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Molecular detection of antibiotic and disinfectant resistant genes in *Salmonella* and *E. coli* caused omphalitis in chicks

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Abstract

Omphalitis is a major factor responsible for early chick mortality during the first few days after hatching. Therefore, the aim of this study is to investigate the role of omphalitis in the productive traits of broiler chicks and to determine the occurrence of antibiotic and disinfectant resistance genes in *Escherichia coli* and *Salmonella* isolated from chicks with reference to the gross lesion and histopathological changes of the affected organs. Random samples (100 newly hatched chicks showed depression, diarrhoea and an indurated yolk sac) were collected from different farms in Sharkia Governorates, gross lesions were recorded, yolk sac and affected organs were collected and examined for the presence of *Salmonella and E. coli* by standard microbiological techniques and for histopathological studies. Both isolates were tested for their sensitivity against nine antibiotics and subjected to resistant and disinfecting genotyping by polymerase chain reaction (PCR). Results showed that *Escherichia coli* (*E. coli*) in examined sample represented 35% while *Salmonella* represented 20% and the antimicrobial susceptibility patterns showed that both isolates of *E. coli* and *Salmonella* were multidrug resistant and were sensitive to ciprofloxacin and gentamycin, respectively. Histopathology of the affected chicks with both *E.coli* and *Salmonella* revealed several pathological alteration in visceral organs. Concerning antibiotic resistance genes most strains showed phenotypic resistance harbored resistance genes moreover, some isolates contained disinfectant resistance genes. This study proved that early and accurate detection of bacteria is important to undertake appropriate control measure, good management and sanitation as well as use of suitable antibiotics help in reducing mortality.

Keywords: *Omphalitis, E. coli, Salmonella, Antibiotic and disinfectant resistant gene, PCR, Pathology, Sensitivity test, Chicks.*

Introduction

Omphalitis is an infectious and non- contagious condition of yolk sac accompanied by unhealed navels in chicks **(Kahn *et al*., 2008).** Yolk sac infection caused chick mortalites during the first week of the post-hatching period **(Yassin *et al*., 2009)**. *Escherichia coli* (*E. coli*) and Genus *Salmonella* were bacteria that have been isolated from yolk sac infections in chicks in different locations all over the world **(Walker *et al*., 2002).**

Moreover, it has been demonstrated that *E. coli* and *Salmonella* spp. show high incidences of resistance of commercially available antimicrobial agents (**Hyytiä-Trees *et al.*, 2007**). Therefore, these facts imply high risks of disseminating antimicrobial resistant genes between bacterial species and ultimately to human. The generation and transmission mechanisms of the drug resistance genes have become a hot research topic in order to control the spread of these multidrug-resistant bacteria (**Yanhong and Wei, 2009**). In poultry farms antibiotic resistance occurs frequently and can

be spread to humans through food or water chain and also by routes such as environmental contamination by poultry waste and direct interaction with animals (**Velhner *et al.*, 2010)**.
There are several classes of antibiotic resistance genes that confer resistance to different groups of antibiotics. Bacteria can use different mechanisms of resistance to antibiotics, however, many bacterial efflux pumps are able to extrude several unrelated classes of antimicrobial compounds from the cell promoting the development of MDR phenotypes **(Alekshun and Levy, 2007)**.
The Quaternary ammonium compound (QAC) were named after one of their substrates (QACs) they had much wider spectrum of activity **(Hassan *et al*., 2006).** Generally (QACs) used for the control of microorganisms in clinical and industrial environ-ments plus used in the disinfection of hard surfaces **(Ioannou *et al.*, 2007)**. QAC genes in Gram negative bacteria were usually linked to plasmid- mediated class 1 integrons. They were found in combination with genes coding for resistance to Sulphonamides, Trimethoprim, Chloramphenicol, Aminoglycosides and ß- lactams **(Zhao *et al*., 2012).**
It is now accepted that the widespread use of antimicrobials in both animal and human populations has led to the emergence of antimicrobial resistant *Salmonella* and *E. coli* isolates. Furthermore, overuse of antiseptic agents has led to decreased susceptibility to QAC and the emergence of alarming antiseptic resistance which is attributable to *qac* determinants that are mainly plasmid borne and confer resistance by a means of proton motive force-de-pendent multidrug efflux **(Chapman., 2003)** and facilitate the selection of strains that exhibit acquired disinfectant resistance and can carry genes encoding cross-resistance to antibiotics, thereby creating a new threat to public health **(Long *et al*., 2016).** Virulence in these microorganisms is associated with the capacity to attach and colonize at the site of infection with subsequent damage to the host and is promoted by aggressions that interfere with the host defense **(Burrows, 1985).**
Therefore, the aim of this study was to explain the failure of treatment of *E. coli* and *Salmonella* infections in poultry farms that may disseminate the infections to the newly hatched chicks causing high mortalities. Besides that the antibiotic resistance with yolk sac inflammation and the pathological changes occur in infected chicks.

Materials and Methods

Samples Collection:

A total of 100 diseased chicks of one to seven days were collected from different farms in Sharkia Governorates were subjected to clinical and postmortem (P.M) examination for pathological study as well as for isolation and identification of *Escherichia coli* and *Salmonella*, from organs including liver, heart, spleen, intestine, thymus and yolk sac. All samples were collected and handled aseptically to prevent cross contamination using sterile sampling materials (Bags, knifes, flasks, scissors and forceps).

Bacterial isolation

The standard microbiological techniques for isolation and identification of the two isolates. *E.coli* isolation was carried out according to **(*Quinn et al*., 2002)**. *Salmonell*a isolation was done according to the International Organization for Standardization (**ISO 6579, 2002)**.

Antimicrobial susceptibility testing

The in vitro susceptibility of all *E.coli* and *Salmonella* isolates to various routine antimicrobial drugs was tested by the Kirby-Bauer standard agar disk diffusion technique as described earlier **(Bauer *et al.*, 1966)**, using Mueller Hinton agar and commercial antibiotic disks (Oxoid, Basingstoke, Hampshire, England, UK). The tested antibiotics and their concentrations in µg/disk were as following: amoxicillin (Ax;10) amoxicillin/clavulanic acid (AMC; 20/10), ceftriaxone (CTX; 30), doxycycline (DO; 30), sulfamethoxazole/trimethoprim (SXT; 25), chloramphenicol (C; 30), streptomycin (S; 10), gentamicin (CN; 10) and ciprofloxacin (CIP; 5). The inhibition zones, in millimeters, were measured in duplicate and scored as sensitive, intermediate, and resistant categories in accordance with the critical breakpoints recommended by the Clinical and Laboratory Standards Institute **(CLSI, 2011)**. *Salmonella* and *E.coli* isolates serotyped in the Serology

Unit, Animal Health Research Institute, Dokki, Giza, Egypt using commercial antisera (Difco, Detroit, MI, USA) according to the manufacturer's instructions.

