes is the "O" antigen determined based on oligosaccharides associated with lipopolysaccharide. Then the "H" antigen is determined based on flagellar proteins (H is short for the German Hauch meaning "breeze"). Since Salmonella typically exhibit phase variation between two motile phenotypes (Chiou *et al*., 2006), different "H" antigens may be expressed. Salmonella that can express only one "H" antigen phase consequently have motile and non-motile phenotypes and are termed monophasic The emergence of antimicrobial resistance among Salmonella strains of poultry origin has important public health implications similar to food poisoning, such as diarrhea and acute gastroenteritis. Several studies resulted in Salmonella infections in human showing drug resistance were caused by strains from poultry (Mondal *et al*., 2008b; Husain, 2010).

It is caused chiefly by a bacterium *Salmonella* Typhimurium and to a less extent by other species of motile Salmonella. The virulence of Salmonella species is associated with a combination of chromosomal and plasmid factors (Oliveira *et al*., 2003). Salmonella produces both endotoxins and exotoxins (Zou *et al*., 2012). The endotoxin is lipid A of the outer membrane lipopolysaccharide (LPS) of Salmonella. The exotoxins are of two type *viz*., cytotoxins and enterotoxins. Differences in virulence among Salmonella serovars have been attributed to the variable acquisition and evolvement of virulence genes (VanAsten and Van Dijk, 2005). Several Salmonella specific virulence genes which takes an important role in the pathogenicity have been identified, that are known to be

* Corresponding author: Ibtehal M. S. Abd El- Ghaffar , ibtehalsaeed31@gmail.com

involved in adhesion and invasion, like sefC; *fim*H; *inv*A; *pef* (Murugkar *et a*l., 2003;Singh *et al*., 2013; Akeem *et al*., 2017) and other genes associated with toxin production *viz*., *stn* (Marcus *et al*., 2000; Singh *et al*., 2013a).According the prevalence and characterization of Salmonella in ducks farms , Salmonellosis is one of the most important diseases facing duck industry in Egypt. Therefore, this study was conducted to throw light over the isolation, identification and characterization of Salmonella species in duckling farms at Kaliobia Governorate, which may provide beneficial information for the development of the duck industry and public health.

2. MATERIAL AND METHODS

2.1. Samples

A total of 21 commercial duckling farms (1- 20 days old) were inspected for Salmonella infection from different localities at Kaliobia Governorate. Samples were taken from internal organs of 105 ducklings from different breeds; 33 diseased and 72 freshly dead ones of different breeds after clinical and postmortem examination. Each organ was taken alone in sterile plastic bags, kept in icebox and transferred with minimum delay to the laboratory.

2.2. Bacteriological examination

The surface of the examined organs was seared by hot spatula, small pieces of them were taken under aseptic condition and putted in sterile Stomacher bag with 45 ml sterile buffered peptone water, then prepared for bacteriological examination following (**APHA, 2001**).

2.2.1. Isolation and identification of Salmonella strains following ISO 6579 (2002) and Markey *et al*. (2013):

The suspected colonies were sub-cultured into nutrient agar plate and incubated at 37°C for 24 hours. Then, the purified colonies were identified morphologically by Gram stain then biochemically and serologically using Salmonella antiserum**.** Typical Salmonella colonies grown on XLD agar medium had a pink color with black center; while on MacConkey's agar the colonies appeared as pale, colorless smooth and transparent; grey- reddish / pink and slightly convex colonies on Brilliant Green agar plate and pale color colonies indicated non-lactose fermenting with or without black centers on Salmonella- Shigella agar. (DENKA SEIKEN Co., Japan) according to Kauffman (1973) and Markey *et al*. (2013).

2.2.2. In-Vitro anti-microbial sensitivity test

In-Vitro sensitivity test was done on each isolated Salmonella isolates to study its antibiotic sensitivity using disc diffusion test according to the recommendation of the Clinical Laboratory and Standards Institute (CLSI, 2018).

2.2.3. Detection of virulence genes in Salmonella isolates by Polymerase Chain Reaction

Genotyping detection of invasin gene (*inv*A); the heat-labile Salmonella enterotoxingene (*stn*); plasmid encoded fimbriae (*pef*A) and *S*. Enteritidis fimbriae (*sef*C) genes**,** in five random Salmonella isolates using PCR, following QIAamp® DNA Mini Kit instructions (Qiagen, Germany, GmbH), Emerald Amp GT PCR mastermix (Takara, Japan) and 1.5% agarose gel electrophoreses (Sambrook *et al.*, 1989) by using the Primers sequences, target genes, amplicons sizes and cycling conditions showed in Table (1).

