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FREE RANGING HOUSEHOLD DUCKS, AN OVERVIEW ON ENTERIC BACTERIAL AND PARASITIC INFECTIONS

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ABSTRACT

Ducks are one of the popular household raised poultry in Egypt. Many Egyptian families in Upper and Lower Egypt are adopting the free range raising system where ducks are confined at night and let free at day time for swimming and foraging in the near canals and ponds. Thus, the present study aimed to survey a sample of free ranging ducks (100 birds) and *Tilapia zillii* fish (100 fish) for bacterial infection (E.*coli, Salmonella spp*., and *Pseudomonas spp*.), and for parasitic infection (*Clinostomum species, Echinostoma species, ligula intestinalis species* and *Contraceacum species*). E.*coli* was isolated with prevalence rates (47%) and (59%) from ducks, and *Tilapia zillii*, respectively. *Pseudomonas spp.* was isolated with prevalence rates of (15%, and 34%) from ducks, and *Tilapia zillii, respectively*. *Salmonella spp* was isolated with prevalence rates of (25%, and 9%) from ducks, and *Tilapia zillii*, respectively. Studying the prevalence of helminthes infestations revealed the isolation of *Echinostoma species* with prevalence rates of (13%, and 17%) from ducks, and *Tilapia zillii*, respectively. *Clinostomum species* was isolated with prevalence rates of (15%, and 39%) from ducks, and *Tilapia zillii*, respectively. Parasitic examination also revealed the isolation of *Ligula intestinalis* cestode with prevalence rates of (19%, and 22%) from ducks, and *Tilapia zillii*, respectively. Moreover, *Contraceacum species* was isolated with prevalence rates of (30%, and 46%) from ducks, and *Tilapia zillii*, respectively. Antimicrobial susceptibility patterns of bacterial isolates were studied by disc diffusion and the results revealed the high prevalence of multidrug resistance phenotypes among isolates. Studying genotypic attributes of isolates by PCR revealed the high prevalence of virulence, antimicrobial resistance and biofilm formation genes. The alarming findings highlighted the importance of conducting more detailed monitoring and investigation in the duck / fish interface and to raise public awareness for biosecurity practices to mitigate the imposed public health risk.

Key words: *Clinostomum species*, *Contraceacum species*, duck, E.*coli, Pseudomonas spp, Salmonella spp., and Tilapia zillii.*

INTRODUCTION

Recently great attention has been brought to fish production from natural waters and aquaculture as fish stands for relatively an affordable source of animal protein. Moreover, free ranging water fowls and fish-eating birds are commonly share the habitat in fish communities. Interestingly, scientific studies have reported the detection of an overlap between parasitic and bacterial pathogens among different hosts potentiating their transmission to humans through food. Therefore, transmission of pathogens from fish to fish-eating birds is not far away, consequently raises the importance of studying the potential circulating bacterial and parasitic pathogens in this host range. To enhance the production of safe food recommendations were raised to monitor fish health in aquaculture establishments (Hernandez *et al*., 1998).

The role of ducks raised in fish farms, as reservoir hosts, has not been adequately investigated. Feeding poultry on snails and fish remains, either intentionally or by discharge of slaughter wastes of ducks and chickens into ponds was identified as one of the risk factors for trematodes infection, (Anh *et al.*, 2010). Furthermore, (Hurlbert *et al*., 2007) concluded that *Pseudomonas, Salmonella* and E.*coli* species are considered major bacterial problems in duck-fish aquaculture development. In the same instances, (Sugita *et al.*, 1994) recorded that these bacteria are widely distributed in fresh water and bottom sediments containing organic materials as well as in the intestinal tract of fish. Furthermore, (Aoki, 1999) reported that infections by these bacterial pathogens may primarily cause massive mortalities, reduce the production and decrease the quality of aquatic

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organisms, moreover, these bacteria may also be encountered as secondary infecting pathogens after primary viral or parasitic infection, as well as causing opportunistic infections after environmental stresses.

Öztürk and Altınok, (2014) concluded that the best way to prevent fish diseases is to prevent both infectious and noninfectious outbreaks, to determine the correct diagnosis, and to apply economically acceptable treatment.

Thus, the present study aimed to investigate the prevalence of some bacterial and parasitic pathogens implicated in enteric infections of household free ranging ducks and fish that share the same environmental habitat.

MATERIALS AND METHODS

I- Samples' collection

One hundred diseased ducks that were raised in free range duck-fish backyards, and one hundred freshly dead harvested fish (*Tilapia Zilli*) were collected from locations at the bank of Mowase canal branched from Nile River at Zagazig city during the period from July 2018 to February 2019. Samples were transferred to RLQP, Sharkia and AHRI Zagazig laboratories. Bacteriological examinations involved samples from intestine, swabs from skin and gills of collected fish, and internal organs of backyard ducks (liver, trachea, lung, heart, and intestine). Parasitological examinations involved the skin, fins, gills, alimentary tract and body cavity of fish (*Tilapia Zilli*), and (intestinal contents, buccal cavity and esophagus) of ducks. Samples were left for a few minutes into a petri dish containing saline solution, then opened, scraped and examined under light microscope.

I.A- Bacterial examination

Isolation of E.*coli, salmonella species* and *Pseudomonas spp* were applied according to (Kreig *et al*., 1984), (ISO /IEC 6579:2017), and (Cheesbrough, 2000), respectively.

I.B- Identification of bacterial isolates

Salmonella, and E. *coli* isolates were serotyped according to (Patrick and Francois, 2007), and (MacFaddin, 2000), respectively. *Pseudomonas* isolates were biotyped according to (Cheesbrough, 2000).

I.C- Parasitic examination

1- Fixation and staining of parasites:

Trematodes and cestodes were put between two slides, preserved in 10% formalin for fixation. Nematodes were fixed by using ethyl alcohol (70%) directly after collection. Trematodes and cestodes were stained in Alum carmine, immediately after staining, the specimens were washed several times in distilled water. Nematodes gave the best results without staining but cleaning in lacto-phenol. This was done as slowly as possible using a dilute solution of acid alcohol (1.0 ml conc. HCL in 1000 ml of 70% alcohol) using ascending grades of ethyl alcohol (50%, 60%, 70%, 80%, 90%, 95%, and absolute alcohol). Clove oil was used as a good clearing material for trematodes and cestodes, while lactophenol gave good results as a clearing material with nematodes. Canada balsam for trematodes and cestodes, and polyvol for nematodes were used as mounting materials, (Lucky, 1977).

2- Identification of Parasites

The isolated parasites were identified according to (Bray *et al.*, 2005) for trematodes, and (Bunkley-Williams and Williams, 1996; and Vernon, 2006) for cestodes and nematodes, respectively.

II- Antimicrobial susceptibility testing

Antimicrobial susceptibility profiles of isolates were tested against four antimicrobial agents from different antimicrobial groups of the commonly used in ducks' treatment (Neomycin, sultamethoxazole trimethoprim, chlortetracycline, and florfenicol). According to the standard Kirby–Bauer disc diffusion method (Quinn *et al*., 1994) and the results were interpreted according to the criteria recommended by (CLSI, 2015).

III- Molecular detection of antibiotic resistance, biofilm and virulence genes

DNA extraction

DNA extraction was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH). Briefly, 200 µl of sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56°C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's instructions. Nucleic acid was eluted with 100 µl of elution buffer.

Oligonucleotide Primer

Primers were supplied from Metabion (Germany) as listed in Table (1).

PCR amplification

Primers were utilized in a 25- µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an applied biosystem 2720 thermal cycler.

***Stx*1** and ***stx*2 duplex PCR**

Primers were utilized in a 50 µl reaction containing 25 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 15 µl of water, and 6 µl of DNA template.

Analysis of the PCR Products

The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of the uniplex PCR products and 40 µl of the duplex PCR products were loaded in each gel slot. Gelpilot 100 bp and 100 bp plus DNA ladders (Qiagen, Germany, GmbH) and gene ruler 100 bp ladder (Fermentas, Germany) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra).

Table 1: Primers' sequences, target genes and amplicon sizes.

Test Target	Target gene	Primers' sequences (5´-3´)	Amplified segment (bp)	References
E. *coli* *Conserved virulence gene*	***pho*A**	CGATTCTGGAAATGGCAAAAG CGTGATCAGCGGTGACTATGAC	720	**Hu *et al.*, 2011**
Salmonella spp.* *Conserved virulence gene	***inv*A**	GTGAAATTATCGCCACGTTCGGGCAA TCATCGCACCGTCAAAGGAACC	284	**Oliveira *et al.*, 2003**
Pseudomonas* *Conserved gene	**16*S* *r*RNA**	GACGGGTGAGTAATGCCTA CACTGGTGTTCCTTCCTATA	618	**Spilker *et al.*, 2004**
Salmonella spp* *Virulence gene	***stn***	TTG TGT CGC TAT CAC TGG CAACC ATT CGT AAC CCG CTC TCG TCC	617	**Murugkar *et al.*, 2003**
E. coli* *Virulence gene	***stx*1**	ACACTGGATGATCTCAGTGG CTGAATCCCCCTCCATTATG	614	**Dipineto *et al.*, 2006**
E. coli* *Virulence gene	***stx*2**	CCATGACAACGGACAGCAGTT CCTGTCAACTGAGCAGCACTTTG	779	
Pseudomonas* *Virulence gene	***tox*A**	GACAACGCCCTCAGCATCACCAGC CGCTGGCCCATTCGCTCCAGCGCT	396	**Matar *et al.*, 2002**
Pseudomonas* *Virulence gene	***las*I**	ATGATCGTACAAATTGGTCGGC GTCATGAAACCGCCAGTCG	606	**Bratu *et al.*, 2006**
E. coli ,Salmonella and pseudomonas* *Florophenicol resistance gene	***flo*R**	TTTGGWCCGCTMTCRGAC SGAGAARAAGACGAAGAAG	494	**Doublet *et al.*, 2003**
E. coli and Salmonella* *Biofilm gene	***adr*A**	ATGTTCCCAAAAATAATGAA TCATGCCGCCACTTCGGTGC	1113	**Bhowmick *et al.*, 2011**
Pseudomonas* *Biofilm gene	***psl*A**	TCCCTACCTCAGCAGCAAGC TGTTGTAGCCGTAGCGTTTCTG	656	**Ghadaksaz *et al.*, 2015**

