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Original article

## Investigation of many bacterial and viral infections circulating in pigeons showing nervous symptoms



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## ABSTRACT

Pigeon's flocks have shown several neurological symptoms including circling, torticollis, tremors, paralysis, which caused suspicion for viral or bacterial natural infections. *Pigeon paramyxovirus* type-1 (PPMV-1) is a notifiable disease-causing high morbidity and mortality with severe nervous symptoms. Clinical represented tissue specimens were collected from 50 infected pigeon flocks in eight governorates. All samples were examined bacteriologically (isolation, identification and serotyping) for *E. coli*, *Salmonella* spp., *S. aureus* and *Pseudomonas aeruginosa*. Antimicrobial susceptibility test (AST) was accomplished for all isolates using a disk-diffusion test. For viral identification, RT-PCR specific oligonucleotide primers were used for distinguishing of *Avian influenza virus*, PPMV-1 and PPMV-3. Neurological manifestations were observed in pigeon's flocks mainly in winter and autumn. The mortality rate in eight governorates was about 50% in 10 flocks and other houses mortality rate was ranged from 10 to 20%. Post mortem examination have shown hemorrhagic enteritis, soft and friable brain tissues and/or hemorrhages. The percentage of isolated bacteria *E. coli*, *Salmonella* spp., *S. aureus* and *Pseudomonas aeruginosa* were 75%, 75%, 50% and 18.75%; respectively. The antibiotic resistance pattern for bacterial isolates showed resist to ampicillin, amoxicillin- clavulanic acid, tetracycline, ceftriaxone, doxycycline, sulfamethoxazole-trimethoprim and ceftazidime with different result for each type of bacteria, while *Salmonella* spp., isolates showed only a highly intermediate result for ciprofloxacin. Eight samples are positive with 16% to PPMV-1. Also, sample No.5,6,9 was co-infected with different types of bacterial isolates in addition to NDV. In conclusion, we reported several neurological symptoms in pigeon's flocks mainly of bacterial infections (*E. coli*, *Salmonella* spp., *S. aureus* and *Pseudomonas aeruginosa*).

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## 1. Introduction

Meningitis, encephalitis and encephalomyelitis are types of inflammation occurred in the meninges, brain, or brain and spinal cord, respectively. In addition, meningoencephalitis and meninge encephalomyelitis are the inflammatory processes occurred in such conditions have long been associated with bacteria, virus, fungi, rickettsial agents, parasites which are finding in people and animals so, various causative agents as *Salmonella* spp., *E. coli*, *Staphylococcus* spp., were isolated from meningitis and encephalitis (Callanan, 2021). Moreover, nervous signs can be

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caused by bacteria as *Salmonella* spp., *E. coli*, *Staphylococcus* spp., and *Pseudomonas aeruginosa* (Shivaprasad, 2014). Newcastle disease (ND), caused by *Newcastle disease virus* (NDV), in pigeons is responsible for severe highly infectious diseases (Alexander, 2000; Wan et al., 2004; Alexander, 2009) (See Table 1).

Salmonellosis is one of the significant diseases infecting aviaries as well as in racing pigeons (Vereeken et al., 2000) and it was caused by *Salmonella* spp., family *Enterobacteriaceae* (De Sousa et al., 2010; Abd El-Hack et al., 2021). *Salmonella* can reproduce and keep alive in contaminated feces for over one month so that, the free pigeon plays a critical role in *Salmonella* spread (Kobayashi et al., 2007; Hughes et al., 2008; Dutta et al., 2013). Pigeons are common carriers of *Salmonella* as susceptible reservoirs and the infections could be enteric, non-enteric (abscesses, pneumonia, osteomyelitis, septic arthritis, endocarditis, and meningitis) and systemic (bacteremia) (Nadeem et al., 2019).

*E. coli* strains can produce septicemia in a variety of tissues; causing joints, brain and ovarian infections. Also, it can cause shell embryos death, and sudden death in young or adult birds (Gordon, 2010; Khudair, 2012; Swelum et al., 2021).

In pigeon flocks, most of staphylococcal infections are caused by *Staphylococcus aureus* (Kizerwetter-Swida et al., 2015), which lived in the choanal slit of healthy birds. Pigeons may be a staphylococcal infection source and may also possess a reservoir of resistance and virulence genes (Chrobak-Chmiel et al., 2021).

*Pseudomonas aeruginosa* is one of the most widespread virulent opportunistic pathogens. The natural coinfection by *Pseudomonas aeruginosa* and *E. coli*. *Pseudomonas aeruginosa* was recognized commonly in intestine, liver, spleen, heart and lung in free-living pigeons (Vasconcelos et al., 2017).

The NDV also termed as *pigeon paramyxovirus* type-1 (PPMV-1) and it is belonged to genus *Avulavirus*, family *Paramyxoviridae* (Amarasinghe et al., 2018). ND was detected in the domestic pigeons and occasionally spreads to a wide variety of other avian species, as feral pigeons, doves, and exotic birds (Alexander, 2000) causing a high mortality from 40 to 80% in infected pigeons (Perec-Matysiak et al., 2017; Qiu et al., 2017) with nervous, respiratory, and digestive symptoms, ranging from moderate to severe depression with neck twisting, ataxia, paralysis, diarrhea, and greenish feces (Vindevoegel et al., 2006; Cattoli et al., 2011; Wang et al., 2015).