Table 1 Primers sequences, target genes, amplicons sizes and cycling conditions

Target gene		Primer sequence (5'-3')	Amplified segment (bp.)	Amplification (25; 35 cycles) Primary Denaturation	Secondary denaturation	Annealing	Extension	Final extension	References
invA	F	GTGAAATTATCGCCACGTTCGGGCAA	284 bp.	94˚C 5 min.	94˚C 30 sec.	55˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	Oliveira *et al.* (2003)
	R	TCATCGCACCGTCAAAGGAACC							
Stn	F	TTG TGT CGC TAT CAC TGG CAA CC	617 bp.	94˚C 5 min.	94˚C 30 sec.	59˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Murugkar *et al.* (2003)
	R	ATT CGT AAC CCG CTC TCG TCC							
*pef*A	F	TGT TTC CGG GCT TGT GCT	700 bp.	94˚C 5 min.	94˚C 15 sec.	55 ˚C 45 sec.	72˚C 45 sec.	72˚C 10 min.	
	R	CAG GGC ATT TGC TGA TTC TTC C							
*sef*C	F	GCG AAA ACC AAT GCG ACT GTA	1103 bp.	94˚C 5 min.	94˚C 60 sec.	55 ˚C 60 sec.	72˚C 60 sec.	72˚C 10 min.	
	R	CCC ACC AGA AAC ATT CAT CCC							

3. RESULTS

1. Isolation of Salmonella

The recovered results in Table (2) cleared that, 94 from 630 samples (14.9%) were positive for Salmonella isolation, where 28 isolates (14.1%) were from internal organs of 33 diseased ducklings and 66 isolates (15.3%) from 72 freshly dead ducklings. Moreover, Salmonella spp. isolates were isolated mostly from 71 intestine samples (75.5%) succeeded by 9 from heart blood samples (9.6%) then 7 from liver samples(7.4%) ; 4 from lung samples (4.3%);2 from spleen samples (2.1%) and 1 from kidney samples (1.1%) .

2. Identification of Salmonella isolates

Biochemically, all 94 isolates had characteristic biochemical features as that of Salmonella, where, they were positive for Methyl red test; citrate utilization test; H2S production test; lysine iron agar and nitrate reduction test.

Meanwhile, they were negative for indole; Voges-Proskauer; oxidase and urease tests. Moreover, the serological examination of five random Salmonella isolates Table (3) appeared that, they were serotyped as *Salmonella* Typhimurium (2/5); *Salmonella* Enteritidis (2/5) and *Salmonella* Blegdam (1/5).

3. Antimicrobial susceptibility of isolated Salmonella

The recorded results in (Table, 4) revealed that, the isolated Salmonella were highly sensitive to gentamycin and norfloxacin (84.0% for each) followed by ciprofloxacin (81.9%) then enrofloxacin and florphenicol (76.6% for each) and cefotaxime (61.7%). Meanwhile, they were

intermediate sensitive to neomycin (68.1%) and doxycycline (67.0%). Moreover, they were highly resistant for oxytetracycline (87.2%) followed by amoxicillin (80.8%); ampicillin (79.8%); streptomycin (68.1%); erythromycin (61.7%) and trimethoprim/sulphamethoxazol (59.6%).

Table 2 Prevalence of isolated Salmonella from studied duckling samples

Duckling cases		No. of ducklings	No. of samples	No. of Positive organ samples						Total Positive samples		
				Heart Blood	Lung	Liver	Intestine	Kidney	Spleen	NO.	%[1]	%[2]
Diseased		33	198	2	1	2	23	0	0	28	14.1	29.8
Freshly dead		72	432	7	3	5	48	1	2	66	15.3	70.2
TOTAL	NO.	105	630	9	4	7	71	1	2	94	14.9	100.0
	%[2]			9.6	4.3	7.4	75.5	1.1	2.1	100.0		