Conserved genes for species confirmation, ***pho*A*:*** Alkaline phosphastase gene**,** ***inv*A:** invasion of the host epithelial cells**, 16S rRNA:** conserved pseudomonas genus-specific gene. Toxin encoding genes, ***stn*:** salmonlla enterotoxin gene, ***stx*1, *stx*2:** E.*coli* shiga toxins 1 and 2 genes, ***tox*A gene:** pseudomonas exotoxin A gene, ***las*I gene**: quoram sensing transcriptional activator. Antimicrobial resistance genes, ***flo*R**: Florophenicol resistance gene. Biofilmm formation encoding genes, ***adr*A gene:** attenuator of drug resistance of E.*coli* and *salmonella spp*, ***Psl*A**: polysaccharide synthesis locus of *pseudomonas spp.*

RESULTS

I.A. Isolation of bacterial pathogens

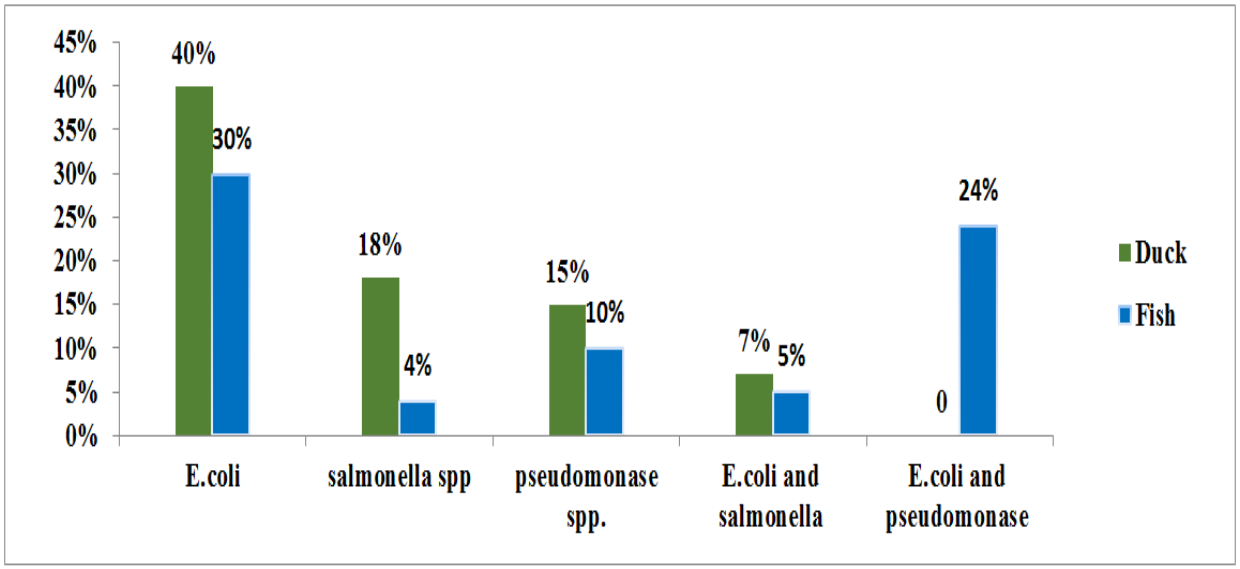
Table 2: Prevalence rate of bacterial isolates.

Source of samples	Prevalence rate of bacterial isolates					
	E. *coli*		*Salmonella spp*		*Pseudomonas spp*	
	Number	*Prevalence*	*Number*	*Prevalence*	*Number*	*Prevalence*
Duck	47/100	47%	25/100	25%	15/100	15%
Fish	59/100	59%	9/100	9%	34/100	34%
Total	106/200	53%	34/200	17%	49/200	24.5%

One hundred and six out of total two hundred examined samples (53%) were positive for E.*coli*, of which 47/100 (47%) were isolated from ducks and 59/100 (59%) from fish *(Tilapia Zillii*). Moreover, examination of samples also revealed the isolation of 34/200 (17%) *Salmonella* isolates, of which 25/100 (25%) isolates were isolated from ducks, 9/100 (9%) from fish (*Tilapia Zillii*). The study also revealed the isolation of 49/200 (24.5%) *pseudomonas spp* of which 15/100 (15%) from ducks, and 34/100 (34%) from fish (*Tilapia Zillii*), respectively, Table (2).

Table 3: Distribution of bacterial isolates in different samples

Source of samples	Ducks (100 birds)			Fish (100 *Tilapia Zillii*)		
Type of infection	**Bacterial *species***	**Number of isolates**	**Prevalence rate**	**Bacterial *species***	**Number of isolates**	**Prevalence rate**
Single bacterial infection	E.*coli*	40/100	40%	E.*coli*	30/100	30%
	Salmonella spp	18/100	18%	*Salmonella spp.*	4/100	4%
	Pseudomonas spp	15/100	15%	*Pseudomonas spp*	10/100	10%
Mixed bacterial infection	E.*coli and Salmonella spp.*	7/100	7%	E.*coli and Salmonella spp.*	5/100	5%
				E.*coli and pseudomonas spp*	24/100	24%



Graph (1): Distribution of bacterial isolates in different samples

Bacteriological examination of ducks revealed that 7/100 (7%) of examined birds were positive for mixed infection by E.*coli* and *salmonella spp.* In the same regards, conventional bacteriological examination also revealed that 40/100 (40%), 18/100 (18%), and 15/100 (15%) of examined ducks suffered from single bacterial infection by E.*coli*, *Salmonellla spp*., and *pseudomonase spp*, respectively. Bacteriological examination of fish *(Tilapia Zillii*) samples, revealed that 5/100 (5%), 24/100 (24%) of examined fish *(Tilapia Zillii*) samples were positive for mixed infection by (E.*coli* and *salmonella spp.)*, and (E.*coli and pseudomonase spp)*, respectively. Examination also revealed that 30/100 (30%), 4/100(4%) and 10/100 (10%) of examined fish *(Tilapia Zillii*) samples were positive for single bacterial infection by E.*coli*, *Salmonellla spp*. and *pseudomonase spp*., respectively, Graph (1), and Table (3).

Confirmation of Isolates by PCR

PCR was applied to confirm the results of conventional isolation through targeting the relevant species conserved genes as demonstrated in Figures (1, 2 and 3).

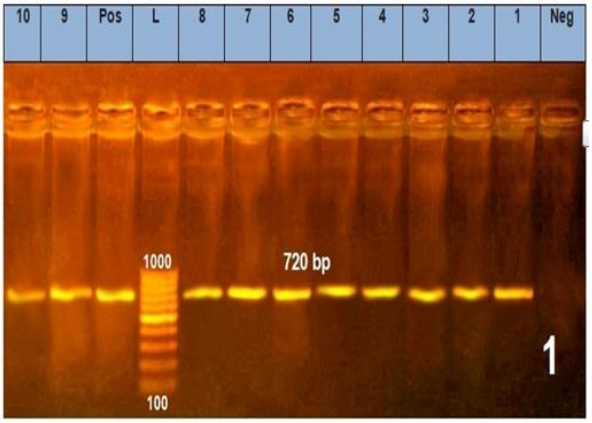


Figure (1): *pho*A gene for E.*coli spp*.
Lane L: DNA molecular size marker 100-1000bp
Lane (Pos): Positive control
Lane (Neg): Negative control
Lane 1-10: Positive for *pho*A gene at 720 bp.