Histopathological examination:
The macroscopic as well as the microscopic findings were recorded in the collected samples from liver, heart, spleen, intestine, thymus and yolk sac and were fixed in 10% neutral buffer formalin solution. Samples were dehydrated and embedded in paraffin wax, then sectioned to 4-5 micron thickness and stained by hematoxylin and eosin for histopathological examination **(Suvarna *et al*., 2013).**

Molecular detection of antibiotic and antiseptic resistance genes
Polymerase Chain Reaction (PCR) was used to detect the presence of antimicrobial resistance genes, isolates, which showed resistance to each category of antimicrobial agent, were examined for the presence of resistance genes.
1-DNA Extraction: Genomic DNA of the strains was obtained using the genomic DNA Extraction Kit (QIAamp DNA mini kit; Qiagen, Hilden, Germany) following the manufacturer's instructions. Finally, the template for amplification was obtained, and stored at −20 ° C for use. The presence of genes associated with aminoglycosides (*aad* A) tetracycline (*tet*A), florfenicol (*flo*R), sulfonamide (*sul*1) and Quaternary ammonium compound (*qac* A/B) were detected by PCR amplification. The primer sequences and predicted sizes for PCR amplification of different resistance genes are listed in Table (1). PCR amplification was performed using a DNA thermal cycler (Bio-Rad, S-1000, USA) and were summarized in table (2).

2- Primer selection: PCR was used to detect the presence of antimicrobial resistance genes, primers were prepared in Sigma Company.

3) PCR amplification and cycling protocol (thermo scientific):
DNA samples were amplified in a total of 50 µl of the following reaction mixture: 25µl Thermoscientific Master Mix (2X)**,** 1µl of each primers**,** 3µl template DNA and completed to 50 µl by water, nuclease-free**.** PCR cycling program was performed in Bio-Rad S1000 programmable thermal cycler (USA) as in table (2).

Table (1). Primers used in PCR reactions for the detection of resistance genes.

Gene	Oligonucleotides primer (5'-3')	Fragment size (bp)	Annealing temp. (C)	Ref.
aadA	(F)GTGGATGGCGGCCTGAAGCC (R) AATGCCCAGTCGGCAGC	528	58	**Gebreyes and Thakur (2005)**
tetA	(F)GCTACATCCTGCTTGCCTTC (R) CATAGATCGCCGTGAAGAG	210	58	**Olesen *et al.*, (2004)**
Sul1	(F)GTGACGGTGTTCGGCATTCT (R) TTTACAGGAAGGCCAACGG	668	58	**Levings *et al.*, (2005)**
floR	(F)AACCCGCCCTCTGGATCAAGTCAA (R) CAAATCACGGGCCACGCTGTATC	549	*55*	**Ghoddusi *et al.*, (2015)**
qacA/B	(F)GCAGAAAGTGCAGAGTTCG (R)CCAGTCCAATCATGCCTG	361	*53*	**huanchuen *et al.*, (2007)**

Table (2). PCR cycling program for antimicrobial resistance genes.

Amplified DNA	Initial denaturation	Actual cycles °C/second	Final extension
Tet (A)	95°C for 10 minutes	35 cycles of : Denaturation: 94/45 Annealing: 58/50 Extension: 72/50	72°C for 10 minutes
Sul1 *Aad*A	95°C for 5 minutes	30 cycles of : Denaturation: 95/60 Annealing: 54/60 Extension: 72/60	72°C for 7 minutes
Qac A/B	94°C for 5 minute	35 cycles of : Denaturation: 94/30 Annealing: 53/40 Extension: 72/40	72°C for 7 minutes
FoR	95°C for 6 minute	35 cycles of : Denaturation: 95/60 Annealing: 58/15 Extension: 72/60	72°C for 4 minutes

Detection of PCR products:
Five µl of each amplicon were loaded on 1.5% agarose gel containing 0.5 µg of ethidium bromide. A 100 bp DNA ladder was used as a molecular weight standard (Pharmacia). The samples were electrophoresed at 90 V for 90 minutes on a mini horizontal electrophoresis unit (BIO-RAD, USA); the gel was visualized under UV trans illuminator (Spectroylyne Model TR-312 A) and photographed.

Oligonucleotide primers
Primers used were supplied from Metabion (Germany) and they listed in Table (1).

DNA extraction
DNA extraction from samples was performed using the QIAamp DNA Mini kit (**Qiagen**, **Germany**, **GmbH**) with modifications from the manufacturer's recommendations.

Results and Discussion

Bacterial incidence in examined samples
E. coli was isolated from 35 of 100 examined chicks (35%). They were identified by standard microbiological techniques similar results obtained by **Ashraf *et al*., (2014)** who isolated *E. coli* in Egypt at 38%. On the other hand, higher incidence of *E. coli* 83.9% was mentioned by **Iqbal *et al*., (2006)**. Herein, three serotypes were obtained, including O111, which accounted for 56.25% as the most predominant serotype of total *E.coli* isolates, other serotypes isolated (43.75%) were O158 and O26. These results were agreed to some extent with that obtained by **Ashraf *et al*., (2014)** who reported serotypes O111, O78. Also **El-Sayed *et al.*, (2015**) found the most dominant serotypes of *E. coli* are O111and O55.

Concerning to *Salmonella* it represented 20%, that was close to that (21.99%) reported earlier in Bangladesh by **Rahman *et al.*, (2004)** and that reported in Egypt by **Ammar *et al.*, (2016)**. Many studies showed different prevalence rates of *Salmonella* isolates in broilers worldwide: in Brazil, a low rate of 2.7% was reported by **Medeiros *et al.*, (2011)**. While in China, a high rate of 52.2% was reported by **Yang *et al*., (2011).** These differences in prevalence rates may reflect considerable disparity in the sampling scheme, sample types, *Salmonella* detection protocol, and geographic location.

Serotyping of salmonella isolates revealed that *S. Typhimurium* was the most prevalent serotype (50%) followed by *S..Enteritidis* (35%), *S. Virchow* (15%) **(WHO, 2006).** Poultry are commonly infected by a wide variety of *Salmonella* serovars; one serovar may be a predominant isolate in a country for several years before it is replaced by another serovar. Serovars vary geographically, but clinically significant *S. Typhimurium* and *S. Enteritidis* were identified as the most common serovars reported globally **(WHO, 2006**). Another study in Egypt reported a predominance of *S. Enteritidis* and *S. Typhimurium* from chicken (58.33% and 41.66%, respectively) **(Rabie *et al*., 2012).**

Drug resistance analysis of bacterial isolates
Antibiotic resistance has become a world-wide problem, and the vast consumption of antibiot-

ics by both humans and animals leads to the development and spread of a large number of antibiotic resistances among bacterial populations consequently creating critical public health problems. Different resistance patterns of *Escherichia coli* and *Salmonella* isolates to antibiotics tested in the current study are in consistence with previous related studies on antimicrobial resistance **(Abbassi *et al.*, 2017** and **Kariuki *et al*., 2014)**. The obtained data demonstrated the widespread resistance of *E. coli* and *Salmonella* species to amoxicillin (100%) followed by amoxicillin-clavulanic acid (85% and 90%, respectively as shown in Table (3), as reported previously by **Sultan *et al.*, (2012**), which is most likely resulted from the long-term and widespread abuse of these antimicrobials in animal farms. These results agreed with **Subedi *et al*., (2018)** who showed that the maximum resistance of 50 *E. coli* strains to ampicillin (98%) and similarly **Ashraf *et al.*,(2016)** found that *E. coli* was 93.02% resistant to amoxicillin. Furthermore, **Eid *et al*., (2015)** reported that the highest resistance rates were recorded against doxycycline, tetracycline, and amoxicillin (93.2%, 92.9%, and 92.3%, respectively). On the contrary **Bakheet *et al*., (2017)** recorded that resistant of *E. coli* to sulfamethoxazole/ trimethoprim was 100%.