Table 3 Serological typing of Salmonella isolates

Serial No.	Serotyping Isolate	Group	O Antigen	H Antigen
1	*Salmonella* Typhimurium	B	1,4,5,12	I: 1,2
2	*Salmonella* Enteritidis	D1	1,9,12	g, m:-
3	*Salmonella* Enteritidis	D1	1,9,12	g, m:-
4	*Salmonella* Blegdam	D1	9,12-:	g, m, q:-
5	*Salmonella* Typhimurium	B	1,4,5,12	I: 1,2

Table 4 Anti-microbial Sensitivity test for isolated Salmonella in vitro

Antimicrobial agents	Disk concentrations	Sensitive		Intermediate		Resistant		AA
		No.	%	No.	%	No.	%	
Amoxicillin	25 µg	1	1.1	17	18.1	76	80.8	R
Ampicillin	10 µg	2	2.1	17	18.1	75	79.8	R
Cefotaxime	30 µg	58	61.7	28	29.8	8	8.5	S
Ciprofloxacin	5 µg	77	81.9	12	12.8	5	5.3	S
Doxycycline	30 µg	17	18.1	63	67.0	14	14.9	IS
Enrofloxacin	5 µg	72	76.6	16	17.0	6	6.4	S
Erythromycin	15 µg	8	8.5	28	29.8	58	61.7	R
Florphenicol	30 µg	72	76.6	14	14.9	8	8.5	S
Gentamicin	10 µg	79	84.0	11	11.7	4	4.3	S
Neomycin	30 µg	17	18.1	64	68.1	13	13.8	IS
Norfloxacin	10 µg	79	84.0	14	14.9	1	1.1	S
Oxytetracycline	30 µg	1	1.1	11	11.7	82	87.2	R
Streptomycin	S/10	3	3.2	27	28.7	64	68.1	R
Trimethoprim/ Sulphamethoxazol	(1.25/23.75) mcg	20	21.3	18	19.1	56	59.6	R

No.: Number of isolates. AA: Antibiogram activity

4. Detection of virulence genes

Five random Salmonella isolates were screened by PCR for the identification of virulence-associated genes (*inv*A, *stn*, *pef*A and *sef*C) and all were positive for at least two of the screened genes. The recovered PCR results in Fig. (1) indicated that, *inv*A gene was amplified in all Salmonella isolates giving product of 284 bp. and also, the *stn* gene was amplified in all five studied Salmonella isolates giving product of 617 bp. (Fig., 2).

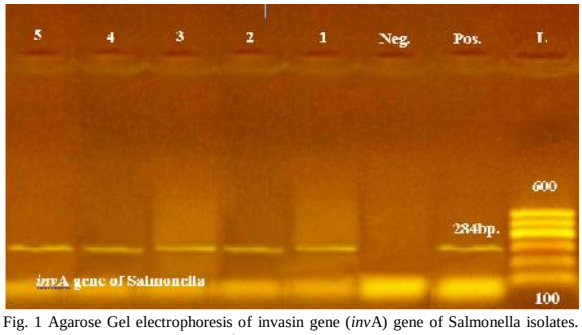

Fig. 1 Agarose Gel electrophoresis of invasin gene (*inv*A) gene of Salmonella isolates. Pos.: Positive control (Salmonella form Ahri .at 284bp.). Lane 1- 5: Positive salmonella isolates

Meanwhile, Fig. (2) showed that, the *pef*A gene was amplified in four out of five studied Salmonella isolates giving product of 700 bp. Moreover, the *sef*C gene was amplified in two out of five studied Salmonella isolates only giving product of 1103 bp. (Fig**.** 4**)**.

4. DISCUSSION

The recovered results in Table (2)were nearly similar to those recorded by Ismail, Rehab(2013); Badr, Heba and Nasef, Soad (2016) and Rahman *et al*. (2016). Meanwhile, they disagree with those of Osman; Kamelia *et al*. (2013); Lebdah *et al*. (2017) and Enany *et al*. (2018) who isolated Salmonella species from internal organs of duck and duckling with lower incidence.