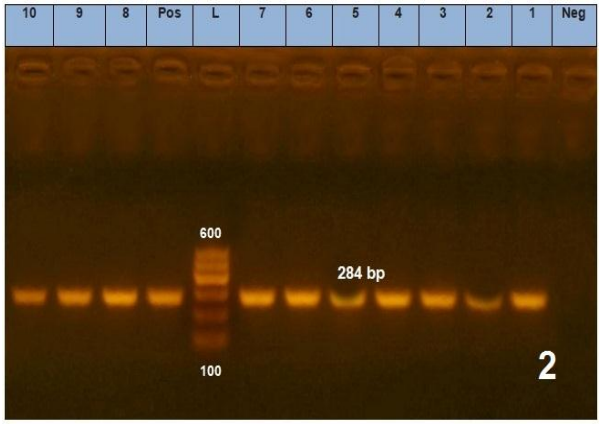


Figure (2): *inv*A gene for *salmonella spp*
Lane L: DNA molecular size marker 100-600bp
Lane (Pos): Positive control
Lane (Neg): Negative control
Lane 1-10: Positive for *inv*A gene at 284 bp

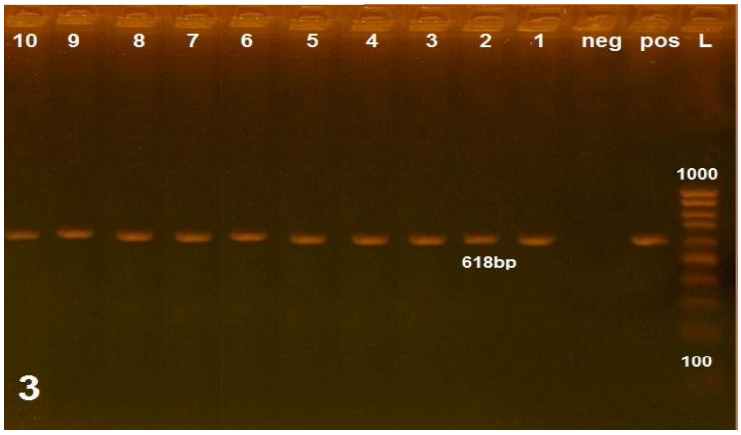


Figure (3):16S *rRNA* gene of *pseudomonas spp*.
Lane L: DNA molecular size marker 100-1000 bp
Lane (Pos): Positive control
Lane (Neg): Negative control
Lane 1-10: Positive for *16S rRNA* gene at 618 bp

Ten E.*coli* isolates and 10 *salmonella* isolates isolated from ten cases of mixed bacterial infections (5 ducks, and 5 fish) produced the target specific amplicons for *pho*A gene, *inv*A gene, the conserved genes for E.*coli* spp, genus *salmonella*, respectively. Moreover, a total of 10 *pseudomonas* isolates with multidrug resistance phenotypes (5 isolates from ducks and 5 isolates from fish) were positive for 16S *rRNA* gene the conserved gene for genus *pseudomonas*., figures (1,2,3).

I.B. Typing of bacterial isolates

Table 4: Serotyping of *E. coli* isolates.

Serotype	No. of isolates		Total
	Ducks	Fish	
O146 : H21	10/47	0/59	10/106
O124	1/47	0/59	1/106
O55 : H7	6/47	0/59	6/106
O158	2/47	0/59	2/106
O127	3/47	15/59	18/106
O26 : H11	7/47	22/59	29/106
O125 : H21	6/47	9/59	15/106
O17 : H18	12/47	13/59	25/106
Total	47/47	59/59	106/106

Serotyping of E.*coli* isolates revealed their distribution in eight serotypes, from which four serotypes were isolated from ducks and *fish (Tilapia Zillii*) as follows, O26:H11 (29/106), O17:H18 (25/106), O127 (18/106), and O125: H21 (15/106), respectively, Table (4).

Table 5: Serotyping of *salmonella spp.* serovars

Serovars	Antigenic structure		Number of isolates		Total
	O	H	Ducks	Fish	
Salmonella Molade	8,20	Z10 : Z6	4/25	0/9	4/34
Salmonella Papuana	6,7	r : e,n,Z15	2/25	0/9	2/34
Salmonella Enteritidis	1,9,12	g,m : -	3/25	1/9	4/34
Salmonella Kentucky	8,20	i : Z6	5/25	0/9	5/34
Salmonella Saintpaul	4,12	i : Z6	2/25	2/9	4/34
Salmonella Anatum	6,7	r : 1,5	3/25	2/9	5/34
Salmonella Typhimurium	1,4,5,12	i : 1,2	2/25	3/9	5/34
Salmonella Heidelberg	1,4,5,12	r : 1,2	4/25	1/9	5/34
Total			25/25	9/9	34/34

Serotyping of salmonella isolates revealed the detection of 8 serotypes, of which five serotypes were isolated from both ducks and fish *(Tilapia Zillii)* as follows, *Salmonella* Enteritidis and *Salmonella* Saintpaul (4/34) isolates each, *Salmonella* Anatum, *Salmonella* Typhimurium and *Salmonella* Heidelberg (5/34) isolates each, Table (5).

Table 6: Biotyping of *pseudomonas spp.* Isolates

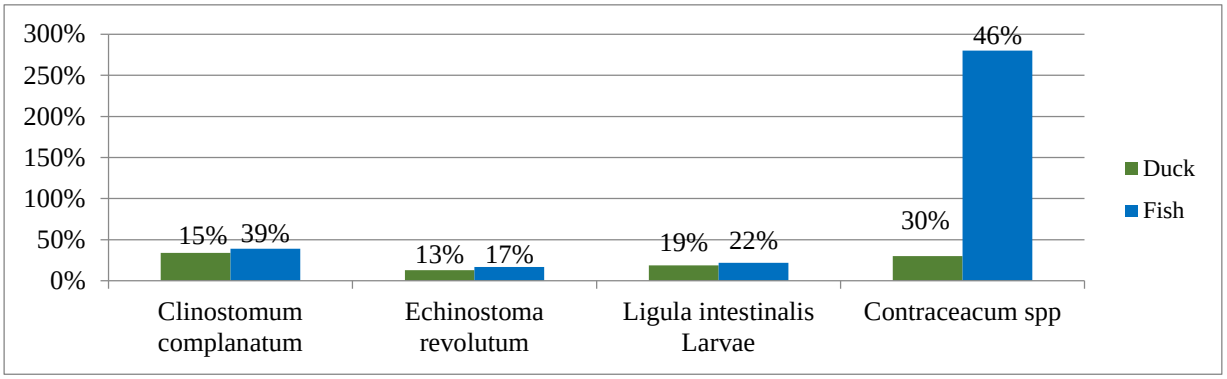
	No. of isolates		Total
	Ducks	Fish	
Pseudomonas fluorescens	9/15	25/34	34/49
Pseudomonas aeruginosa	6/15	9/34	15/49
Total	15/15	34/34	49/49

Biotyping of *pseudomonus* isolates revealed the identification of two biotypes which were isolated from ducks and fish *(Tilapia Zillii)* as follows, *Pseudomonas fluorescens* in (34/49) isolates, *and pseudomonas aeruginosa* in (15/49) isolates, Table (6).

I.C. Parasitological Examination

Table 7: Prevalence rate of helminthes parasites in ducks and fish

Source of samples	Prevalence rate of helminthes			
	Trematodes		Cestodes	Nematodes
	Clinostomum spp.	*Echinostoma spp.*	*Ligula intestinalis Larvae*	*Contraceacum spp*
Ducks	15/100 (15%) adult	13/100 (13%) adult	19/100 (19%)	30/100 (30%)
Fish	39/100 (39%) metacercaria	17/100 (17%) Metacercaria	22/100 (22%)	46/100 (46%)
Total	54/200 (27%)	30/200 (15%)	41/200 (20.5%)	76/200 (38%)



Graph (2): Prevalence rates of helminthes parasites in ducks and fish

In the present study, a total of 100 ducks and 100 *Tilapia zillii* fish were examined for helminthes infestations. Two species of trematodes were isolated, first of which *Echinostoma species* with a total prevalence rate of 30/200 (15%) from total examined 200 ducks and fish, out of which 13/100 (13%) were isolated from ducks and 17/100 (17%) were isolated from *Tilapia zillii*, respectively. Secondly, *Clinostomum species* was isolated with a total prevalence rate of 54/200 (27%) of the total 200 examined ducks and fish, of which 15/100 (15%) and 39/100 (39%) were isolated from ducks and *Tilapia zillii*, respectively. Examination also revealed the isolation of one species of cestodes, *Ligula intestinalis* larvae with a total prevalence rate of 41/200 (20.5%) from the 200 examined ducks and fish, out of which (19/100 (19%) were isolated from ducks and 22/100 (22%) were isolated from *Tilapia zillii*. Examination also revealed the isolation of one species of nematodes, *Contraceacum species* with a total prevalence rate of 76/200 (38%) of examined ducks and fish, out of which 30/100 (30%) were isolated from ducks and 46/100 (46%) were isolated from *Tilapia zillii*, respectively, graph (2), and table (7).

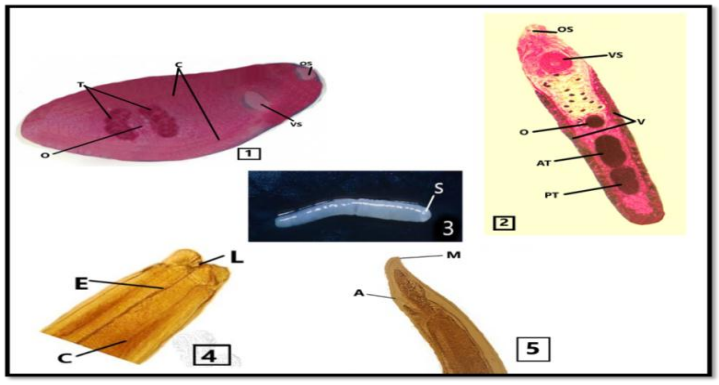


Figure (4), figure (4.1) *Clinostomum spp.* (**OS:** Oral Sucker; **VS:** Ventral Sucker; **C:** Ceca; **T:** Testis; **O:** Ovary), **figure (4.2)** *Echinostoma spp.* (**OS:** Oral Sucker; **VS:** Ventral Sucker; **O:** Ovary; **V:** Vitellaria; **AT:** Anterior Testis; **PT:** Posterior testis)**, figure (4.3**) *Ligula intestinalis* (**S:** Scolex), **figure (4.4**) Anterior end of *Contraceacum spp*. (**L:** Lips; **E:** Esophagus; **C:** Ceca) **figure (4.5)** Posterior end of *Contraceacum spp*. (**M:** Mucron; **A:** Anus).