All NDV strains belong to APMV-1 and 20 additional serotypes have been identified (ICTV, 2018). Regarding pathogenicity to domestic birds, *Pigeon paramyxovirus* type-3 (APMV-3) possibly is second in importance to NDV. There is a high degree of amino acid sequence variation between APMV-3 and APMV-1 (NDV), Haemagglutination test showed a cross reaction between APMV-3 and APMV-1, which often leads to misdiagnosis of APMV-3 as APMV-1. APMV-3 caused encephalitis with high mortality in caged birds and nervous signs in Psittacine and Passerine birds (Beck et al., 2003).

*Influenza A* viruses, family *Orthomyxoviridae*, are segmented negative-sense ssRNA viruses. *Avian influenza virus* (AIV) is divided into various subtypes depended on its surface glycoproteins;

haemagglutinin (HA) and neuraminidase (N) (Fouchier et al., 2005). AIV can infect a wide range of hosts, such as chickens, wild birds, aquatic birds, humans, cats, and dogs (Zhang et al., 2020). Particularly, nevertheless, pigeon flocks were be resistant to AIV-H5 in the previous studies (Liu et al., 2007), but H5N1 viruses were identified in sparrows, and pigeons (Liu et al., 2010; Yao et al., 2015). Other studies have mentioned that the H5N1 *Influenza viruses* could infect pigeons with a various mortality rate (Mansour et al., 2014). Regarding novel H7N9 *Influenza virus* which isolated from pigeons so, the host may be either susceptible to infection or may spread the virus (Shi et al., 2013).

The infected pigeons exhibited an indicative clinical sign for AIV as respiratory, nervous symptoms and greenish diarrhea (Elgendy et al., 2016; Mansour et al., 2017). The aim of our study was to identify the most common infectious agents (bacterial and viral) causing nervous manifestations in pigeons in Egypt during 2020–2021 using classical and molecular methods.

## 2. Materials and methods

### 2.1. Clinical examination and sampling

Between 2020 and 2021, samples were collected from eight governorates (Cairo, Giza, EL-Gharbia, EL- Fayum, EL-Menofia, EL-Kalubia, Kafer El-Shaigh, and El-Behera) in Egypt (See Table 1). The clinical represented specimens were collected from 50 affected pigeon flocks suffered from severe nervous manifestation as trembling wings, circling, lameness, torticollis, neck twisting, leg paresis or paralysis, greenish diarrhea and deaths. The pigeons from several houses ranged between 9 and 100 with mean of 50 pigeons/house. The age of the pigeons varied from two weeks to 3 years. Internal organs (liver, heart, spleen, lung, cecum, brain and bone marrow) were collected from affected flocks for viral and bacterial detection and isolation.

### 2.2. Bacterial isolation and identification

Fifty samples were examined bacteriologically for various bacterial species as *E. coli*, *Salmonella* spp., *S. aureus* and *pseudomonas aeruginosa*.

#### 2.2.1. Isolation and identification of *Salmonella* spp.

It was performed according to ISO 6579–1: 2017/ Amd1: 2020, the pre-enrichment of tested samples was done in buffer peptone water (Oxoid®) and incubated at 37°C for 16–18 h. Then transfer 0.1 ml of the incubated pre-enrichment medium to Modified Semisolid Rappaport-Vassiliadis medium (MSRV, Oxoid®) and incubated for 24 h. at 41.5°C as well as 1 ml to Muller-Kauffmann Tetrathionate (MKTTn broth, Oxoid®) and incubated aerobically at 37°C for 24 h. After that, it streaked onto Xylose Lysine Deoxycholate agar (XLD, Oxoid®) and Hektoen Enteric (HE, Liofilchem®) agar plates and incubated aerobically at 37 °C for 24 h. the selected colonies distinguished by biochemical tests (Urea agar, Triple sugar iron, and Lysin iron) (LabM, Oxoid & Liofilchem®; respectively).

#### 2.2.2. Isolation and identification of *E. Coli*

The isolation was performed according to Lee and Nolan, (2008); all the collected samples were pre-enriched in buffered peptone water (Oxoid®) and incubated under aerobic conditions at 37C for 24 h. Then a loopful from broth cultures were inoculated onto MacConkey agar (Neogen®) and Eosin methylene blue agar plates (LabM®) and incubated at 37 °C for 24 h. The isolated colonies were detected morphologically and biochemically (“oxidase stripe, TSI oxid®”, “urea, Simmon Citrate, peptone water LabM®” and “Kovacs reagent Himedia®”).

**Table 1**

History of samples collected from pigeon flocks.

Governorate	Breed	No. of samples
Giza	Domestic pigeons	10
Cairo	Foreign pigeon, domestic pigeon	8
El-Fayom	Domestic pigeons	5
El-Dakahalia	Domestic pigeons, foreign pigeon	6
El-Gharbia	Domestic pigeons, foreign pigeon	7
El Menofia	Domestic pigeons, foreign pigeon	6
El-Behera	Domestic pigeons	4
EL Kalubia	Domestic pigeons	4

### 2.2.3. Isolation and identification of *Staphylococcus aureus*

It was done according to standard methods [BAM, 2016](#) and [ISO 6888–1, 2018](#). Pre-enriched buffer inoculated on Baird Parker ager (Oxoid®) and incubated at 37 °C for 24–48 h. selected colonies were identified morphologically “black colony surrounded with double zones showed the tellurite reduction with lipase activity”, microscopically “gram positive grape like structure” and biochemically; positive for slide catalase test, mannitol fermentation, tube coagulase test and acetoin production (VP) while, negative for oxidase test.