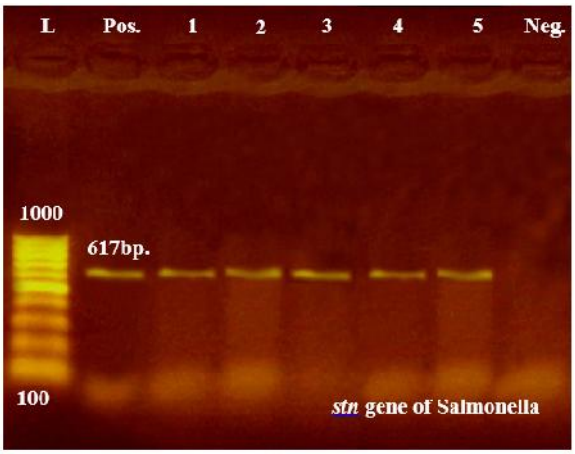

Fig. 2 Agarose Gel electrophoresis of heat-labile Salmonella enterotoxin (*stn*) gene of Salmonella isolates Pos.: Positive control (Salmonella form Ahri .at 617 bp.). Lane 1- 5: Positive Salmonella isolates

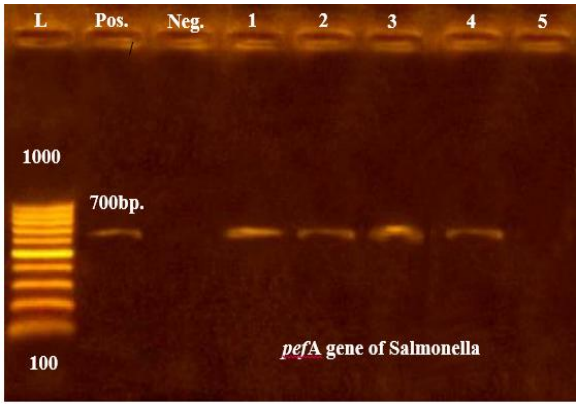

Fig. 3 Agarose Gel electrophoresis of plasmid encoded fimbriae (*pef*A) gene of Salmonella isolates. Pos.: Positive control (Salmonella form Ahri positive .at 700 bp.). Lane 1- 4: Salmonella (Positive for *pef*A gene at 700 bp.). Lane 5: Salmonella (Negative for *pef*A gene at 700 bp.)

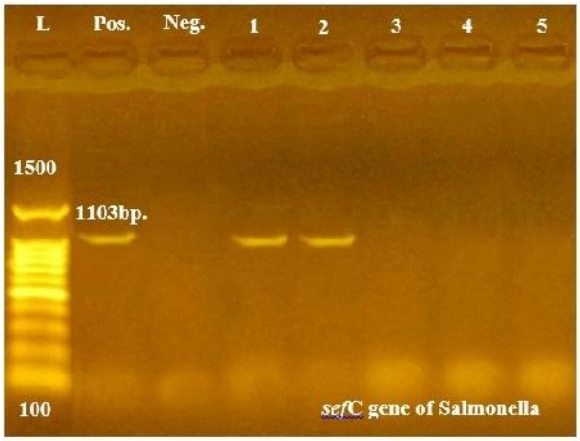

Fig. 4 Agarose Gel electrophoresis of *S.* Enteritidis fimbriae (*sef*C) gene of Salmonella isolates. Pos.: Positive control (Salmonella form Ahri .at 1103 bp). Lane 1- 2: Salmonella (Positive for *sef*C gene at 1103 bp.). Lane 3- 5: Salmonella (Negative for *sef*C gene at 1103 bp.)

The obtained results indicated the acute nature of the disease and the predominant role of Salmonella in causing enteritis and death of ducklings. Moreover, the variation of isolation rates in different localities may be due to the prophylactic and therapeutic use of antibiotics, vaccination against viruses and immune status of ducklings or variation in degree of hygiene and overloading in the farms. Regarding to the phenotypic characters of isolated Salmonella, the colonial appearance and the biochemical profile were similar to those previously reported such as the fermentation of certain sugars or enzymatic reaction that might be characteristic of highly virulent strains associated with Salmonellosis (Markey *et al*., 2013; Rahman *et al*., 2016; Ahmed *et al*., 2019). Moreover, the serogroups obtained Table (3) were came in consistent with those of Adzitey *et al*. (2012); Ismail, Rehab (2013); Osman, Kamelia *et al*. (2013); Lebdah *et al*. (2017), and Enany *et al*. (2018), who recorded the same serotypes from ducklings affected with Salmonellosis. The results of in-vitro antimicrobial sensitivity tests Table (4) were nearly similar to that reported by Adzitey *et al*. (2012); Ismail, Rehab (2013); Badr, Heba and Nasef, Soad (2016); Rahman *et al*. (2016) and Enany *et al*. (2018). High resistance of Salmonella isolates to oxytetracycline, amoxicillin, ampicillin, streptomycin and erythromycin in this finding, indicated that, they are multidrug-resistant strains and of clinical serious concern as these drugs are still considered the most recommended for the treatment of bacterial infections in duck farms in Egypt.