Description and identification of Helminthes Parasites

The helminthic fauna isolated in this study represented the most helminthes species that were commonly found in all habitats and ducks as the main final host encounter the infection by eating snails or fish containing helminthes parasites.

1. Trematodes

1.1. *Clinostomum complanatum*

Habitat

Metacercaria are found on the gills and skin of *Tilapia zillii* (fish), while adults are found in buccal cavity and esophagus of ducks.

Clinostomum complanatum adult is described as having narrow anterior end, small oral sucker, ceca are bifurcated, extended from ventral sucker to posterior end, triangular median testis, oval ovary, figure (4.1).

1.2. *Echinostoma revolutum*

Habitat

Metacercaria are found in the intestine of *Tilapia zilli* (fish) and the adult are found in the intestine of ducks. The adults were found with well-developed head collar, oral sucker sub terminal, sub spherical. Ventral sucker is sub spherical, with deep cavity, located fairly close to anterior extremity of the short esophagus; intestinal bifurcation in second half of fore body; caeca blind, narrow, reach close to posterior extremity of body. Testis are two, tandem, separated, located in third quarter of body. Ovary is sub-globular. Eggs are not very numerous, small. Vitellarium follicular, forming 2 lateral fields of small follicles overlapping caeca, figure (4.2).

2. Cestodes

2.1. *Ligula intestinalis* Larvae

Habitat

Ligula intestinalis are found in the body cavity of *Tilapia zillii* (fish) and the adults are found in the intestine of ducks.

The body of these plerocercoids was elongated, solid, whitish and flat, measured 1 -14 cm in length and 0.3-0.85 cm in width. The external segmentation of strobila was absent. Two bothridial depressions were hardly visible on the anterior end representing of scolex, figure (4.3).

3. Nematodes

3.1. *Contraceacum species* Larvae

Habitat

Contraceacum spp larvae were found in the intestine of *Tilapia zilli* (fish) and ducks.

The body is whitish and its length is 2-6 mm, relatively thick with maximum width of 0.19-0.23 mm at the middle of the body. The isolated nematodes have 3 lips around the mouth opening, without ridges. Intestinal cecum is well developed with one esophageal appendix. Esophagus ends in short. Intestine is filling the remainder of body. Tail sharply pointed, with spine, figure (4.4 and 4.5).

II- Studying the phenotypic antimicrobial resistance profiles of bacterial isolates

Table 8: Phenotypic resistance profiles

Chemotherapeutic Group	Bacterial isolates	E. *coli* isolates		*Salmonella* isolates		*Pseudomonas* isolates	
	Chemotherapeutic agents	No. of Resistant isolates	Resistance rate	No. of Resistant isolates	Resistance rate	No. of Resistant isolates	Resistance rate
Aminoglycosides	**Neomycin(N), 30ug**	103/106	97.1%	34/34	100%	49/49	100%
Diaminopyrimidine	**Sulfamethoxazole-trimethoprim (SXT), 25ug**	97/106	91.5%	31/34	91.2%	45/49	91.8%
Tetracycline	**Chlortetracycline (CL), 15ug**	100/106	94.3%	30/34	88.2%	46/49	93.9%
Quinolones	**Florfenicol (FL), 30ug**	90/106	84.3%	29/34	85.3%	44/49	89.8%

Resistance rates were calculated with regards to the total number of isolated species, the results revealed that 97 /106 (91.5%) of E. *coli*, 30/34 (88.2%) of *Salmonella spp.* and 45/49 (91.8%) *pseudomonas spp.* demonstrated multidrug resistance phenotypes, table (8).

III-Investigation of genotypic virulence attributes of isolates by PCR

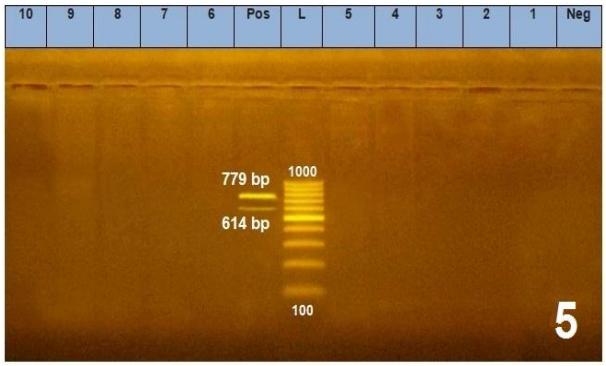


Figure (5): *stx*1, *stx* 2 gene for E.*coli spp.*
Lane L: DNA molecular size marker 100-1000 bp
Lane (Pos): Positive control
Lane (Neg): Negative control
Lane 1-10: Negative for *stx*1,2 genes at 614,779 bp, respectively

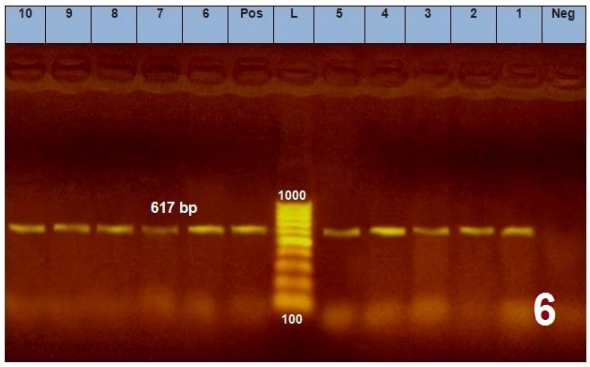


Figure (6): *stn* gene for *salmonella spp*.
Lane L: DNA molecular size marker 100-1000 bp
Lane (Pos): Positive control
Lane (Neg): Negative control
Lane 1-10: positive for *stn* gene at 617 bp

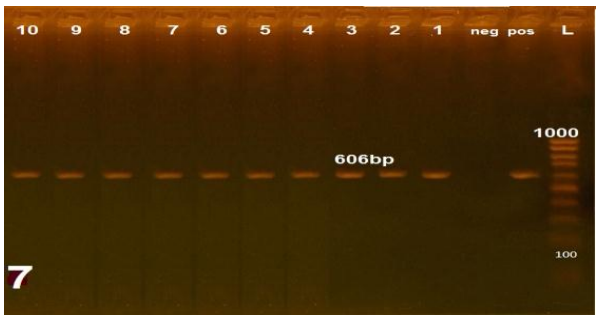


Figure (7): *las*I gene for *pseudomonas spp*
Lane L: DNA molecular size marker 100-1000 bp
Lane (Pos): Positive control
Lane (Neg): Negative control
Lane 1-10: positive for *las*I gene at 606 bp

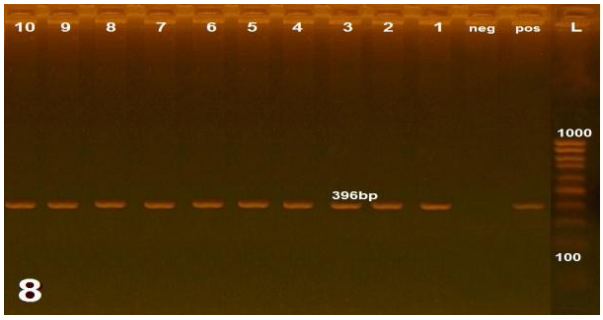


Figure (8): *tox*A gene for *pseudomonas spp*
Lane L: DNA molecular size marker 100-1000 bp
Lane (Pos): Positive control
Lane (Neg): Negative control
Lane 1-10: positive for *tox*A gene at 396 bp

E.*coli* and *Salmonella spp.* isolated from ten cases of mixed bacterial infections (5 in ducks, and 5 in fish) and that demonstrated multidrug resistance phenotypes were tested for genotypic virulence attributes. In this regards, PCR failed to detect neither *stx*1, nor *stx*2 virulence genes of E.*coli*, figure (5). On the other hand, PCR confirmed the detection of *stn* gene of *Salmonella spp. in* 10/10 (100%) of tested isolates, figure (6).

The presence of *las*I and *tox*A virulence genes was by confirmed in PCR in 10 randomly selected *pseudomonas spp* isolates that demonstrated multidrug resistance phenotypes, figure (7, 8).