### 2.2.4. Isolation and identification of *Pseudomonas aeruginosa*

It was done according to [Shukla and Mishra, \(2015\)](#) and [UK Standards for Microbiology Investigations, \(2015\)](#). Inoculated pre-enriched buffer onto selective medium MacConkey agar and incubated at 37 °C for 24 h. to notice the non-lactose fermenting colonies and sub- cultured onto nutrient agar plate to notice the *Pseudomonas* colonies pigmentation (nearly colourless), with yellow to green colony, large, oval, convex and rough, sometimes surrounded by serrated growth. The suspected colonies were picked up and subjected to further detections based on: morphology non-spore forming, Gram negative rods which are straight or slightly curved and are (0.5 – 1.0 µm by 1.5 – 5.0 µm), pigment production, detection of musty smell, catalase positive and oxidase test positive.

## 2.3. Serotyping of isolated *Salmonella* species and *E. Coli*

### 2.3.1. Serotyping of isolated *Salmonella* species

It was performed according to [ISO 6579–3: 2014](#) using slide agglutination method. Firstly, mixing of the fresh culture and normal saline after give negative agglutination continued with testing polyvalent (O) group followed by polyvalent (H) group then checking the monovalent (O) and (H) to can detect the formula of *Salmonella* strains and reading of *Salmonella* species by Kauffman–White scheme ([Grimont and Weill, 2007](#)) using *Salmonella* antiserum (Sifin Co., Germany®).

### 2.3.2. Serotyping of isolated *E. Coli*

By using Somatic (O) antigens of *E. coli* according to kit (DENKA SEIKEN Co., Japan® and Sifin Co., Germany) antiserum. Using slide agglutination method, mixing of the fresh culture and normal saline after give negative agglutination continued with testing polyvalent (O) group from 1 to 8 Denka Seiken and group 1 to 3 of Safin antisera followed by checking the monovalent (O) included in the positive polyvalent group.

## 2.4. Antimicrobial susceptibility test (AST)

An AST was conducted for all isolates of *E. coli*, *Salmonella* spp., *S. aureus* and *pseudomonas aeruginosa* using a disk-diffusion test, according to a method described by ([CDC and WHO, 2003](#)) against 15 antibiotics (Himedia®), which were as follows: “amoxicillin-clavulanate (AMC 30 µg), ampicillin (AMP 10 µg), aztreonam (AT 30 µg), ciprofloxacin (CIP 5 µg), ceftriaxone (CTR 30 µg), cef-tazidime (CAZ 30 µg), cefepime (CPM 30 µg), colistin sulfate (CT 10 µg), doxycycline (DO 30 µg), fosfomycin (FO 200 µg), gentamycin (GEN 10 µg), norfloxacin (NX 10 µg) streptomycin (S 10 µg), sulfamethoxazole-trimethoprim (SXT 25 µg), and tetracycline (T 30 µg),” and interpreted according to [CLSI/NCCLS, \(2017\)](#).

## 2.5. Virus isolation and detection

### 2.5.1. Virus isolation

After clinical examination, pigeons were euthanized for necropsy. The collected brain issues were homogenized into 10%

sterile PBS (phosphate buffered saline) suspension then centrifuged at 2,000xg for 10 min. The supernatant of homogenized organs was inoculated into the allantoic sac of 9–11-day-old specific-pathogen-free (SPF) of embryonated chicken eggs (ECEs), Then incubated at 37 °C for 5 days. After that, the fresh allantoic fluids were harvested from the ECE then tested for hemagglutination (HA) activity. Negative HA samples were inoculated for another two blind passages ([OIE, 2012; Dodovski et al., 2017](#)).

### 2.5.2. Viral RNA extraction and polymerase chain reaction (PCR)

Viral RNA was extracted by using QIAamp viral RNA mini kit (Qiagen, Germany) in according to manufacturer’s instructions. AgPathID One-Step RT-PCR (Thermo scientific, USA) and specific oligonucleotide primers and taqman probes assays were applied for detection of AIV and for AIV-H5 and H7 ([Löndt et al., 2008](#)), F gene of NDV ([Wise et al., 2004](#)). RT-PCR was done using Step One Plus Real-Time PCR machine (Applied Biosystem Thermo scientific, USA). While for *Avian Paramyxovirus*, specific oligonucleotide primers were used according to ([Tong et al., 2008](#)). The specific gene amplification was performed using ProFlex thermal cycler - PCR machine (Applied Biosystem Thermo scientific, USA). The gene-specific PCR amplicons were detected by agarose gel electrophoresis.

## 3. Results

### 3.1. Clinical signs and post mortem examination

Examination of internal organs showing neurological manifestations of circling, torticollis, head and neck tremors, leg and wing paralysis were noticed in affected pigeons’ flocks mainly in winter and autumn ([Fig. 1](#)). All examined pigeons showed greenish diarrhea, dullness, and lethargy. The mortality rate in 10 of 50 houses was ~ 50% and the other houses mortality rate was ranged from 10 to 20%. Post mortem examination (PM) which performed on visceral organs of pigeon’s flocks showing the presence of severe hemorrhagic enteritis in the intestine, soft brain tissues and/or hemorrhages with congested blood vessels, petechial hemorrhage in the sub mucosae of the gizzard, and congested liver ([Fig. 2](#)).

### 3.2. Bacterial isolation and identification

Isolation of different bacteria as *E. coli*, *Salmonella* spp., *S. aureus* and *Pseudomonas aeruginosa* with percentage 75%, 75%, 50% and 18.75%; respectively as described on ([Table 2](#)).

#### 3.2.1. *Salmonella* serotypes

The 36 isolated *Salmonella* strains were serotyped separately revealed 18 (50%) strains were *S. Stanley* {O 1,4, [5], 12,[ 27]; d; 1, 2}, 12 strains (33.3%) were

*S. Typhimurium* {O 1,4,[5],12; i; 1,2} and 6 strains (16.6%) were *S. Virchow* {O 1,7, 14; r; 1,2}.