Polymerase chain reaction (PCR) is capable of identifying the pathogenic Salmonella isolates in duckling and duck farms (Gong *et al*., 2014; Lebdah *et al*., 2017; Yang *et al*., 2019).The invasin gene (*inv*A) encodes a protein in the inner membrane of bacteria, which is necessary for invasion of the intestinal mucosa of the host (Singh *et al*., 2013a) and a common unique marker gene in all strains of Salmonella spp. (Liu *et al*., 2012). The results of *inv*A gene amplification in Fig. (1) clarified that, they were Salmonella strains and PCR confirmed the conventional tests performed. Similar detection was recorded by Osman, Kamelia *et al*. (2014); Elgohary, Amany *et al*. (2017) and Yang *et al*. (2019) who detected *inv*Agenes in all Salmonella serovars isolated from duckling and duck farms. Meanwhile, for the heat-labile Salmonella enterotoxin gene (*stn*) serve as effector proteins, which are involved in the pathogenesis of Salmonellosis and diarrhea (Murugkar *et al*., 2003; Singh *et al*., 2013a), the obtained results came in parallel with those of Ismail, Rehab (2013); Elgohary, Amany *et al*. (2017); Lebdah *et al.* (2017) and Enany *et al*. (2018) who detected *stn* genes in all Salmonella serovars isolated from duckling and duck farms. Fimbriae play an important role in the pathogenicity of bacteria, because they promote their attachment to intestinal epithelial cells and encoded by the *pef* operon located in plasmid (Castilla *et al*., 2006).Similar findings of plasmid encoded fimbriae (*pef*A) gene (Fig. 3) in Salmonella strains isolated from duckling and duck farms were recorded by Gong *et al*. (2014) and Elgohary, Amany *et al*. (2017).The fimbriae 14 (SEF14) of Salmonella Enteritidis is encoded by the *sef* operon, which contains *sef*C gene. It contains four major protein subunits *Sef*A, *Sef*B, *Sef*C, and *Sef*D. SEF14 plays important role in the ability of Salmonella to colonize Peyer's patches and in the adhesion and invasion of epithelial cells of the host intestine (Castilla *et al*., 2006). The obtained results for PCR amplification of *S.* Enteritidis fimbriae (*sef*C) gene in Salmonella isolates(Fig., 11) appeared that, it was amplified in two Salmonella isolates only and as this gene is considered to be specific for serovar *Salmonella* Enteritidis (Murugkar *et al*., 2003), so, the two positive isolates were *Salmonella* Enteritidis strains. These results were similar to those obtained by Murugkar *et al*. (2003);Castilla *et al*. (2006); Das *et al*. (2012); Ammar *et al*. (2016) and Akeem *et al*., 2017), who detected *sef*C gene

in *Salmonella* Enteritidis strains isolated from different sources.

5. CONCULSION

Finally the present work concluded that, Salmonella is a serious bacterial pathogen responsible for disease in ducklings resulting in high mortality and morbidity, as they are isolated with high percentages; multiple antibiotic resistances are widely spread among them, but gentamycin, norfloxacin and ciprofloxacin are the most effective against the isolated Salmonella in vitro and for treatment of Salmonellosis in duck farms. Moreover, all studied Salmonella by PCR were pathogenic as they had two virulence genes at least that play a role in pathogenicity and virulence of the Salmonella.