Interestingly, *Salmonella* was found to be 90% resistant to amoxicillin/cluvulanic acid followed by cefotaxime and doxycycline 50% for both. That result partially agreed with **Ashraf *et al.*, (2016)** and **Ammar *et al*.,(2016).**

On the other hand, the two isolates (*E. coli, Salmonella*) were sensitive for ciprofloxacin (86%, 100%), followed by gentamycin (72%, 75%)), respectively. Similar results obtained by **Ammar *et al.*, (2016)** who found that higher rates of sensitivity of *salmonella* were to ciprofloxacin, gentamicin and chloramphenicol, (100%, 88.24% and 82.35%, respectively). Also, *E. coli* iso-lates were sensitive to levoflox-acin with the percentage of 38.23% **(Waleed *et al.*, 2019**).

Resistance to traditional antibiotics was high in *Salmonella* isolates, previously reported by **Deekshit *et al.*, (2012)**. Herein, most *Salmonella* serovars and *E. coli* were resistant to at least three of the tested antimicrobial agents, making them MDR. This finding was in agreement with that reported in Morocco **Khallaf *et al*., (2014)**, where 42.1% of *Salmonella* isolates showed multiple-drug resistance **Ammar *et al.*, (2016**) who found that nine *Salmonella* strains (52.94%) were resistant to at least three of the nine antimicrobial agents tested, making them MDR, Hence, functional surveillance of antimicrobial resistance and appropriate effective measures geared towards curbing indiscriminate and unregulated use of antibiotics are urgently needed to prevent outbreaks of MDR bacteria in Egypt.

Table (3). Resistance percentage of *E. coli* and *Salmonella* to different antimicrobial agents.

AMA	No. of resistant *E.coli* / Total No. (35)	Resistant percent (%)	No. of resistant *salmonella* / Total No (20).	Resistance percent (%)
AX	35/35	100%	20/20	100%
AMC	30/35	85%	18/20	90%
CTX	28/35	80%	11/20	55%
DO	21/35	60%	10/20	50%
SXT	18/35	50%	7/20	35%
C	18/35	50%	6/20	30%
S	11/35	31%	6/20	30%
CN	10/35	28%	5/20	25%
CIP	5/35	14%	0/20	0%

AMA: antimicrobial agents, AX: Amoxicillin, AMC: Amoxicillin/Clavulanic acid, CTX: Ceftriaxone, DO: Doxycycline, SXT: Sulfamethazol / Trimethoprime, C: Chloramphenicol, S: Streptomycin, CN: Gentamycin, CIP: Ciprofluxacin.

Clinical signs and gross lesions:
Mortality occurs in newly hatched chicks. The affected chicks manifest depression, dropping of the head and huddling near to the heat source. Chicks with Yolk sac infection revealed thickened, inflamed, prominent necrotic navel, poorly healed, open navels or enlarged navels, distended abdomen, unabsorbed yolk materials with putrid smell, subcutaneous edema and bluish colour of abdominal muscles. Often yolks are ruptured and peritonitis is common **Plate. 1: (Fig. A & B).** These results were nearly in accordance with **Rahman *et al*., (2004) and Khan *et al*., (2008).** Omphalitis is a bacterial infection of the navel, just before a chick is hatched, it absorbs the yolk sac, this is done through the navel, once complete, the navel will heal over and dry out, it is at risk of becoming infected with bacteria this finding was in conformity with the reports of **Khalil and El-Shamy, (2012).** Several bacteria such as *E. coli* and *Salmonella* spp have been isolated from the infected chicks our findings are in accordance with that recorded by **white *et al*., (2003), Cortes *et al*., (2004) and Khan *et al*., (2004).** Omphalitis is one of the leading causes of early chick mortalities in newly hatched chicks during the first week post hatching this approved with the results obtained by **Rai *et al*., (2005)**, **Yassein *et al*.**, **(2009)** and **cortes *et al*., (2010).** Similar results were reported by **Rosario *et al*., (2004)** who indicated that the YSI mortality curve lasts (7-10) days, its peak at (4-5) days and decreases during the following (3-5) days and yolk sac infection mortality was highly correlated with E. coli isolation. **Kim and Kim, (2010)** reported that the infection is aggravated by poor hygiene in breeding farms and faulty management at the hatchery, this finding was in conformity with the reports of **Rahman *et al*., (2007)** that assessed 31,45% and 28,42% mortality in chicks due to yolk sac infection, poor hatchery management and hygienic activities adopted in the farm. **Amare *et al.,* (2013)** mentioned that the major gross lesions observed in chicks died of yolk sac infection were unabsorbed yolk sac, congestion and discoloration of the yolk (greenish yellow , dark brown to bright yellow), retained caseous yolk sac and edematous yolk especially in (3-7 -days old chicks) , the yolk sac infection was usually associated with peritonitis, pericarditis, peticheal and ecchymotic hemorrhages on the serosal surface of visceral organs similar results were also in conformity with the reports of different workers, **Sainsbury, (1992)**, **Anjum, (1997)** and **Rai *et al*., (2005)** who reported that putrefaction and offensive odor was observed as characteristic clinical signs of yolk sac infection (omphalitis), unabsorbed yolk sac can be palpated externally at the caudo-ventral aspect of the abdominal cavity, yolk sac content changed from viscid yellow green to watery yellow brown due to denaturation of yolk by bacteria, abdomen of chick appear soft and distended with thickened, inflamed and spoiled vent region. Subcutaneous blood vessels were dilated and engorged with blood , hemorrhagic spots and peritonitis were agreed with **Jordan and Pattison, (1996)** and **cortes *et al*., (2004)**. This study revealed that chicks survived more than four days develop peritonitis, pericarditis or perihepatitis, peticheal and ecchymotic hemorrhage on the serosal surface of visceral organs in addition to omphalitis indicating local and systemic spread of the organism from the yolk sac, this result is potentiated with what had been reached by **Barns and Gross, (1997)**, **Yassein *et al*.**, **(2009) and Amare *et al*., (2013)**. In severe cases the body wall and overlying skin undergo lysis this was in accordance with that recorded by **Saif *et al*., (2003).**

Histopathological findings:
Yolk sac of *E. coli* infected chicks showed inflammatory response which represented by infiltration of inflammatory cells and congested blood vessels. Furthermore, foamy cells which represent enterocyte of the yolk which filled with colloidal material were also seen **Palte II: (Fig. 1, 2 & 3)**, however, Yolk sac of *Salmonella* infected chicks revealed thickened wall by edematous fluid with presence of some inflammatory cells, necrotic areas in addition to presence of congestion and foam cells **(Fig. 4 & 5)**.
Plate III: liver of *E. coli* infected chicks showed replacement of necrotic hepatic area by round cells infiltration **(Fig. 6).** The latter also observed in the perivascular area beside moderate degenerative changes were also seen in some hepatocytes **(Fig. 7).** Moreover, periportal leucocytic infiltration mainly hetero-