#### 3.2.2. *E. Coli* serotypes

The 36 *E. coli* strains were serotyped showed 12 (33.3%) strains were (O27), 9 (25%) strains were (O25), 6 (16.6%) strains were (O78) while 3 (8.3%) strains were serotyped for each (O63, O103 and O111).

### 3.3. Antimicrobial susceptibility test (AST)

Antimicrobial susceptibility pattern was carried for all isolates (*Salmonella* spp., *E. coli*, *S. aureus* and *Pseudomonas aeruginosa*) as shown in ([Table 3](#) & [Table 4](#)). The results of antimicrobial susceptibility test reported that the majority of antimicrobial showed





**Fig. 1.** Pigeon populations suffered from severe nervous manifestation as neck twisting, and leg paresis or paralysis.



**Fig. 2.** PM lesions of pigeon flocks (4-week-old pigeons). **A and B:** Congested brains. **C:** severe hemorrhagic enteritis.

resistance was ampicillin with 100% for all types of strains, also amoxicillin clavulanic acid and tetracycline showed 100% for all types of strains except *S. aureus* only with percentage 62.5% and 50%; respectively. While, *Pseudomonas aeruginosa* showed 100% also for ceftriaxone, doxycycline and Sulfamethoxazole-trimethoprim but, *E. coli* isolates were resistant to ceftriaxone with

percentage 91.7% and 83.3% for Sulfamethoxazole-trimethoprim. On the other hands, ceftazidime was 100% resistant for *S. aureus* isolates but, showed 58.3% for *Salmonella* spp., while, *E. coli* showed intermediate with percentage 66.7%, [Figs. 3a and 3b].

### 3.4. Virus detection and isolation

#### 3.4.1. Virus isolation

After three blind passages in 9–11-day-old SPF-ECEs to isolate NDV, they manifested pigeon embryo mortality within 72 h. The haemagglutinating titer of allantoic fluid was (1024 HA units/0.05 ml) that obtained after the 3 passages in ECE. Based on HA and lesion in embryo eight samples suspected viral infection.

#### 3.4.2. Polymerase chain reaction:

The positive allantoic fluid was confirmed by RT-PCR by amplification of the viral RNA using specific oligonucleotide primers for detection of AIV, F gene of NDV, and Avian Paramyxovirus serotype-3. Eight samples are positive to NDV (F gene) in PCR reaction with percentage 16% in young pigeon (below 1 months,) than in older birds mainly in (Cairo, Giza, EL Kalubia) governorates, Egypt in winter and autumn and in foreign breed than native one (Fig. 4). Regarding NDV, sample No. five were co-infected with *E. coli*, *Salmonella* spp., *S. aureus* and *Pseudomonas* in addition to NDV. Also, samples No. six were co-infected with NDV, *E. coli*, *Salmonella*

**Table 2**  
Incidence of bacterial species from pigeon samples suffered from several nervous manifestations.

Samples	Examined Samples (120)	Positive bacterial isolates (%)			
		<i>Salmonella</i> spp.	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>
Diseased (24 cases)	Organs*	12	18	9	6
	Brain	21	18	9	6
	Cecum	15	–	–	–
Total positive diseased cases		21 (87.5)	18 (75)	9 (37.5)	6 (25)
Dead (24 cases)	Brain	15	18	15	3
	Bone marrow	15	18	15	0
Total positive dead cases		15 (62.5)	18 (75)	15 (62.5)	3 (12.5)
Total positive		36 (75)	36 (75)	24 (50)	9 (18.75)

\* Organs (liver, heart, spleen, lung).

**Table 3**  
Antimicrobial susceptibility pattern of the isolated *Salmonella* and *E. coli* from infected pigeons.

Antimicrobial agent	<i>Salmonella</i> (n = 36) isolates			<i>E. coli</i> (n = 36) isolates		
	Resistant No. (%) <sup>*</sup>	Intermediate No. (%) <sup>*</sup>	Sensitive No. (%) <sup>*</sup>	Resistant No. (%) <sup>*</sup>	Intermediate No. (%) <sup>*</sup>	Sensitive No. (%) <sup>*</sup>
Amoxicillin-clavulanic acid (AMC <sup>30</sup> )	36 (100%)	0	0	36 (100%)	0	0
Ampicillin (AMP <sup>10</sup> )	36 (100%)	0	0	36 (100%)	0	0
Aztreonam (AT <sup>30</sup> )	12 (33.3%)	0	24 (66.7%)	0	0	36 (100%)
Cefepime (CPM <sup>30</sup> )	15 (41.7%)	21 (58.3%)	0	3 (8.33%)	27 (75%)	6 (16.7%)
Ceftazidime (CAZ <sup>30</sup> )	21 (58.3%)	15 (41.7%)	0	0	24 (66.7%)	12 (33.3%)
Ceftriaxone (CTR <sup>30</sup> )	18 (50%)	9 (25%)	9 (25%)	0	0	36 (100%)
Ciprofloxacin (CIP <sup>5</sup> )	3 (8.3%)	33 (91.7%)	0	0	0	36 (100%)
Colistin sulphate (CL <sup>10</sup> )	6 (16.7%)	0	30 (83.3%)	0	0	36 (100%)
Doxycycline (DO <sup>30</sup> )	9 (25%)	0	27 (75%)	33 (91.7%)	3 (8.3%)	0
Fosfomycin (FO <sup>200</sup> )	9 (25%)	0	27 (75%)	0	0	36 (100%)
Gentamycin (GEN <sup>10</sup> )	6 (16.7%)	0	30 (83.3%)	6 (16.7%)	0	30 (83.3%)
Norfloxacin (NX <sup>10</sup> )	3 (8.3%)	6 (16.7%)	27 (75%)	0	0	36 (100%)
Streptomycin (S <sup>10</sup> )	9 (25%)	15 (41.7%)	12 (33.3%)	12 (33.3%)	3 (8.3%)	21 (58.3%)
Sulfamethoxazole-trimethoprim (SXT <sup>25</sup> )	15 (41.7%)	0	21 (58.3%)	30 (83.3%)	0	6 (16.7%)
Tetracycline (TE <sup>10</sup> )	9 (25%)	0	27 (75%)	36 (100%)	0	0

\* Percentage calculated by dividing the result to total number of isolates.