IV-Investigation of genotypic resistance attributes of isolates by PCR

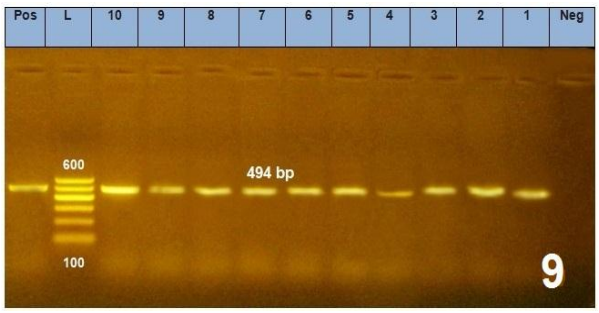


Figure (9): *floR* resistance gene for E.*coli spp*
Lane L: DNA molecular size marker 100-600 bp
Lane (Pos): Positive control
Lane (Neg): Negative control
Lane 1-10: positive for *flo*R gene at 494 bp

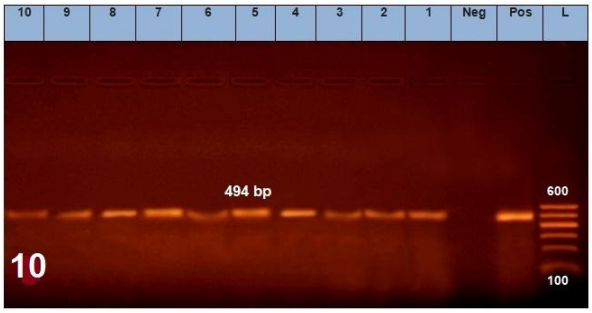
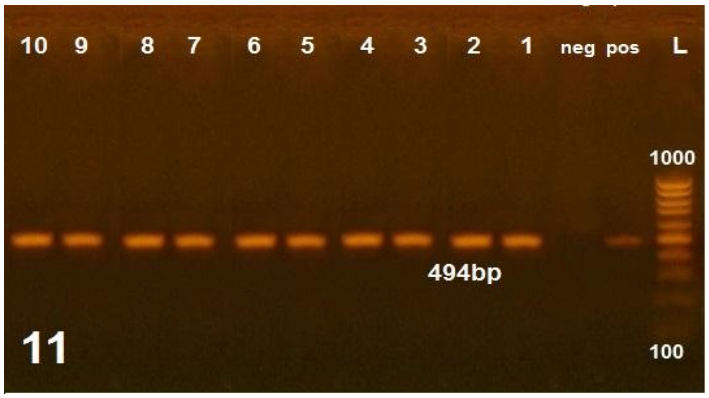


Figure (10): *flo*R gene for *salmonella spp*
Lane L: DNA molecular size marker 100-600 bp
Lane (Pos): Positive control
Lane (Neg): Negative control
Lane 1-10: positive for *flo*R gene at 494 bp



Fguire (11): *flo*R resistance gene for *pseudomonas spp*
Lane L: DNA molecular size marker 100-1000 bp
Lane (Pos): Positive control
Lane (Neg): Negative control
Lanes 1-10: positive for *flo*R gene at 494 bp

Florophenicol resistance genotypic attributes was tested by PCR for the presence of *flo*R gene and was confirmed in 10/10 (100%) of each of the tested E.*coli*, *salmonella spp*. and *pseudomonas spp isolates*, figure (9.10 and 11).

V-Investigation of genotypic biofilm attributes of isolates by PCR

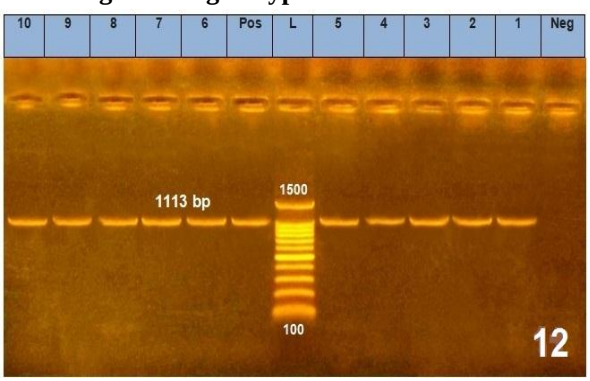


Figure (12): *adr*A biofilm gene for E.*coli spp*
Lane L: DNA molecular size marker 100-1500 bp
Lane (Pos): Positive control
Lane (Neg): Negative control
Lane 1-10: positive for *adr*A gene at 1113 bp

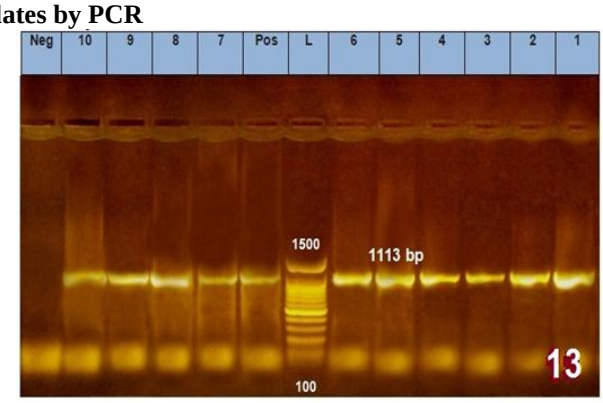


Figure (13): *adr*A biofilm gene for *salmonella spp*
Lane L: DNA molecular size marker 100-1500bp
Lane (Pos): Positive control
Lane (Neg): Negative control
Lane 1-10: positive for *adr*A gene at 1113 bp

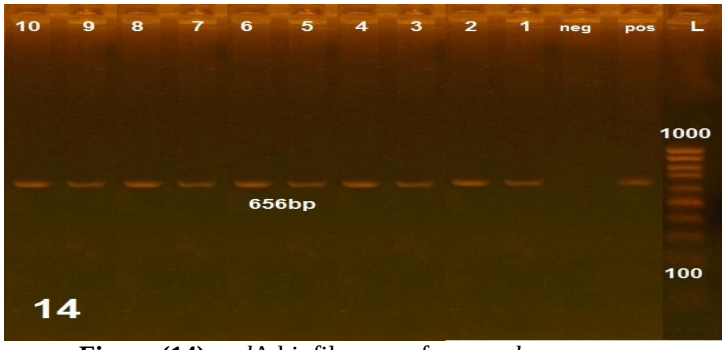


Figure (14): *psl*A biofilm gene for *pseudomonas spp*
Lane L: DNA molecular size marker 100-1000 bp.
Lane (Pos): Positive control
Lane (Neg): Negative control
Lane 1-10: positive for *psl*A gene at 656 bp

Genetic attributes for biofilm formation were tested by PCR for the presence of *adr*A gene and were confirmed in 10/10 (100%) of the tested E.*coli* and 10/10 (100%) of the tested *salmonella* isolates, figure (12, 13). PCR also confirmed the detection of *psl*A gene for in 10/10 (100%) of the tested *pseudomonas spp isolates*, figure (14).

Table 9: Phenotypic, and genotypic resistance and biofilm profiles of E. *coli* isolates from cases of mixed infection with parasites.

No	Source	Serotype	Helminthes	Antimicrobial resistance profile	Confirmatory gene (*pho*A)	Virulence genes		Antibiotic resistance genes (*flo*R)	Biofilm genes (*adr*A)
						*Stx*1	*Stx*2		
1	D*, Mixed	**O146 : H21**	***Clinostomum spp., Ligula intestinalis Larvae***	N^1,SXT^2, CL^3,FL^4	+	-	-	+	+
2	D*, Mixed	**O124**	***Echinostoma spp., Contraceacum spp.***	N^1,SXT^2, CL^3,FL^4	+	-	-	+	+
3	D*, Mixed	**O55 : H7**	***Clinostomum spp.+ Contraceacum spp***	N^1,SXT^2, CL^3,FL^4	+	-	-	+	+
4	D*, Mixed	**O158**	***Clinostomum spp., Ligula intestinalis Larvae***	N^1,SXT^2, CL^3,FL^4	+	-	-	+	+
5	D*, Mixed	**O127**	***Echinostoma spp., Contraceacum spp.***	N^1,SXT^2, CL^3,FL^4	+	-	-	+	+
6	F**, Mixed	**O26 : H11**	***Clinostomum spp., Contraceacum spp***	N^1,SXT^2, CL^3,FL^4	+	-	-	+	+
7	F**, Mixed	**O125 : H21**	***Clinostomum spp., Ligula intestinalis Larvae***	N^1,SXT^2, CL^3,FL^4	+	-	-	+	+
8	F**, Mixed	**O17 : H18**	***Echinostoma spp., Contraceacum spp***	N^1,SXT^2, CL^3,FL^4	+	-	-	+	+
9	F**, Mixed	**O125 : H21**	***Clinostomum spp., Contraceacum spp***	N^1,SXT^2, CL^3,FL^4	+	-	-	+	+
10	F**, Mixed	**O17 : H18**	***Clinostomum spp., Ligula intestinalis Larvae***	N^1,SXT^2, CL^3,FL^4	+	-	-	+	+

D*: Ducks, F**: Fish, N^1: Neomycin, SXT^2: Sulfamethoxazole-trimethoprim, CL^3: Chlortetracycline, FL^4: Florophenicol

Table 10: Phenotypic, genotypic resistance and biofilm profiles of *salmonella* isolates from cases of mixed infection with parasites:

No.	Source	Serotype	Helminths	Antimicrobial resistance profile	Confirmatory gene (*inv*A)	Virulence genes (*stn*)	Antibiotic resistance genes (*flo*R)	Biofilm genes (*adr*A)
1	D*, Mixed	***S. Molade***	***Clinostomum spp., Ligula intestinalis Larvae***	N[1],SXT[2], CL[3],FL[4]	+	+	+	+
2	D*, Mixed	***S. Papuana***	***Clinostomum spp., Contraceacum spp.***	N[1],SXT[2], CL[3],FL[4]	+	+	+	+
3	D*, Mixed	***S. Enteritidis***	***Clinostomum spp., Ligula intestinalis Larvae***	N[1],SXT[2], CL[3],FL[4]	+	+	+	+
4	D*, Mixed	***S. Kentucky***	***Echinostoma spp., Contraceacum spp.***	N[1],SXT[2], CL[3],FL[4]	+	+	+	+
5	D*, Mixed	***S.saintpaul***	***Clinostomum spp., Contraceacum spp.***	N[1],SXT[2], CL[3],FL[4]	+	+	+	+
6	F**, Mixed	***S. Anatum***	***Clinostomum spp.,Contraceacum spp.***	N[1],SXT[2], CL[3],FL[4]	+	+	+	+
7	F**, Mixed	***S. Typhimurium***	***Clinostomum spp., Ligula intestinalis Larvae***	N[1],SXT[2], CL[3],FL[4]	+	+	+	+
8	F**, Mixed	***S. Heidelberg***	***Echinostoma spp., Contraceacum spp.***	N[1],SXT[2], CL[3],FL[4]	+	+	+	+
9	F**, Mixed	***S. Typhimuriur***	***Clinostomum spp., Contraceacum spp.***	N[1],SXT[2], CL[3],FL[4]	+	+	+	+
10	F**, Mixed	***S. Heidelberg***	***Clinostomum spp., Ligula intestinalis Larvae***	N[1],SXT[2], CL[3],FL[4]	+	+	+	+

D*: Ducks, F**: Fish, N[1]: Neomycin, SXT[2]: Sulfamethoxazole-trimethoprim, CL[3]: Chlortetracycline, FL[4]: Florophenicol

Table 11: Phenotypic, genotypic resistance and biofilm profiles of *pseudomonas spp*. isolates from cases of mixed infection with parasites.