philes were also seen **(Fig. 8).** Disseminated thrombi which formed from fibrin threads, RBCs and leukocytes were observed within hepatic blood vessels of *Salmonella* infected chicks **(Fig. 9 & 10).** In addition some cases showed focal coagulative necrosis with diffuse congestion of the hepatic blood vessels **(Fig. 11**). **Plate IV:** heart of *E. coli* infected chicks showed degenerative changes in some cardiomyocytes specially hyaline degeneration **(Fig. 12**). Heart of *Salmonella* infected chicks showed focal apoptotic myocytes and interstitial edema (**Fig. 13).** Furthermore the spleen of *E. coli* and *Salmonella* infected chicks showed depleted lymphoid populations of white pulp and perivascular edema **(Fig. 14 & 15).** Thymus of *E. coli* and *Salmonella* infected chicks revealed depleted lymphoid contents of thymic lobules **(Fig. 16).** Presence of normal Hassal's corpuscle within medullary lymphoid populations was also seen **(Fig. 17**). **Plate V:** intestine of *E. coli* and *Salmonella* infected chicks showed lymphocytic cell infiltration with degeneration of submucosal gland **(Fig. 18)**. The cecum of *E. coli* infected chicks revealed desquamation of lining epithelium and with degenerative and necrotic changes with mild submucosal leukocytic infiltration **(Fig. 19).** However, cecum from *Salmonella* infected chicks showed moderate leukocytic infiltration within lamina propria and submucosa **(Fig. 20).** The importance of life of broilers among the major problems during this period is omphalitis, retained yolk sac or infectious yolk sac (IYS). Diverse species of bacteria cause natural cases of yolk sac infection, *E. coli* is continuously reported as the main pathogen involved in many intestinal and extraintestinal disease conditions in poultry including omphalitis, these results agree with that obtained by **Iqbal *et al.*, (2006), Oh *et al*., (2011) and Amare *et al.*, (2013)**. **El-sawah *et al*., (2018)** recorded that unvaccinated 1-day old male broiler chicks orally inoculated with O27 and O157 containing 9.0 x 10^8 cfu / ml showed congestion of the liver and spleen, later on, severe pericarditis, perihepatitis and gas distended caeci were observed, also liver of chicks orally inoculated at 7^{th} day PI showed severe leucocytic infiltration, severe degenerative changes of hepatocytes and congestion, the cecum showed moderate leucocytic infiltration with degenerative changes**. Richard, (2013)** mentioned that infection with bacteria of the genus *Salmonella* are responsible for both acute and chronic poultry diseases characterized by hemolysis and presence of necrotic lesions in the heart and alimentary tract. The cardiac lesions are characterized by necrosis of myofibers with infiltration of heterophils, lymphocytes and plasma cells being replaced by histocytes as disease progresses into a more chronic phase of infection. Chicks in peracute cases , only severe vascular congestion in various organs especially liver and spleen. In acute to subacute cases, there is multifocal necrosis of hepatocytes with accumulation of fibrin and infiltration of heterophils in the hepatic parenchyma, periportal infiltration of heterophils mixed with a few lymphocytes and plasma cells. The spleen may have severe congestion or fibrin exudation of vascular sinuses in acute stages and severe hyperplasia of the mononuclear phagocytic system cells in later stages. The caeca in young chicks may have extensive necrosis of the mucosa and submucosa with accumulation of necrotic debris mixed with fibrin and heterophils in the lumen, other changes such as serositis of various organs including the pericardium, pleuroperitoneum, synovium and serosa of the intestinal tract can be seen, also **Desmidt *et al*., (1998)** reported that oral dose of 500 CFU of *Salmonella* hadar (SH) sufficient to infect all chicks at 1-day old, serous typhlitis and ompalitis were the main lesions. Hyperemia was observed in the lamina propria of caecal tonsils. The number of macrophages in the lamina propria of the villi was slightly increased from 18h until 2-weeks PI. In the liver inflammation was observed in the portal triads and in the sinusoids. Infection with S. hadar lead to intense colonization of the gut and extensive faecal shedding and it may also cause invasive infections in 1-day old chicks. This rather low dose of infection (500 CFU) which might approximate the doses of Salmonella that lead to natural infection of 1-day old chicks in hatcheries, these findings are in accordance with that recorded by **Cox *et al*., (1991)** who found that the samples from hatcheries had more than 10^2 *Salmonella* per gram of hatchery fluff. The yolk sac is regarded as a

diverticulum of small intestine, this mean that material is absorbed directly from the sac into the blood stream, consequently retained yolk sac will predispose for maternal antibodies, absorption of toxins and spread of *E. coli* by extension into the body, this as reported by **Khan *et al*., (2008)** and **Khalaf *et al*., (2015).** There are many causes for omphalitis in chicks that include colibacillosis and *Salmonellosis,* the result is parallel to that reported by **Khalil and El-Shamy, (2012).** Similar result also reported by **Oh *et al*., (2011)** who reported that the isolated *E. coli* and *Salmonella* spreads into several internal organs of chicks and causes systemic fatal disease which is charactarized by septicemia with multiple organ lesions, pericarditis, perihepatitis, peritonitis and other extra- intestinal lesions.

Detection of disinfectant and antibiotic resistance genes of isolated *E. coli* and *Salmonella* by PCR:

Screening the presence of *tetA*, *aad*A, *sul*1, *flo*R. *qac A/*and *qac B* genes in the most MDR *E. coli* and *Salmonella* after DNA extraction by PCR technique as showed in **Fig** (**21**:**28**) revealed that distribution of resistance genes among five phenotypically resistant *E. coli* were as the following: two out of five subjected isolates to PCR were positive for *flo*R, *Sul1* and *tetA* genes with percentage of (40%) while four and three isolates were positive for *aadA* (80%) and *qacA/B* (60%), respectively.

The *tetA* gene was tested for isolated *E. coli* to assess its resistance to tetracycline. Interestingly, the positive PCR percentage was (40%). However, the phenotypic antibi-otic susceptibility test was 60%, which may be related to more genes than *tetA* gene contributing for tetracycline resistance in *E. coli*. This finding agreed with previously illustrated by **Abo-Amer *et al.,* (2018)** who found that *tetA* gene was represented by (65%). Higher percent of genotypic resistant were recorded by **Sengelov *et al*., (2003**) who found that *tetA* was 72.2%. Concerning to *flo*R gene it represented about 40%, Meanwhile, **Kathleen *et al.,* (2000)** recoded that three of the four isolates contained *flor* on high-molecular-weight plasmids of 186 and 204 kb. The florfenicol resistance determinant appears to be present in a variety of large-molecular-weight DNA fragments in avian *E. coli*.

Regarding to the subjected isolates of *Salmonella* to PCR, results were relatively in accordance with those of antimicrobial susceptibility test as they were positive for *aadA* (100%), but *Sul1 genes* and floR were (80%). Meanwhile, three isolates were (60%) and two (40%) show positive results for, *tetA* and *qacA/B* genes, respectively. Similarly another study reported that twelve of the 17 sulfonamide resistant isolates contained a *sul*-type gene (70%) **(Leila *et al.,* 2012)** while he found that among the 33 streptomycin-resistant isolates, the *strA-strB* and *aadA* genes were found in 6 and 17 isolates, respectively, and also found that among the 21 tetracycline- resistant isolates, 15 of them carried the *tet* (A) gene (71.4%), but *tet* (B) and *tet*(C) were not detected. The *tet* (A) gene may be genotypic detected lower than the phenotypic results as this gene associated with tetracycline efflux pumps that was reported to be the predominant one in *Salmonella* and *E. coli* isolates from livestock and food animals. Conversely, a discrepancy between genotypic and phenotypic detection of antimicrobial sensitivity patterns was evidenced by the absence of these genes in the five resistant isolates. The reason for this difference is the possibility of carrying other drug resistance genes or harboring some other antimicrobial resistance phenomenon **(Miko *et al.*, 2005)** and **(Jouini *et al.,* 2009)**.