6. REFERENCES

1. Adzitey, F.; Rusul, G. and Huda, N. (2012): Prevalence and antibiotic resistance of Salmonella serovars in ducks, duck rearing and processing environments in Penang, Malaysia. Food Research International, 45: 947–952.
2. Ahmed, A.O.; Raji, M.A.; Mamman, P.H.; Kwanashie, C.N.; Raufu, I.A. ;Aremu, A. andAkorede, G.J. (2019): Salmonellosis: Serotypes, prevalence and multi-drug resistant profiles of *Salmonella enterica* in selected poultry farms, Kwara State, North Central Nigeria. Onderstepoort J. Veterinary Research, 86(1):1-8.
3. Akeem, A.O.; Mamman, P.H.; Raji, M.A.; Kwanashie, C.N.; Raufu, I.A. and Aremu, A. (2017): Distribution of virulence genes in Salmonella serovars isolated from poultry farms in Kwara State, Nigeria. Ceylon J. Science, 46(4): 69-76.
4. Ammar, A. M.; Mohamed, A. A.; Abd El-Hamid, M.I. and El- Azzouny, M. M. (2016): Virulence genotypes of clinical Salmonella Serovars from broilers in Egypt.J. Infect .Des., 10 (4): 337-346.
5. APHA "American Public Health Association" (2001): Compendium of Methods for the Microbiological examination of Foods. 4th Ed. F.P. Downes and K. Ito (editors), APHA. Washington D.C., USA.
6. Badr, Heba, M. and Nasef, Soad A. (2016): Pathogenicity of *Salmonella* Typhimurium isolated from Pekin duck. 3rd Conf. of Sci. Assoc. of An. Health Res. Inst., 10-13/3/2016:267-277.
7. Castilla, K.S.; Ferreira, C.S.A.; Moreno, A.M.; Nunes, I.A. and Ferreira, A.J.P. (2006): Distribution of virulence genes *sef*C, *pef*A and *spv*C in *Salmonella* Enteritidis phage type 4 strains isolated in Brazil. Brazilian J. Microbiology, 37:135-139.
8. Chiou, C. S.; Huang, J. F.; Tsai, L. H.; Hsu, K. M.; Liao, C. S. and Chang, H. L. (2006): "A simple and low-cost paper-bridged method for Salmonella phase reversal". Diagnostic Microbiology and Infectious Disease, 54 (4): 315–317.
9. CLSI" Clinical and Laboratory Standards Institute"(2018): Performance Standards for Antimicrobial Disk Susceptibility Tests.28th Ed. CLSI standard M02. Wayne, Pennsylvania, USA.
10. Das, A.; Hari, S.S.; Shalini, U.; Ganeshkumar, A. and Karthikeyan, M. (2012): Molecular Screening of Virulence Genes from Salmonella enterica Isolated from Commercial Food Stuffs. Biosci., Biotech. Res. Asia, 9(1), 363-369.
11. Elgohary, Amany, H.; AbuElnaga, Azza, S.M.;Ibrahim, Hala, S.; Hedia, Riham, H.; Dorgham, Sohad, M. and Elgabry, E.A. (2017): Detection of virulence genes of Salmonella in diarrhoeic ducks by using Polymerase Chain Reaction (PCR). Egypt. J. Vet. Sci., 48(1): 11-21.
12. Enany, M.E.; Wafaa, M.M. and Reem, M.M. (2018): Comparison between different methods for detection of Salmonella species in imported and local duckling. SCVMJ, XXIII (1):171-180.
13. Gong, J.; Zhang, J.; Xu, M.; Zhu, C.; Yu, Y.; Liu, X.; Kelly, P.; Bu Xu, B. ; Wang, C, (2014): Prevalence and Fimbrial Genotype Distribution of Poultry Salmonella Isolates in China (2006 to 2012). Applied and Environmental Microbiology, 80(2): 687–693.
14. Husain, A. (2010): "Prevalence and antimicrobial resistance of Salmonella serovars isolated from poultry and meat in Hyderabad, Pakistan". Turk. Vet. Anim. Sci., 34(5): 455-460.
15. Ismail, Rehab, H.I. (2013): Some studies on bacterial causes of respiratory troubles in ducklings. M. V. Sci. Thesis (Avian and Rabbit Medicine) Fac. Vet. Med., Zagazig University.
16. ISO "International Organization of Standardization", 6579 (2002): Microbiology of food and animal feeding stuffs- Horizontal method for the detection of Salmonella spp. International standard. (4th edition).
17. Kauffmann, F. (1973): Serological diagnosis of Salmonella spp., Kaufmann White Scheme, Copenhagen, Denmark. J. Acta. Path. Microbiol. Sci., 61:385.
18. Lebdah, M.A.; Eid, Amal, A.M.; Nasef, Soad, A. and Hamad, Enas, M. (2017): Phenotypic and genotypic characterization of Paratyphoid Salmonellae isolated from Poultry in Delta area- Egypt. Zagazig Veterinary J., 45(3): 262-272.
19. Liu, B.; Zhou, X.; Zhang, L.; Liu, W.; Dan, X.; Shi, C. and Shi, X. (2012): Development of a novel multiplex PCR assay for the identification of Salmonella Enterica, Typhimurium and Enteritidis. Food Control, 27(1): 87-93
20. Mondal, T., Khan M. S. R., Alam M. and Purakayastha M. (2008a). "Molecular Characterization of Salmonella Isolates of Duck in Comparison to Salmonella Isolates of Chicken and Ruminants." Bangladesh J. Microbiology, 25(2): 91-94.
21. Mondal, T.; Khan, M. S. R.; Alam, M.; Purakayastha, M. Das, M. and Siddique, M. P. (2008b): Isolation, identification and characterization of Salmonella from duck. Bangladesh J. Vet. Med., 6 (1): 07-12.
22. Murray, P. R.; Baron, E. J.; Pfaller, M. A.; Tenover, F. C. and Yolken, R. H. (1995):"Manual of Clinical Microbiology". Washington, DC: ASM Press.
23. Marcus, S. L.; Brumell, J. H.; Pfeifer, C. G. and Finlay, B. B. (2000): Salmonella pathogenicity islands: big virulence in small packages. Microbial Infection, 2: 145-156.
24. Markey, B.K.; Leonard, F.C.; Archambault, M.; Cullinane, A. and Maguire, D. (2013): Clinical Veterinary Microbiology. Second edition. MOSBY. Elsevier Ltd. Edinburgh London New York Oxford Philadelphia St Louis Sydney Toronto.
25. Murugkar, H.V.; Rahman, H. and Dutta, P.K. (2003): Distribution of virulence genes in Salmonella serovars