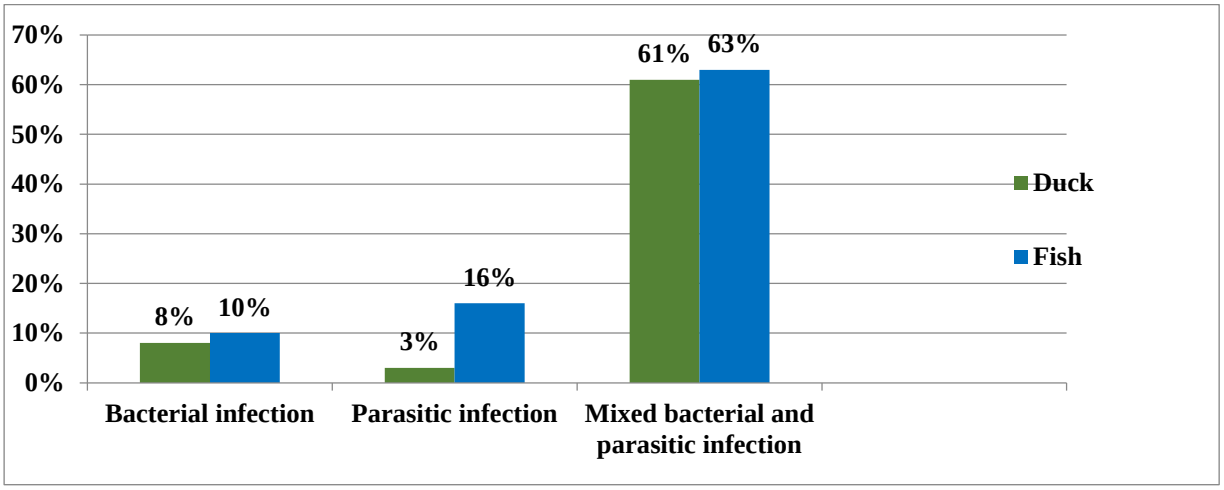
No	Source	Serotype	Helminths	Antimicrobial resistance profile	Confirmatory gene (*16S rRNA*)	Virulence genes (*stn*)		Antibiotic resistance genes (*flo*R)	Biofilm genes (*pslA*)
						lasI	*toxA*		
1	D*,single	***P.fluorescens***	***Echinostoma spp.+ Contraceacum spp.***	N[1],SXT[2], CL[3],FL[4]	+	+	+	+	+
2	D*,single	***P.fluorescens***	***Clinostomum spp.+ Ligula intestinalis Larvae***	N[1],SXT[2], CL[3],FL[4]	+	+	+	+	+
3	D*,single	***P.fluorescens***	***Echinostoma spp.+ Contraceacum spp.***	N[1],SXT[2], CL[3],FL[4]	+	+	+	+	+
4	D*,single	***P.aeruginosa***	***Clinostomum spp.+ Contraceacum spp.***	N[1],SXT[2], CL[3],FL[4]	+	+	+	+	+
5	D*,single	***P.aeruginosa***	***Clinostomum spp.+ Contraceacum spp.***	N[1],SXT[2], CL[3],FL[4]	+	+	+	+	+
6	F**,single	***P.fluorescens***	***Clinostomum spp.+ Ligula intestinalis Larvae***	N[1],SXT[2], CL[3],FL[4]	+	+	+	+	+
7	F**,single	***P.fluorescens***	***Echinostoma spp.+ Contraceacum spp.***	N[1],SXT[2], CL[3],FL[4]	+	+	+	+	+
8	F**,mixed	***P.fluorescens***	***Clinostomum spp.+ Contraceacum spp.***	N[1],SXT[2], CL[3],FL[4]	+	+	+	+	+
9	F**,mixed	***P.aeruginosa***	***Clinostomum spp.+ Ligula intestinalis Larvae***	N[1],SXT[2], CL[3],FL[4]	+	+	+	+	+
10	F**,mixed	***P.aeruginosa***	***Clinostomum spp.+ Ligula intestinalis Larvae***	N[1],SXT[2], CL[3],FL[4]	+	+	+	+	+

D*: Ducks, F**: Fish, N[1]: Neomycin, SXT[2] : Sulfamethoxazole-trimethoprim, CL[3]: Chlortetracycline ,FL[4]: Florophenicol

VI- Association between bacterial and parasitic infection in ducks and fish

Table 12: Bacterial infection associated with parasitic infection in examined ducks and fish**.**

Sources of isolates	Bacterial infection	Parasitic infection	Mixed bacterial and parasitic infection	Total
Ducks	8/100 (8%)	3/100 (3%)	61/100 (61%)	72/100 (72%)
Fish	10/100 (10%)	16/100 (16%)	63/100 (63%)	89/100 (89%)
Total	18/200 (9%)	19/200 (9.5%)	124/200 (62%)	161/200 (80.5%)



Graph (3): Bacterial infection associated with parasitic infection in examined ducks and fish

Cases of mixed bacterial and parasitic infections were detected with rates of 61/100 (61%) and 63/100(63%) of examined ducks, and fish, respectively. The results also revealed that 8/100 (8%) and 10/100(10%) of examined ducks and fish suffered only form bacterial infections with no parasitic infestations. Moreover, parasitic infestations were isolated singly without bacterial infections in 3/100 (3%) and 16/100(16%) of examined ducks and fish, respectively, graph (3) and table (12).

DISCUSSION

A wide range of bacterial infections occur in free-ranging birds. Such infections are regarded as important causes of morbidity and mortality (Davis *et al*., 1971, Steele and Galton, 1971). Egypt is considered the largest farmed tilapia producer in Africa and the second globally after China (FAO, 2013).

High prevalence rates of E.*coli* and *Salmonella* were recorded from birds thus clearly demonstrated poor hygiene and sanitary condition, (Adeyanju and Ishola, 2014). In the current study, the results of E.*coli* isolation revealed a total of 106/200 E.*coli* isolates with an overall prevalence rate of (53%) among the total 200 studied ducks and fish, out of which 47/100 (47%) isolates were isolated from household free range ducks. Higher prevalence rates were recorded by (Kissinga *et al*., 2018) who isolated E.*coli* from ducks with prevalence rate (91%). In this regards, (Roshdy *et al*., 2012) attributed the differences in prevalence rates of E. *coli* among cases of diarrhea and septicemia in ducks to the differences in the pathogenicity, virulence of involved strains, the severity of the cases and the immunological status of the host.

The result of E.*coli* isolation from fish recorded in the present study revealed that 59/100 (59%) of examined *Tilapia Zilli* fish were infected with E.*coli*, this finding was in accordance with that of (Dutta, and Sengupta, 2016) who recorded a prevalence rate of (65%) for E.*coli* from fish.

The current investigation revealed that the isolated E. *coli* belonged to eight serotypes of which four serotypes were isolated from ducks and fish as following, O26:H11, O17:H18 (25/106), O127 and O125: H21. Similar results for sero-identification of pathogenic E.*coli* serotypes (O125, O26, O146, O17, O127 and O153) were reported by (Wang *et al*., 2010 and Roshdy *et al*., 2012) who recorded the isolation of the mentioned serotypes from fish and from ducks, respectively. The results also were in agreement with that of (Gupta *et al*., 2013) who detected O17 and O26 in fish and ducks.

In the present study, bacteriological examination of a total of 200 examined ducks and fish revealed their infection by *Salmoenella* spp with a prevalence rate of 34/200 (17%) of the examined ducks and fish, of which 25/100 (25%) were isolated from ducks. These

results were in agreement with the result of (Tran *et al.,* 2006) who isolated *Salmonella serovars* from ducks with a prevalence rate of (39.0%), while lower prevalence rate (5.57%) for salmonella from cecal content of ducks was recorded by (Adzitey *et al*., 2012). The current result also revealed the isolation of *salmonella spp* from 9/100 (9%) of examined *Tilapia Zilli* fish, higher prevalence rate was recorded by (Jeyasanta *et al.*, 2012) who isolated salmonella with a prevalence rate of (69%) from fish.

In the current study serotyping of the isolated *Salmonella* revealed that isolates belonged to eight serotypes of which 5/8 serotypes were isolated from ducks and fish as follows, *Salmonella* Enteritidis, *Salmonella Saintpaul*, *Salmonella* Anatum, *Salmonella* Typhimurium, and *Salmonella Heidelberg*. Similarly, the same serotypes were isolated by (Heinitz *et al*., 2000, Kumar *et al.,* 2015) and by (Rahman *et al.,* 2016 and Santosa *et al*., 2019) who reported their isolation from fish and ducks, respectively.