Similarities in genetic profiles among *E. coli* and *Salmonella* isolates carrying resistant genes may be linked to clonal expansion of these bacteria. Some resistant genes have been reported to spread through clonal expansion of antimicrobial resistant bacteria, this implies that the resistance genes could have been gained through independent genetic mechanisms, possibly selection for resistance following exposure to antibiotics and through horizontal gene transfer. Previously, resistance caused by expansion of clones and independent strains has been described. This indicated that antimicrobial resistance is a consequence of complex interactions involved in spread of resistance **(Namboodiri *et al*., 2011**).

Most antibiotic resistance genes are found on intregons, plasmids or transposons, which can be transferred and mobilized to other bacteria

of different or the same species. Integrons have been reported to be involved in the acquisition of antibiotic resistance element **(Deekshit *et al.*, 2012).**

Other management strategies for antibiotic resistance includes the following: limiting the non-therapeutic usage of antibiotics for agriculture; improved information to strengthen resolutions on standard therapeutic regimen, education, other actions, coupled with continuous monitoring and validating effectiveness of management strategies **(Global Antibiotic Resistance Partnership-India Working Group, 2011)** and **(Pruden *et al.*, 2013)**. These recommendations could assist in the reduced of antibiotic resistance, directly advance public health, advantageous to the populace and decrease pressure on healthcare system. Finally, enhancing the coverage and types of juvenile vaccines administered by government agencies would enormously decrease the disease burden and circumvent the misuse of antibiotics (**Global Antibiotic Resistance Partnership - India Working Group., 2011)**.

Concerning *qac*A/*B* gene it represented (60%) in *E. coli* however in *salmonella* was (50%)**,** in contrary another study found that *qacA/B* gene was founded only in 14.7% of *E. coli* nevertheless, *qacA/B* was founded in Gram-negative bacteria like *E. coli*. It seems that the presence of the *qac* genes does not necessarily imply increased resistance to antiseptics that could be rele-vant for practice **(Waleed *et al.*, 2019)**. The obtained results were nearly in accordance with **Ashraf *et al.*, (2016)** who found the distribution of *qac*E 1gene was (63.16%) in *E. coli*, as it was detected by PCR in 12 out of the 19 tested isolates. In *Salmonella* as it was detected by PCR in 4 out of the 7 tested isolates (57.14%).

The co-resistance of QAC and antibiotics could be attained by linkage of dif-ferent resistance mechanisms on the similar plasmid, transposon otherwise integrin, or any combination of these (**Hegstad *et al.*, 2010)**. The localization of these QAC determinants on different mobile elements may share in the transmission of resistance to the other bacteria **(Gillings *et al.*, 2009)**. Among Gram-negative bacteria, the *qac* genes are often related with plasmid-mediated class 1 integrons which harbor a diversity of antibiotic resistance genes **(Zhao *et al.*, 2012)**.

Conclusion

The occurrence of multi-drug resistance of bacteria in chicks suffering from omphalitis is alarming as this resistance may gain access to man and animals, which might result in difficulties in treatment of bacterial infection. Since the unabsorbed yolk in the young chicks provides a suitable medium for multiplication of bacteria. So, we recommend strict hygienic measures for receiving new chicks as well as in hatcheries and further investigations for non bacterial causes of omphalitis should be done. Hence, functional surveillance of antimicrobial resistance and appropriate, effective measures geared towards curbing indiscriminate and unregulated use of antibiotics are urgently needed to prevent outbreaks of MDR bacteria in Egypt.

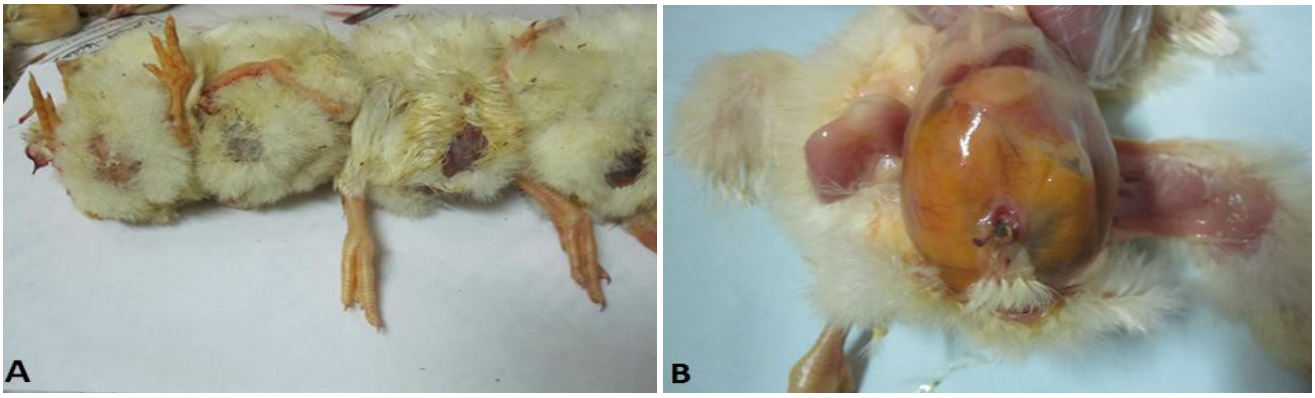

Plate (1): (**A**) yolk sac of infected chick showing inflamation, prominent necrotic navel and (**B**) distended abdomen with unabsorbed yolk materials