**Table 4**  
Antimicrobial susceptibility pattern of the isolated *S. aureus* and *Pseudomonas aeruginosa* from infected pigeons.

Antimicrobial agent	<i>S. aureus</i> (n = 24) isolates			<i>Pseudomonas aeruginosa</i> (n = 9) isolates		
	Resistant No. (%) <sup>*</sup>	Intermediate No. (%) <sup>*</sup>	Sensitive No. (%) <sup>*</sup>	Resistant No. (%) <sup>*</sup>	Intermediate No. (%) <sup>*</sup>	Sensitive No. (%) <sup>*</sup>
Amoxicillin-clavulanic acid (AMC <sup>30</sup> )	15 (62.5%)	0	9 (37.5%)	9 (100%)	0	0
Ampicillin (AMP <sup>10</sup> )	24 (100%)	0	0	9 (100%)	0	0
Aztreonam (AT <sup>30</sup> )	24 (100%)	0	0	0	3 (33.3%)	6 (66.7%)
Cefepime (CPM <sup>30</sup> )	12 (50%)	12 (50%)	0	3 (33.3%)	3 (33.3%)	3 (33.3%)
Ceftazidime (CAZ <sup>30</sup> )	24 (100%)	0	0	0	3 (33.3%)	6 (66.7%)
Ceftriaxone (CTR <sup>30</sup> )	3 (12.5%)	12 (50%)	9 (37.5%)	9 (100%)	0	0
Ciprofloxacin (CIP <sup>5</sup> )	3 (12.5%)	3 (12.5%)	18 (75%)	0	6 (66.7%)	3 (33.3%)
Colistin sulphate (CL <sup>10</sup> )	15 (62.5%)	0	9 (37.5%)	6 (66.7%)	0	3 (33.3%)
Doxycycline (DO <sup>30</sup> )	3 (12.5%)	3 (12.5%)	18 (75%)	9 (100%)	0	0
Fosfomycin (FO <sup>200</sup> )	0	0	24 (100%)	3 (33.3%)	6 (66.7%)	0
Gentamycin (GEN <sup>10</sup> )	3 (12.5%)	3 (12.5%)	18 (75%)	0	0	9 (100%)
Norfloxacin (NX <sup>10</sup> )	0	3 (12.5%)	21 (87.5%)	0	0	9 (100%)
Streptomycin (S <sup>10</sup> )	3 (12.5%)	3 (12.5%)	18 (75%)	6 (66.7%)	0	3 (33.3%)
Sulfamethoxazole-trimethoprim (SXT <sup>25</sup> )	9 (37.5%)	0	15 (62.5%)	9 (100%)	0	0
Tetracycline (TE <sup>10</sup> )	12 (50%)	0	12 (50%)	9 (100%)	0	0

\* Percentage calculated by dividing the result to total number of isolates.

spp., and *Pseudomonas aeruginosa*. While, samples no. 9 was co-infected with NDV and *E. coli* only (Fig. 5) as the result of co-infection in pigeons affected with nervous signs. But all samples are negative to Avian influenza virus and Pigeon paramyxovirus serotype-3.

#### 4. Discussion

Animals' health is affected by bacterial, fungal and viral illnesses, which can be fatal. These diseases are costly to treat, and they can cause high mortality in animals, resulting in significant

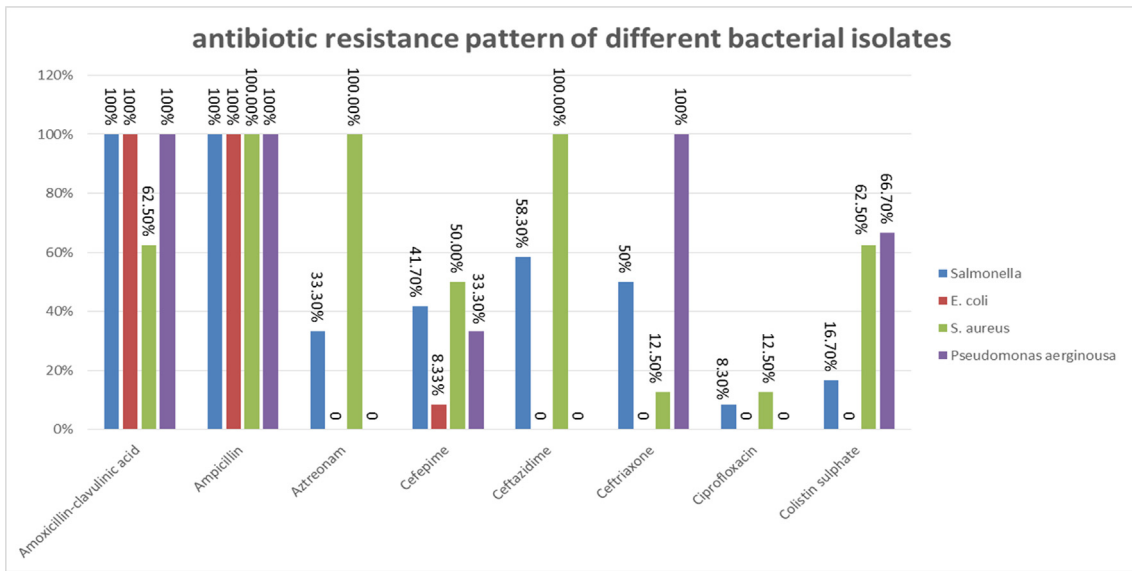


Fig. 3a. Antimicrobial resistance pattern of the different bacterial isolates from infected pigeons.