Öztürk and Altinok, (2014) concluded that *Pseudomonas* species are opportunistic pathogens as mostly are isolated with other bacteria, thus they suggested that *Pseudomonas spp.* can play the role of being secondary infections. In the present study, bacteriological examination, of *Tilapia Zillii*(fish) and backyard ducks revealed the isolation of 49/200 (24.5%) strains of *Pseudomonas spp*, amongst 15/100 (15%) isolates were isolated from ducks and 34/100 (34%) isolates were isolated from *Tilapia Zilli* fish. These results agreed with that of (Aly *et al.,* 2002) who isolated *Pseudomonas spp*. from ducklings with a prevalence rate of (18%), while higher prevalence rate of *pseudomonas* species from fish was recorded by (Khalifa *et al*., 2016) who reported *Pseudomonas aeruginosa* with a prevalence rate (43%) from Sea Bream fish. Lower prevalence rate was recorded by (Abd El-Tawab *et al*., 2016) who reported the isolation of pseudomonas species with a prevalence rate of (17%) from fresh water fish.

In the present study, *Pseudomonas fluorescens and Pseudomonas aeruginosa* were detected form ducks and *Tilapia zilli*. This result was in agreement with that of (Aly *et al.,* 2002) who detected *Pseudomonas fluorescens* from ducks and fish. The result also was in accordance with that of (Benie *et al*., 2017) who isolated *Pseudomonas aeruginosa* from fresh and smoked fish.

In the current study, bacteriological examination revealed the presence of mixed infection by *Salmonella and* E.*coli* in ducks and in fish with prevalence rates (7% and 5%), respectively, similar result was reported by (Sifuna and Onyango 2018) who found cases of mixed infection with *Salmonella spp* and E.*coli* in (82%) of studied ducks and (7.3%) of studied fish, respectively.

Isolation by conventional methods was confirmed by PCR for the detection of the relevant conserved genus or species genes in ten selected isolates from each of the studied bacterial species, the results revealed the positive detection of 16S rRNA gene sequence, *inv*A gene (encodes for protein in the inner bacterial membrane and which is important for invasion of host epithelial cells by pathogenic salmonella species), and *pho*A gene (encodes for enzyme alkaline phosphatase in E.*coli*) in each 10/10 (100%) of the tested isolates from *Pseudomonas isolates*, salmonella isolates, and E.*coli*, respectively. The results accorded with that of (Alagarsamy *et al.,* 2009) who used PCR and confirmed the detection of (*pho*A gene) in all their tested E.*coli* isolates. The results also agreed with those of (Tekale *et al.,* 2015) who targeted and detected (*inv*A gene) to confirm all *salmonella* isolates, and (Khalifa *et al*., 2016) who detected (16S rRNA gene) in all *Pseudomonas* isolates.

Considering the studied helminthes; *Clinostomum species, Echinostoma species, Ligula intestinalis* and *Contracaecum* from ducks, the findings appear to indicate a host-parasite association as a part of the life cycle of these helminthes. Feeding animal reservoir hosts (dogs, cats, pigs, chicken, and ducks) with fish have been recorded as a risk factor for infection by zoonotic helminthes (Phan *et al*., 2010). Parasitic infections can affect a large number of fish species, especially in countries where untreated human and animal sewage and wastes may be drained into fish habitats without or with insufficient treatment, rendering the risk of potential fish-borne parasitic zoonosis quite large, particularly with important parasitic diseases as trematodes, nematodes, cestodes and protozoa, (Uddin *et al.*, 2018).

In the present study, *Clinostomum species* was found with a total prevalence rate of 54/200 (27%) from total 200 examined ducks and fish of which 15/100 (15%) were isolated form ducks and 39/100 (39%) were isolated from *Tilapia zillii*. In the same instances, six *Clinostomum species* were recognized in North America based on detailed morphological descriptions of stained and mounted adults recovered from avian hosts as recorded by (Caffara *et al*., 2011, Pe´rez-Ponce de Leo´n *et al*., 2016 and Rosser *et al*., 2017). Higher prevalence rates were recorded by (Taher, 2009) who recorded a total prevalence rate with one or more species of encysted metacercariae of (84.75%), while the prevalence rate of microscopic encysted metacercariae and *clinostomatid metacercariae* were (78.25% and 62.25%), respectively. Similar but slightly higher result was also recorded by (Arafa *et al.,* 2005) who recorded (42.86%) from fish, and (Abd-El- Rahman, 2005) who recorded a prevalence rate of (45%) from ducks. Moreover, higher prevalence rate of *Clinostomum*

species from fish was recorded by (Khattab, 1990) (87.06%).

In the present study, *Echinostoma species* was isolated with a total prevalence rate of 30/200 (15%) from examined ducks and fish of which 13/100 (13%) was recorded from examined ducks (adults). In this regards, (Nagwa *et al.,* 2013) recorded that (38%) of studied ducks were infected with *Echinostoma species*, (Khater, 1993) recorded a prevalence rate of *Echinostoma species* infection in birds (47.4%). Examination of fish revealed the isolation of *Echinostoma species* (metacercaria) from *Tilapia zillii* fish with a prevalence rate of 17 /100 (17%), similar prevalence rate was recorded by (Tolossa and Tafesse, 2013) who recorded a prevalence rate of (24.53%) from fish.

Fish-borne cestodes are capable of infecting birds. The members of the order Diphyllobothriidea, the large-sized tapeworms have three host life-cycles, in which fish play a role of the second intermediate hosts and represent a source of the infection (Chai *et al.,* 2005).

In the present study, *Ligula intestinalis* larvae (cestode) was isolated with a total prevalence rate of 41/200 (20.5%) of the total examined ducks and fish of which 19/100 (19%) were isolated from ducks and 22/100 (22%) were isolated from *Tilapia zillii*. These results nearly agreed with that reported by (Weliange and Amarasinghe 2001, Amer *et al*., 2007 and Woinishet and Anwar, 2014) who recorded prevalence rates of 39%, 48% and 32% from *Tilapia zillii*, respectively. In the same regards, (Sohn *et al.,* 2016) reported that the plerocercoid larvae of *Ligula intestinalis* recently caused mass fatality of fish in Korea. Hoole, (1994) concluded that *Ligula intestinalis cestode* have a strong host specificity for fish and birds, (Loot *et al*., 2001) concluded that *Ligula intestinalis* is an avian parasite but not a human parasite

Some nematodes have zoonotic significance, among these parasites, *Contraceacum species* larvae have the highest medical importance because of the severe allergic reactions and gastrointestinal symptoms they cause in the final hosts due to eating or handling infected fish (Lima-dos-Santos *et al.,* 2011).

In the present study, *Contraceacum species* larvae was isolated with a total prevalence rate of 76/200 (38%), of examined ducks and fish of which 30/100 (30%) was isolated from examined ducks, higher prevalence rate was recorded by (Ansary *et al.,* 2009) who recorded isolation of *Contraceacum species* larvae from ducks with a prevalence rate of (65%). The current recorded prevalence rate of *Contraceacum species* larvae isolated from *Tilapia zillii* was 46/100 (46%), while this result was in agreement with that of (Al-Moussawi and Mohammad, 2011) who recorded a prevalence rate of (59%) from Tilapia, higher prevalence rate was recorded by (Milad *et al.,* 2013) who recorded a rate of (71%) from Tilapia. Because the life-cycle of *Contraceacum species* larvae involving hosts at different level of the aquatic webs, *Contraceacum species* larvae parasites could be used as possible indicators of trophic web stability and health of aquatic ecosystems (Mattiucci and Nascetti, 2007).

Antimicrobial resistance has become a global concern in public health and veterinary medicine, high incidence of resistance might be attributed to the indiscriminate use of antibiotics (Szmolka and Nagy, 2013).

In the current study, the isolated E. *coli*, *salmonella spp*. and *Pseudomonas spp* demonstrated high phenotypic resistance patterns to neomycin with resistance rates of (91.5%, 100% and 100%), respectively. Moreover, the studied E. *coli*, *salmonella spp*. and *Pseudomonas spp* isolates demonstrated resistance rates for Florfenicol (84.3%, 85.3% and 89.8%), respectively. The studied isolates of E. *coli*, *salmonella spp* and *Pseudomonas spp* also demonstrated phenotypic resistance to trimethoprim–sulphamethoxine with rates of (91.5%, 91.2% and 91.8%), respectively. These results were similar to those of (Kissinga *et al*., 2018) who recorded high resistance rates of E.*coli* isolates from ducks to several antibiotic groups including cephalosporins aminoglycosides and tetracycline and (Alagarsamy *et al.,* 2009) who recorded that their studied E.*coli* isolates demonstrated resistance to trimethoprim–sulphamethoxine with a rate of (62.3%). Similarly, (Cengizler *et al.,* 2017) reported multidrug resistance phenotypes in aquaculture. Furthermore, (Skov *et al.,* 2007) recorded that all their studied *Salmonella* isolates demonstrated multidrug resistance to Florfenicol, neomycin, trimethoprim–sulphamethoxine, and chlortetracycline with rates of (89%, 80%,75%, and 68%), respectively. In the same regards, (Khalifa *et al*., 2016) recorded that all *Pseudomonas aeruginosa* isolates were resistant to trimethoprim/ sulphamethoxazole, and florfenicol with resistance rates of (89% and 65%), respectively.