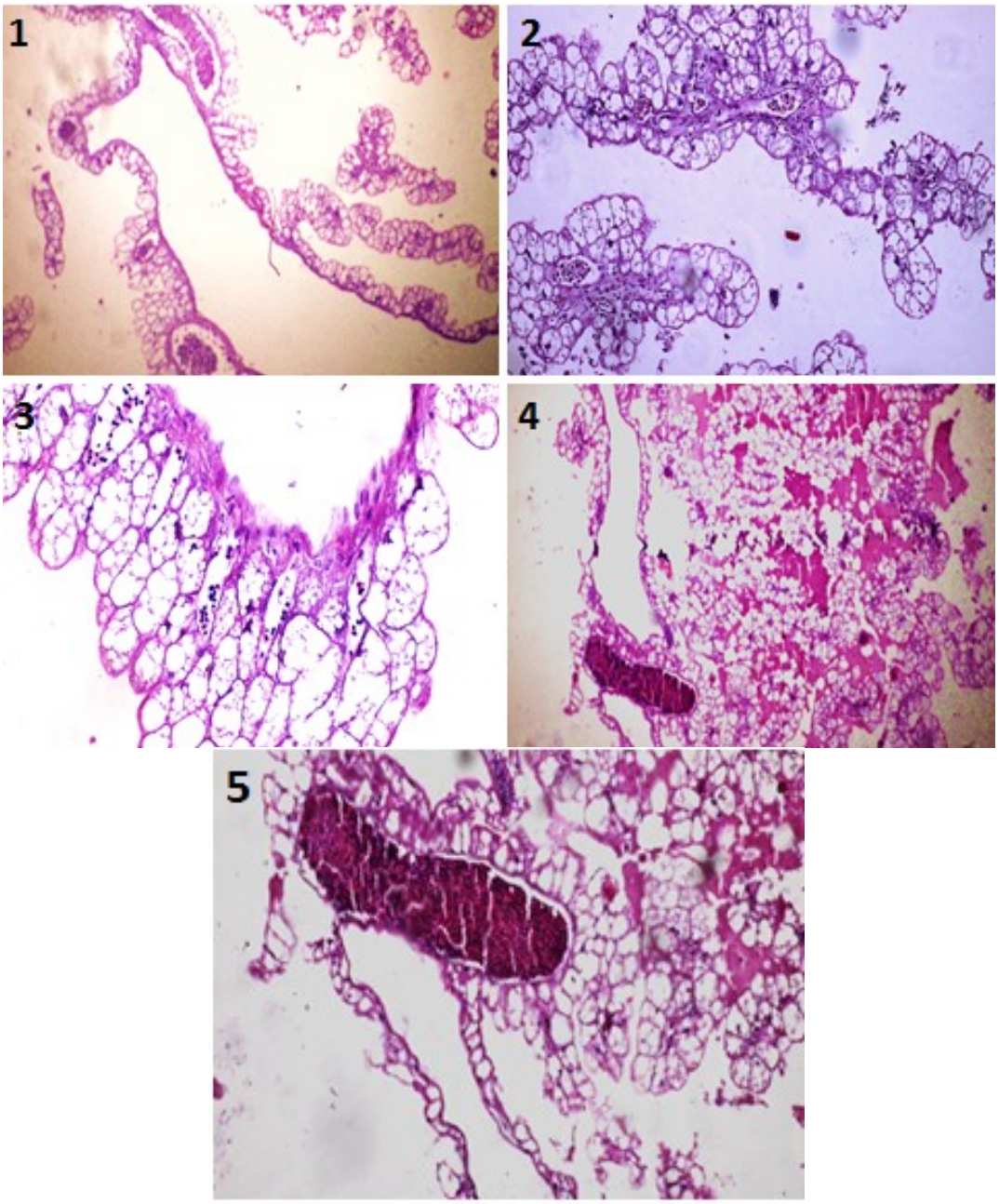

Plate II: **Yolk sac of *E. coli* infected chicks:**
Fig. (1): Showing Congested blood vessels with presence of foam cells. (H&E X100)**.**
Fig. (2): Showing Congested blood vessels with aggregations of inflammatory cells and hyperplasia of foam cells. (H&E X200)**.**
Fig. (3): High power view of the previous figure**.** (H&E X400)**.**
Yolk sac of *Salmonellla* infected chicks:
Fig. (4): Showing severe congestion of the blood vessels and hyperplasia of foam cells. (H&E X100)**.**
Fig. (5)**:** High power view of the previous figure**.** (H&E X400)**.**

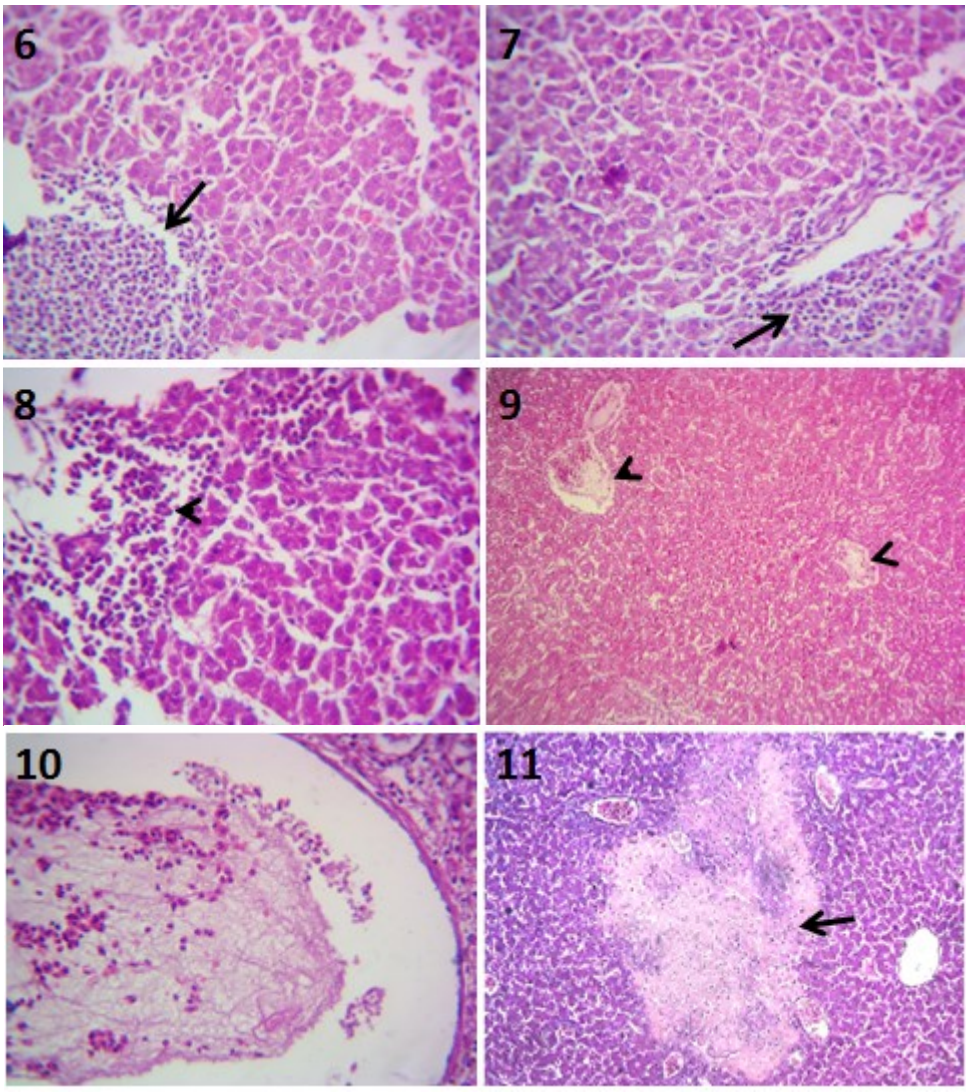

Plate III: Liver of *E. coli* infected chicks:
Fig. (6): Showing round cells infiltration replacing necrotic hepatic area **(arrow)** (H&E X400)**.**
Fig. (7): Showing perivascular round cells infiltration **(arrow)**. (H&E X400)**.**
Fig. (8): Showing periportal heterophilic infiltration **(arrow head).** (H&E X400)**.**
Liver of *Salmonellla* infected chicks:
Fig. (9): Showing disseminated thrombi within hepatic blood vessels **(arrow head).** (H&E X100)**.**
Fig. (10): High power view of the previous figure to show recent thrombus. (H&E X400**).**
Fig. (11): Showing liver of *Salmonella* infected chicks showing focal coagulative necrosis with diffuse congestion of the hepatic blood vesseles (H&E X100)**.**