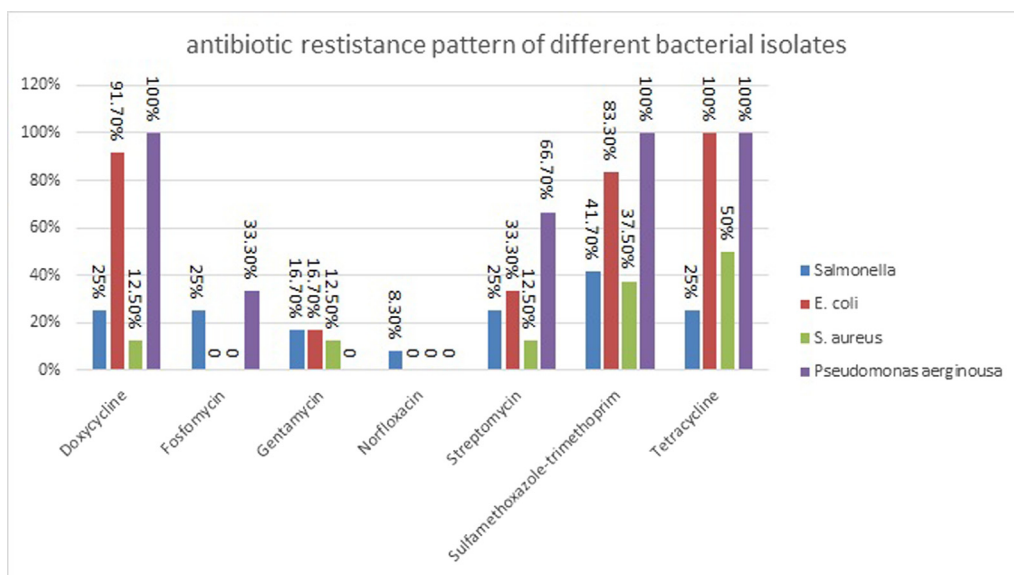


Fig. 3b. Antimicrobial resistance pattern of the different bacterial isolates from infected pigeons.

financial losses for breeders (Swelum et al., 2020; El-Saadony et al., 2021; El-Shall et al., 2022). In this study, pigeon’s flocks have shown several clinical neurological symptoms consisting of circling, torticollis, head and neck tremors, leg and wing paralysis mainly in winter and autumn, which caused suspicion for viral or bacterial natural infections. To the best of our knowledge, no research has been performed mainly on a neurological symptom of viral or bacterial etiology in affected pigeons’ flocks, Egypt.

According to our findings, the most common clinical samples from eight governorates in Egypt, were reported with clinical neurological symptoms in the form of circling, torticollis, head and neck tremors, leg and wing paralysis in affected pigeon’s flocks mainly in winter and autumn. These results were in line with the findings of Badr et al., (2020); Kaczorek-Lukowska et al., (2021); Chang et al., (2021).

In our study, the high mortality rate ~ 50% was detected in 10 affected flocks in three governorates (Cairo, Giza, EL Kalubia),

Egypt, and clinically observed and the other pigeon houses mortality rate was ranged from 10 to 20%. These results came in accordance with Farghaly and Mahmoud, (2011); Mansour et al., (2017) and it was observed mainly in automun and winter as previously mention by Guo et al., (2013); Munmun, et al., (2016); Mansour et al., (2017). Post mortem examination showed the presence of severe hemorrhagic enteritis in the intestine, soft and friable brain tissues and/or hemorrhages, petechial hemorrhage in the gizzard, and congested liver. These findings are in agreement with Kasuya et al., (2017); Dodovski et al., (2017); Apostolakis et al., (2021).

Some affected pigeons have “twisted neck” syndrome commonly associated with PMV; but also, found probability for other infection as salmonellosis and colibacillosis (Paul et al., 2015). Pigeons are common carriers of *Salmonella* and considered as a reservoir (Ammar et al., 2014). *Salmonella* spp., is the most usual cause of bacterial meningitis which commonly causes death