isolated from man & animals. Indian J Med Res., 117:66-70.
26. Oliveira, S.D.; Rodenbusch, C.R.; Ce, M.C.; Rocha, S.L.S. and Canal, C.W. (2003): Evaluation of selective and non-selective enrichment PCR procedures for Salmonella detection. Lett. Appl. Microbiol., 36: 217-221
27. Osman, Kamelia, M.; Marouf, S.H.; Zolnikov, Tara R. and Al-Atfeehy,N. (2013): Isolation and characterization of Salmonella enterica in day-old ducklings in Egypt. Pathogens and Global Health, 1:1-12.
28. Osman, Kamelia, M.; Marouf, S.H.; Erfan, A.M. and Al-Atfeehy, N.(2014):*Salmonella enterica* in imported and domestic day-old turkey poults in Egypt: repertoire of virulence genes and their antimicrobial resistance profiles. Rev. Sci. Tech. Off. int. Epiz., 33 (3): 1017-1026.
29. Rahman, M.; Rahman, M.; Meher, M. M.; Khan, S. I. and Anower, A. K. M. (2016): Isolation and antibiogram of Salmonella spp. from duck and pigeon in Dinajpur, Bangladesh. J. Adv. Vet. Anim. Res., 3(4): 386-391.
30. Singh, S.; Singh, H.; Tiwari, S.C.; Prejit Nambiar P. and Agarwal, R.K.(2013a):Characterization of virulence factors among diverse Salmonella serotypes and sources. Adv. Anim. Vet. Sci., 1(2): 69–74.
31. Sambrook, J.; Fritscgh, E. and Meniates, E. (1989): Molecular cloning. A laboratory manual. (1). Cold spring Harbor Laboratory press, New York.
32. Van Asten, A.J.A.M. and Van Dijk, J.E. (2005): Distribution of ''classic'' virulence factors among Salmonella spp. FEMS. Immunol. Med. Microbiol., 44: 251–259.
33. Yang, J.; Zijing,J.; Yang,Y.; Zhao,X.; Jiang,Z. and Sun,S.(2019): Serotype, antimicrobial susceptibility and genotype profiles of Salmonella isolated from duck farms and a slaughterhouse in Shandong province, China. BMC Microbiology, 19:202-214.
34. Zou, M.; Keelara, S. and Thakur, S. (2012): Molecular Characterization of Salmonella enterica Serotype Enteritidis Isolates from Humans by Antimicrobial Resistance, Virulence Genes, and Pulsed-Field Gel Electrophoresis. Foodborne Pathogens and Disease, 9 (3):232-238.