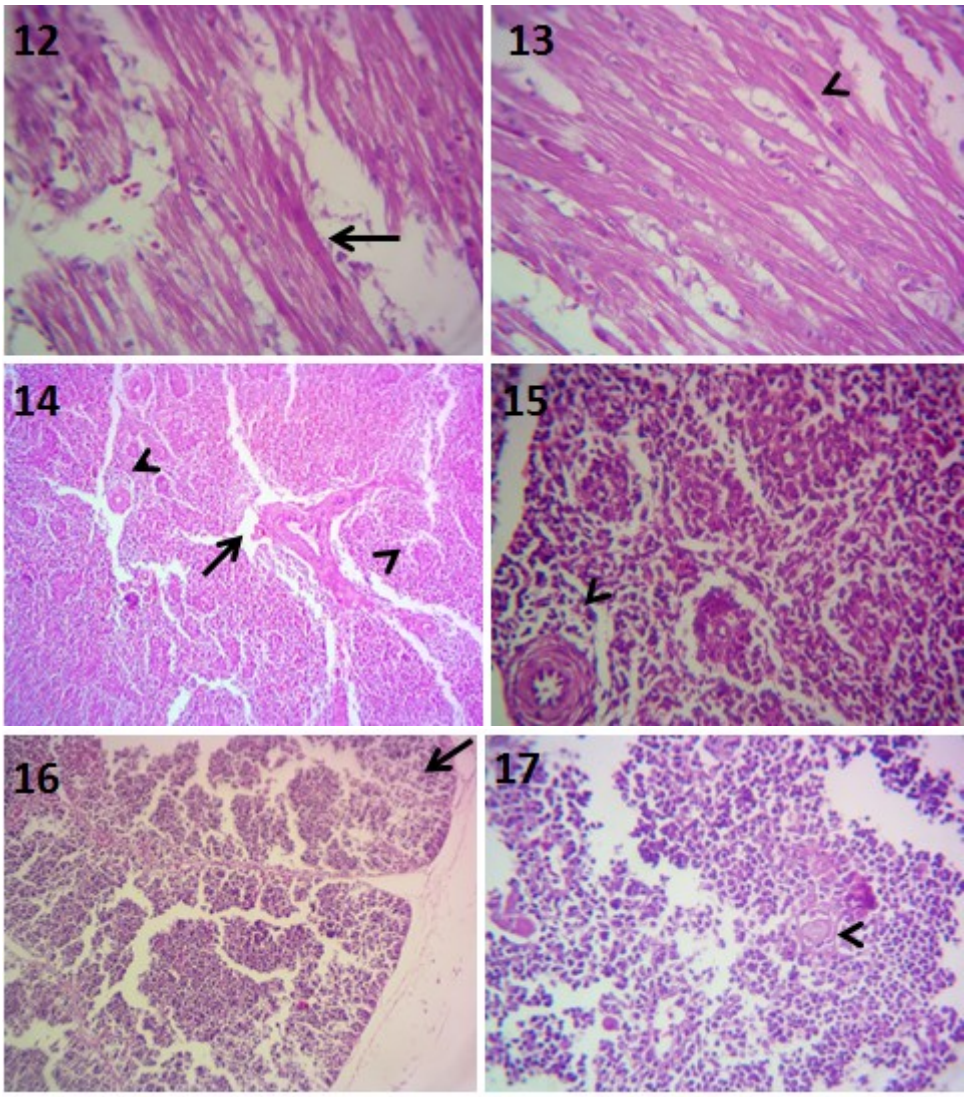

Plate. IV:
Fig. (12): Showing heart of *E. coli* infected chicks showing hyaline degeneration **(arrow).** (H&E X400)**.**
Fig. (13): Showing heart of *Salmonella* infected chicks showing focal apoptotic myocytes **(arrow head).** (H&E X400)**.**
Fig. (14): Showing spleen of *E. coli* infected chicks showing depletion of white pulp lymphoid population **(arrow heads)**. (H&E X100)**.**
Fig. (15): High power view of the previous figure to show lymphocytic depleted areas (**arrow head).** (H&E X400)**.**
Fig. (16): Showing thymus of *E. coli* infected chicks showing depleted lymphocytes **(arrow).** (H&E X100)**.**
Fig. (17): Showing normal hassales corpuscles in medulla **(arrow head)**. (H&E X400**).**

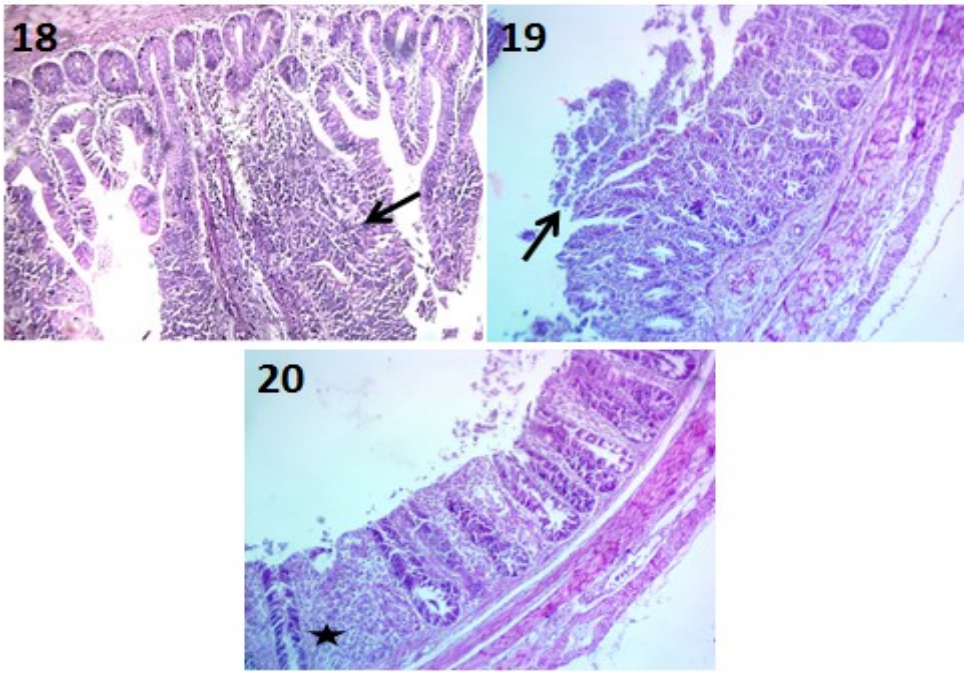

Plate V:
Fig. (18): Showing intestine of *Salmonella* infected chicks showing interstitial lymphocytic cell infiltration **(arrow)** with degeneration of submucosal gland. (H&E X200)**.**
Fig. (19): Showing caecum of *E. coli* infected chicks showing necrotic and desquamated epithelial lining **(arrow).** (H&E X400)**.**
Fig. (20): Showing caecum of *Salmonella* infected chicks showing leukocytic infiltration within lamina propria and submucosa **(star).** (H&E X400)**.**

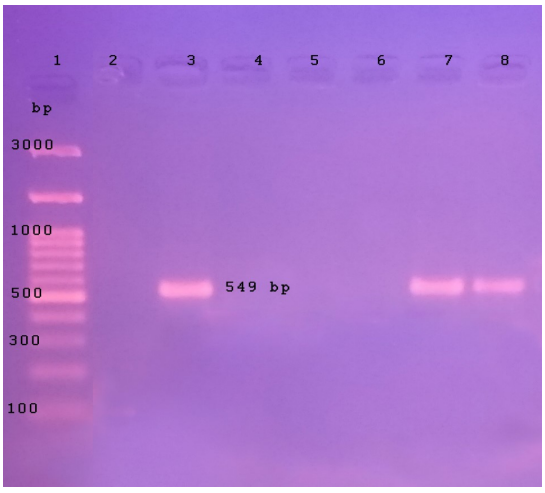

Fig. (21): Results of PCR assays for identification of *floR* gene *E.coli*.
Lane 1: 100 bp ladder,
Lane 2: Negative control,
Lanes 3 Positive control,
Lanes 4-8: Samples
(lanes 7 and 8 give bands at 549 bp).