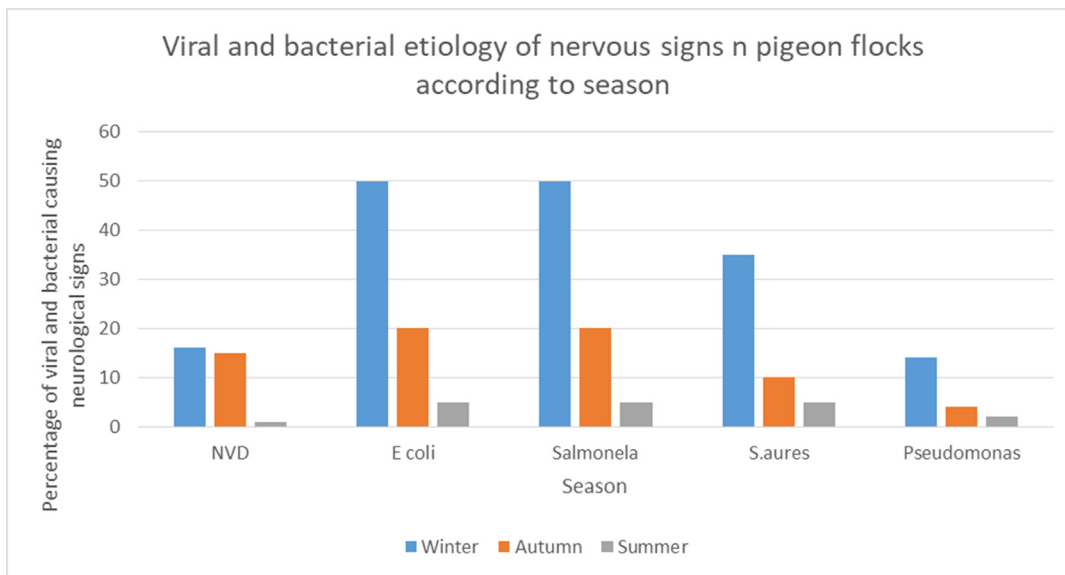


Fig. 4. Viral and bacterial etiology of neurological manifestations in pigeon populations according to season.

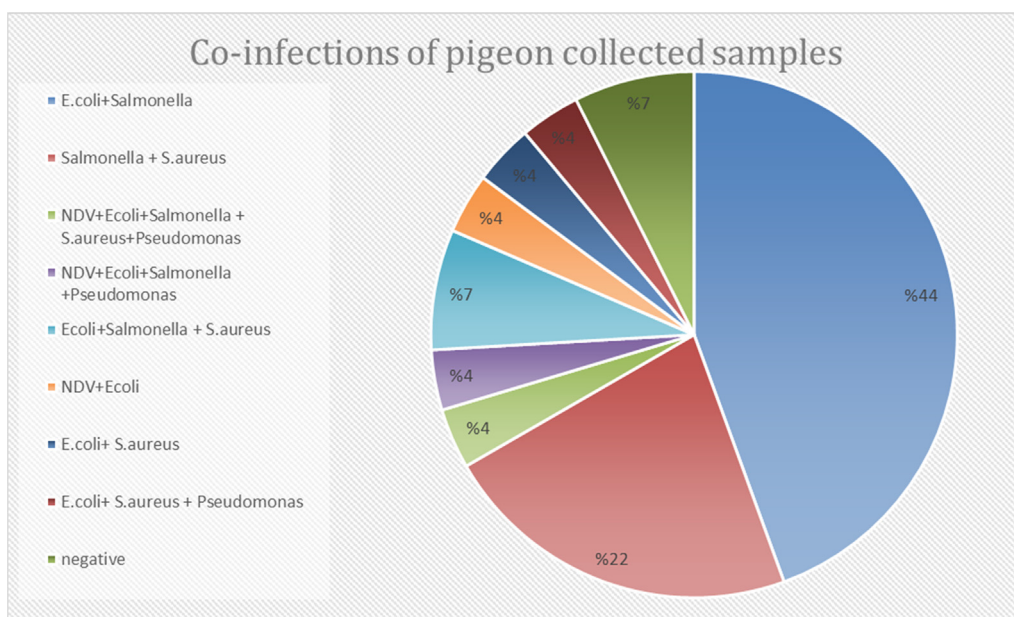


Fig. 5. Co-infection findings of viral and bacterial aetiology from tissue samples of pigeons.

(Badr et al., 2020). The clinical manifestations of pigeon salmonellosis may have torticollis and other neurological signs (Kaczorek-Łukowska et al., 2021).

In our study, *Salmonella* spp., was isolated with percentage of 75% (36/48) from pigeon flocks suffered from nervous system manifestation. But, Osman and Marouf, (2014) detected 12/150 (8 %) *Salmonella* isolates from pigeons, in Egypt.

The *Salmonella* strains in our study were serotyped, revealed 50% were *S. Stanley*, 33.3% were *S. Typhimurium* and 16.6% were *S. Virchow*. Also, Ammar et al., (2014) represented *S. Typhimurium*, *S. Enteritidis* more than *S. Agona*, in Egypt. Osman et al., (2013) isolated *S. Typhimurium*, *Braenderup*, and *Lomita* from pigeon in Egypt. Farghaly and Mahmoud, (2011) isolated 50% *S. Typhimurium* from adult pigeons suddenly died in Egypt. While, (Georgiades and Iordanidis, 2002) stated (75.5%) *S. Typhimurium*

and others as *S. Enteritidis*, *S. Gallinarum* *S. Hadar*, and *S. Abony* were isolated from pigeons, in Thessalonica.

In our study, *E. coli* isolation was 36/48 with percentage of 75%. *E. coli* strains were serotyped showed O27, O25, and O78 with percentage 33.3%, 25%, 16.6%; respectively, while, (O63, O103 and O111) were 8.3%. But *S. aureus* and *Pseudomonas aeruginosa* were isolated with percentage (24/48) 50% and (9/48)18.75%, respectively. Similarly, Farghaly and Mahmoud, (2011) stated some bacterial pathogens (*Salmonella* spp., *E. coli*, *S. aureus*, and *Pseudomonas aeruginosa*) from suddenly death adult pigeons in Egypt, reported 24% *E. coli* which serotyped as O2, O78 and O126.