PCR was proven to be more sensitive and specific for detection of pathogens. In the current study PCR was applied to investigate the genotypic virulence attributes of the isolated *salmonella spp.* through detection of *Salmonella enterotoxin (stn*) gene, and the result revealed the detection of *stn* gene in the tested 10/10 (100%) salmonella isolates. This result was in accordance with that of (Tekale *et al*., 2015) who found *(stn*) gene in all fish and ducks isolates. On the other hand, PCR failed to detect shiga-toxin-producing genes (*stx*1 *and stx*2) in the tested 10 E.*coli* isolates from ducks and *Tilapia Zillii*. The result was nearly in agreement with that of (Wang *et al*., 2010) who detected (*stx*1 *and stx*2) genes with a low

detection rate (2.4%) in E.*coli* isolates from ducks. In contrast, the recorded result disagreed with that of (Cardozo *et al*., 2018) who reported high detection rate of *stx* genes in E.*coli* isolates from fish.

Bacteria communicate with each other to coordinate expression of specific genes in a cell density-dependent fashion, a phenomenon called quorum sensing and response. Quorum sensing in *Pseudomonas aeruginosa* is a complex, multisignal, global regulatory network with control over diverse target functions including virulence factors, exoenzymes, motility, nutrient acquisition, and biofilm formation, two quorum sensing systems of LuxI-type proteins encoded at separate sites within the *Pseudomonas aeruginosa* genome for the transcriptional activators LasI and RhlI that regulate the virulence genes expression in *Pseudomonas aeruginosa*, (Clay Fuqua, 2006). In the current study, PCR was applied for the detection of *las*I gene of pseudomonas species which is functioning as common regulatory feature and participate in specific ways in the infection process. Moreover, *las*I gene may affect host cell signal transduction in ways that enhance the spread of infection, PCR was also applied to detect exotoxin (*tox*A) genes of pseudomonas species, the results revealed that 10/10 (100%) of the tested pseudomonas isolates which demonstrated phenotypic multidrug resistance were positive for both genes. These results accorded with those of (Markey *et al*., 2013) who detected *tox*A gene and *las*I gene of *Pseudomonas aeruginosa* with rates of (66.7% and 81%), respectively from Nile tilapia. The result also was in accordance with that of (Abd El -Tawab *et al*., 2016) who detected *tox*A gene and *las*I gene with rates of (67% and 88%), respectively from ducks.

PCR was applied for studying the antimicrobial genotypic attributes of isolates through the detection of florfenicol resistance gene *flo*R, the antibiotic against which the isolates from different bacterial species have demonstrated the lowest phenotypic resistance rates, the results revealed that the tested 10/10 (100%) *E.coli*, 10/10 (100%) *Salmonella spp.* and 10/10 (100%) *Pseudomonas* isolates were carriers for *flo*R gene, similar result was recorded by (Abd El- Tawab *et al*., 2016) who detected *flo*R gene in *salmonella spp.* isolated from ducks with a prevalence rate of (77.8%) and (Smith, 2008) who detected *flo*R gene in *salmonella spp.* isolated from fish. The results also accorded with that of (Keyes *et al*., 2000) who reported the detection of the florfenicol resistance gene *flo*R in E. *coli* isolates and (Nhung *et al*., 2017) who detected *flo*R gene in *Pseudomonas spp.* isolates from birds.

Biofilm is defined as microbial communities attached to interfaces and to each other. In a biofilm, bacterial cells are embedded into a matrix forming a physicochemical barrier against different environmental conditions. Resistance to antimicrobial agents is the key feature of biofilm infections, thus, infections caused by bacterial biofilms are difficult to be treated. Moreover, biofilm formation was proven to enhance the multidrug resistance attributes of pathogens. In this regards, *adr* genes regulate cellulose production, consequently regulate biofilm formation in E.*coli* and *salmonella spp* (Rui *et al.*, 2014). Moreover, *psl*, the polysaccharide synthesis gene cluster of pseudomonas, was recently identified as being involved in exopolysaccharide biosynthesis and biofilm formation, (Overhage *et al.*, 2005). In this instance, (Bayoumi *et al*., 2012) concluded that pathogens may develop certain strategies to defy harsh conditions such as chemical sanitization, antibiotic treatment pressure, and biofilm formation which represents a prominent one among the adopted strategies, by which pathogens protect themselves against external threats.

In the current study testing the genetic attributes for biofilm formation was studied by PCR, the results revealed that 10/10 (100%) of tested E.*coli* isolates and 10/10 (100%) of tested *salmonella spp.* isolates were positive for *(adr*A) gene, and that 10/10 (100%) of tested *pseudomonas* isolates were positive for (*psl*A) gene, these positive results for biofilm genetic attributes for all examined isolates were in association with the results of the phenotypic antimicrobial resistance profiles that revealed the phenotypic multidrug resistance attributes for 97/106 (91.5%) of E. *coli*, 30/34 (88%) of *Salmonella spp.* and 45/49 (91.8%) *pseudomonase spp* involved in the study by demonstrating phenotypic resistance to antimicrobial agents from 3 and more antimicrobial categories as described by (CLSI, 2015). Similarly, (Hawash *et al.*, 2017) reported the detection (*adr*A) gene encodes for biofilm formation in all *salmonella* isolated from cases of mixed infection in birds.

In the current study, mixed bacterial and parasitic infections were detected with prevalence rates of 61/100 (61%) of examined ducks and 63/100(63%) of examined fish, these results agreed with the results of (Gomes *et al*., 2019) who detected a link between increased parasitic and bacterial infection in fresh water aquaculture with prevalence rate of (56%). The result also agreed with that of (Hollmén and Franson, 2015) who found an association between bacterial and parasitic infection in ducks involved in their study.

Fish-eating birds not only acquire pathogens from environment, but also return them via excretion to the environment, facilitating the dissemination of those pathogens to humans and other animals, especially through water. Livestock on many farms rely on rivers, streams and other untreated water sources for at least part of their drinking water, (Reilly, 1981).

Since many of the pathogens found in bird feces originate from human sewage, it's expected that humans will be most probably highly susceptible to infection when the bacteria or parasite re-enter the food chain through drinking water supplies and bathing water. Although all evidence concerning the life cycle and means of transmission of these helminthes have been obtained through experimentation, there is strong evidence that fish species are an important source of infection, as parasitic eggs fed by fish hatch in the intestine, and larvae from these fish have led to patent infections when reached birds, (Cross, 1990).

CONCLUSION

Free ranging ducks may represent an interface between aquaculture and terrestrial ecosystems for circulation and spreading of bacterial and parasitic pathogens that possessed virulence, antibiotic genotypic attributes and that some of which may also have zoonotic significance. Consequently, they may expose the environment including water resources, animals, fish, and humans to infection. Moreover, the high prevalence rates of bacterial and parasitic pathogens isolated from free range ducks and fish that share the same community and that are mutually exposed highlight the importance of conducting more investigations and studies on the implicated sources of infections, potential, association, and recommended control measures.

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البط المنزلى الحر ، نظرة عامة على المسببات البكتيرية والطفيلية للعدوى المعوية

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يعتبر البط أكثر الطيور انتشارا بالتربية المنزلية في محافظات الصعيد والوجه البحري بجمهورية مصر العربية ، حيث يتم ابقاء البط ليلا بالعشش واطلاقه نهارا للسباحة والتغذي الحر بالترع المتفرعة من نهر النيل. وقد استهدفت الدراسة تقصي عينة من البط (١٠٠ طائر) والسمك البلطي الشبار (١٠٠ سمكة) من مدينة الزقازيق ، محافظة الشرقية في الفترة من يوليو عام ٢٠١٨ إلى فبراير عام ٢٠١٩ . استهدفت الدراسة الأستدلال على وجود العدوى المعوية ببعض المسببات البكتيرية (الاى كولاى ، سالمونيلا والسودوموناس) والطفيلية مثل (Clinostomum ، Echinostoma ، *intestinalis* Ligula و Contraceacum). وقد أظهرت النتائج عزل ميكروب الاى كولاى بنسبة (٤٧٪ من بط و ٥٩٪ من البلطى الشبار)، على التوالي وعزل ميكروب السيدومونس بنسبة (١٥ ٪ من البط و ٣٤ ٪ من البلطى الشبار) ،على التوالي. في حين تم عزل ميكروب السالمونيلا بنسبة (٢٥ ٪ من البط و ٩ ٪ من البلطى الشبار) ، على التوالي. تبين بالدراسة عزل نوعين من التريماتود منها *Echinostoma sp.* بنسبة (١٣ ٪ من البط و ١٧ ٪ من البلطى الشبار) و .Clinostomum sp بنسبة (١٥% من البط و ٣٩ ٪ من البلطي الشبار. تم كذلك عزل يرقات Ligula *intestinalis* بنسبة (١٩٪ من البط و ٢٢٪ من البلطى الشبار) وعزل Nematode Contraceacum spp بنسبة (٣٠ ٪ من البط و ٤٦ ٪ من البلطي الشبار) ، على التوالي. استخدم تفاعل انزيم البلمرة المتسلسل للتحقق من امتلاك المعزولات البكتيرية لجينات ضراوة ،جينات مقاومة المضادات الحيوية وجينات البيوفيلم. وتشير النتائج الى أهمية مراقبة ودراسة الوسط الناقل للعدوى بين البط والسمك وكذلك اهمية رفع الوعي العام بممارسات الأمن الحيوي للتخفيف من المخاطر المحتملة التي قد تسببها الميكروبات المعزولة على الصحة العامة.