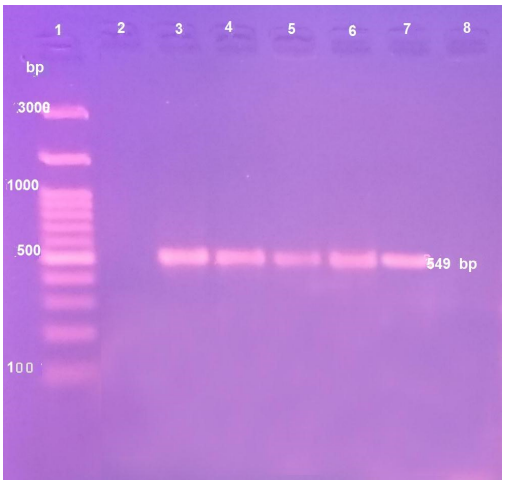

Fig. (22): Results of PCR assays for identification of *floR* gene *Salmonella*.
Lane 1: 100 bp ladder,
Lane 2: Negative control,
Lanes 3: Positive control,
Lanes 4-8: Samples
(lanes 4, 5, 6 and 7 give bands at 549 bp).

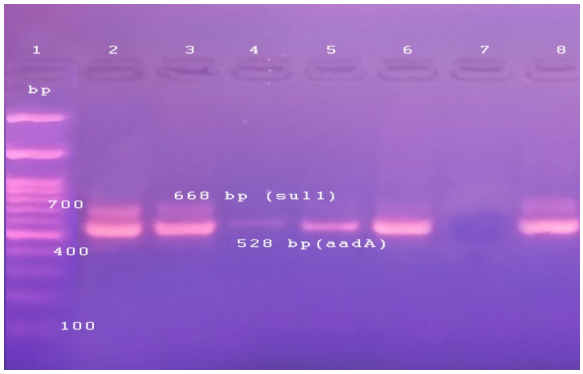

Fig. (23): Results of PCR assays for identification of aadA gene and sul1gene of *E. coli.*
Lane 1: 100 bp ladder,
Lane 2-6: Samples
(lanes 2 and 3 give bands for sul1gene at 668 bp while lanes 2, 3, 5 and 6 give bands for aadA gene at 528 bp),
Lanes 7: Negative control and Lanes 8: Positive control

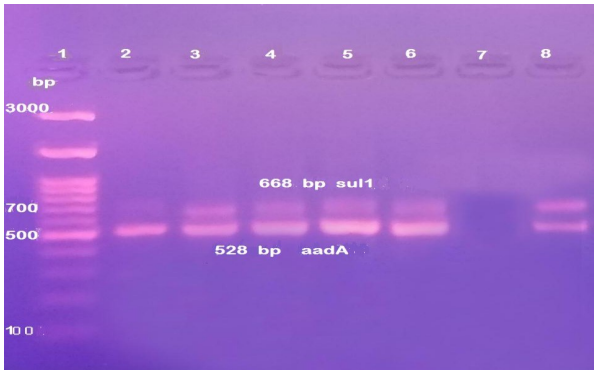

Fig. (24): Results of PCR assays for identification of *aad*A gene and *su*l1gene of *Salmonella*.
Lane 1: 100 bp ladder,
Lane 2-6 : Samples (lanes 3, 4, 5 and 6 give bands for *sul1gene* at 668 bp while lanes 2, 3, 4, 5 and 6 give bands for *aadA* gene at 528 bp), Lanes 7: Negative control and Lanes 8: Positive control

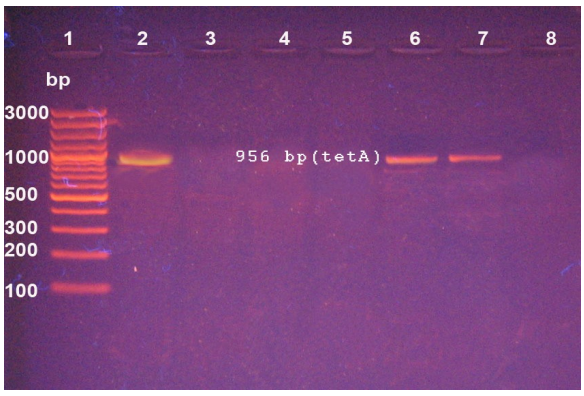

Fig. (25): Results of PCR assays for identification of *tet*A gene of *E.coli*.
Lane 1: 100 bp ladder, Lane 2 : Positive control, Lanes 3: Negative control and Lanes 4-8: Samples (lanes 6 and 7 give bands at 956bp)

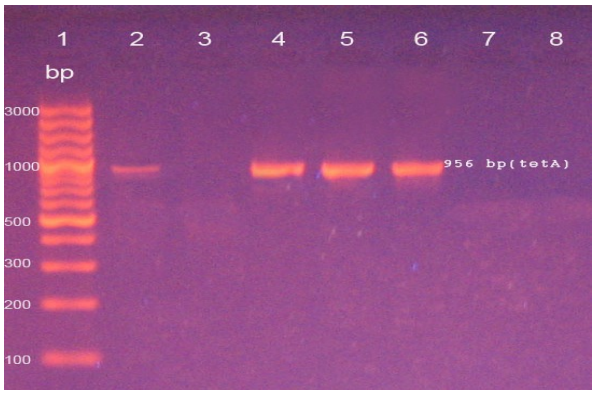

Fig. 26: Results of PCR assays for identification of *tet*A gene of *Salmonella*.
Lane 1: 100 bp ladder, Lane 2 : Positive control , Lane 3:Negative control and Lanes 4-8: Samples (lanes 4,5 and 6 give bands at 956 bp)

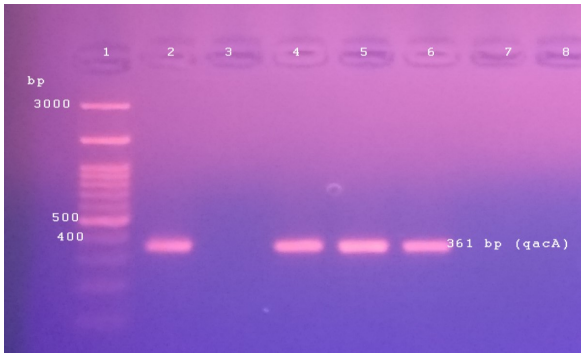

Fig. (27): Results of PCR assays for identification of *qac*A/B gene of *E.coli*.
Lane 1: 100 bp ladder,
Lane 2 : Positive control, Lanes 3: Negative control
Lanes 4-8: Samples (lanes 4, 5 and 6 give bands at 361 bp)

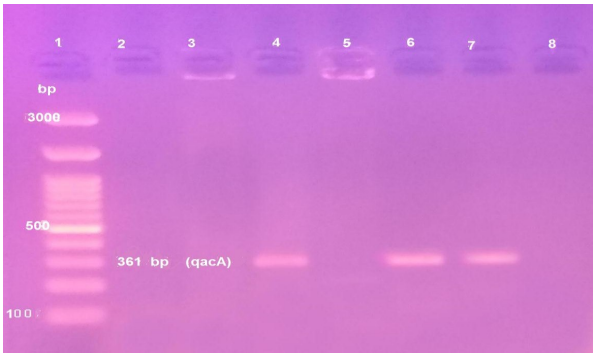

Fig. (28): Results of PCR assays for identification of *qacA/B* gene of *Salmonella*.
Lane 1: 100 bp ladder, Lane 7: Positive control , Lanes 8:Negative control and Lanes 2-6: Samples (lanes 4 and 6 give bands at 361 bp)

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