On the other hands, Hassan and Bakeet, (2014) identified 19 isolates of *E. coli* from pigeons, serotyped as O78 (21.05%) followed by O2 then O1, O128 and O119, while O111, O114 and O44 were 5.26%. Apostolakos et al., (2021) reported 7.2% colibacillosis from



brain of encephalitis lesion. Also, Karim et al., (2020) detected *E. coli* and *Salmonella* spp., was 52.5 and 27.5%; respectively, in Bangladesh. While, Kasuya et al., (2017) reported bacterial meningitis cases of colibacillosis during the quarantine period of imported chicks with serotypes O18 and O161.

Recently, *S. aureus*-associated meningitis and brain abscesses have increased, however the precise mechanism through leave the bloodstream and enter to the central nervous system (CNS) are unknown (Sheen et al., 2010; Chrobak-Chmiel et al., 2021). On the other hands, Vasconcelos et al., (2017) presented that the pigeon reported co-infection by *Pseudomonas aeruginosa* and *E. coli*. These results suggest that pigeons are capable of hosting these pathogens and are susceptible to their infection. Varriale et al., (2020) examined companion birds, isolated *P. aeruginosa* with percentage of 7.8%, whereas *E. coli* was isolated in 30.7%.

The antibiotic therapy is the most common methods to treat diseased pigeons (Kaczorek-Łukowska et al., 2021). In our result, antimicrobial susceptibility test reported that the majority of antimicrobial resistance was ampicillin with 100% for all types of strains, also amoxicillin-clavulanic acid, tetracycline, ceftriaxone, doxycycline, Sulfamethoxazole trimethoprim and ceftazidime showed 100% for one or some isolated strains, but other antibiotic with different percentage.

Near to *Salmonella* result, Farghaly and Mahmoud, (2011) and Osman et al., (2014) who reported *Salmonella* resistant to ampicillin, oxytetracyclin, sulfamethoxazole/trimethoprim and streptomycin. Also, Yousef and Mamdouh, (2016) found that all *Salmonella* isolates were resist to streptomycin, amoxicillin/clavulanic acid, ampicillin and ceftazidime. Ledwoń et al., (2019) detected rising resistance to amoxicillin-clavulanic acid, gentamicin and tetracycline in *Salmonella* strains from two studies.

Parallel to our result, Farghaly and Mahmoud, (2011) and Hassan and Bakeet, (2014) reported that all *E. coli* isolates were resist to trimethoprim/sulphmethoxazol followed by ampicillin, ciprofloxacin, colistin sulphate, tetracyclin and doxycycline, In Egypt. While, Varriale et al., (2020) stated that the *E. coli* isolates were resist with 51.1% to amoxycicillin-clavulanic acid, (55%) to sulfamethoxazole-trimethoprim, (57.1%) to doxycycline, (26.4%) to gentamicin and (63.6%) to oxytetracycline.

Also, Gharajalar and Shahbazi, (2018) mentioned that the isolated *Staphylococcus* spp., were resist to tetracycline with percentage (100%). By examination of Chrobak-Chmiel et al., (2021) to *S. aureus* and *E. coli*, which isolated from oral cavity of racing pigeon found that the *E. coli* isolates revealed resistant to amoxicillin-clavulanic acid, gentamicin, tetracycline, doxycycline, sulfamethoxazole/trimethoprim and ampicillin. Whereas, *S. aureus* isolate was resistant to all beta-lactam antibiotics tested and to gentamicin, in Poland. In addition, Varriale et al., (2020) reported that *P. aeruginosa*, 45/59 (76.3%) strains were resistant to amoxycicillin-clavulanic acid and sulfamethoxazole-trimethoprim, (71.2%) to doxycycline, (28.9%) to gentamicin and (81.3%) to oxytetracycline.

Currently, NDV, AIV and Pigeon paramyxovirus serotype-3 were enzootic in Egypt resulting in huge economic losses due to high morbidity and mortality rates, culling of infected birds, meat and eggs loss, cost of veterinary medications. Pigeons are a major threat for NDV transmission to domestic chickens because of their migratory nature, difficulties in vaccinations, being existed in live bird markets, and backyard houses. In addition, pigeons can simply keep in contact with uncaged birds (Elgendy et al., 2016; Mansour et al., 2014; Kumar et al., 2010). We detected the viral infection by isolation and RT-PCR by amplify the viral RNA using specific oligonucleotide primers to detect AIV-H5 and H7, fusion gene (F gene) of NDV, and Avian Paramyxovirus serotype-3.

In our study, only eight samples were positive to NDV with percentage 16% in young pigeon (below 1 months) than in older birds.

While, all samples are negative to AIV H7, H5 and Avian Paramyxovirus serotype-3. These results were in line with the findings of (Munmun et al., 2016; Mansour et al., 2017; Islam et al., 2020). Terregino and Capua, (2009) and Dodovski et al., (2017) reported that young pigeons are more susceptible to NDV infection with mortality and morbidity rate accompanied with neurological signs. Also, three samples are co-infected with NDV, *Salmonella* spp., *E. coli*, *S. aureus* and *Pseudomonas aeruginosa* with percentage 7%. This finding is nearly similar to Paul et al. (2015) who reported salmonellosis and colibacillosis infection with percentage (20.32% and 4.98 %).

## 5. Conclusion

In conclusion, we reported several clinical neurological symptoms in the current study of circling, torticollis, head and neck tremors, leg and wing paralysis in winter and autumn in pigeon's flocks during 2020–2021 mainly of bacterial natural infections (*E. coli*, *Salmonella* spp., *S. aureus* and *Pseudomonas aeruginosa*), in addition to NDV. With recommendation of increase the study of pigeon's cases with nervous signs regarding to identify the most common diseases affect those cases.

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## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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