

RESEARCH ARTICLE

Virulence -Determinants and Antibiotic Resistance Pattern of Salmonella Species Isolated from Fancy Pigeons in Port-Said Governorate, Egypt

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Abstract

This study was carried out to investigate the prevalence of salmonellosis among fancy pigeons in Port- Said Governorate, Egypt. Two hundred (150 pet stores and 50 lofts) samples were collected from pigeons suffered from general signs of illness, joint lesions and diarrhea. Bacteriological and serological analysis revealed 12 (6%) isolate of *Salmonella* species distributed among eight serotypes, in which *S. Virchow* was more frequently isolated (25%), followed by *S. Typhimurium* and *S. Paratyphi* (16.6% each). Meanwhile, *S. Akay*, *S. Salamae*, *S. Anderlecht*, *S. Magherafelt* and *S. Montevideo* were 8.3%, each. All *Salmonella* isolates showed 100% sensitivity towards norfloxacin followed by ciprofloxacin (83.3%). On the other hand, ten *Salmonella* serotypes showed high resistance to erythromycin and rifampicin by 91.7 %. However, 42.1% of the recovered isolates exhibited Multi drug resistant (MDR) and 16.6% exhibited Extensive drug resistant (XDR) to different antibiotics. Molecular detection of 5 virulence genes (*invA*, *stn*, *sopE1*, *pefA* and *fimH*) among four chosen *Salmonella* serotypes (*S. Paratyphi*, *S. Typhimurium*, *S. Virchow*, *S. Montevideo*) using conventional PCR revealed the presence of *invA*, *stn* and *fimH* genes in all examined *Salmonella* serotypes. Meanwhile, *sopE1* and *pefA* genes were detected only between *S. Virchow* and *S. Typhimurium*, respectively.

Keywords: Pigeons; *Salmonella*; serotypes; virulence genes; MAR index

Introduction

Fancy pigeons are domesticated varieties of the rock pigeon (*Columba Livia*). There are exceeding than three hundred of fancy pigeon breeds that are considered a source of currency due to its high value, which can play a role in national trading and national income. In Egypt, during the past few years, the import and export trading, resulted in the availability of all international fancy pigeon breeds for example, Archangel, Blondinette, Chinese owl, Egyptian Swift, Fantail and messenger pigeon. The breeders of these fancy varieties exhibit their birds at pigeon displays, fairs and other

livestock exhibits, and its export represents several thousands of dollars per year [1].

Salmonella infection is considered a serious medical and veterinary problem worldwide, which causes food safety and zoonotic importance hazards, as well as responsible for considerable losses among cage birds and pigeons [2]. Salmonellosis is considered the major bacterial disease in pigeons; it causes mortality in young chick and occasional deaths in adult pigeons, which is important reason of economic losses in poultry industry. The common clinical signs were differing from acute to chronic as

weight loss, diarrhea, polyuria, lameness and inability to fly. Treatment of infected flocks is difficult since even long-term antibiotic therapy may leave subclinical carriers that keep the salmonella infection in the loft going [3]. The most common Salmonella serotype internationally is *Salmonella* Typhimurium (*S. Typhimurium*) var Copenhagen, which causes enteritis and joint infections [4]. Although *S. Typhimurium* causes septicemia in squabs, of greater clinical significance is the presence of arthritis in pigeons chronically infected with *S. Typhimurium* O:1,4,12:H:I:1,2. Infection with other types of *S. Typhimurium* serovars are also likely to be subclinical and pigeon might be carriers of a wide variety of Salmonella serotypes [5].

Antibiotics are considered the best choice to overcome any microbial infections in poultry farms but lost their effectiveness through progress of multidrug resistance. Many studies discuss the use of different antibiotics, amoxicillin/clavulanic acid, enrofloxacin, flumequine, norfloxacin, doxycycline, nalidixic acid, erythromycin, rifampicin, chloramphenicol, ceftriaxone, gentamicin, ciprofloxacin, ampicillin and oxytetracycline were the most used antibiotic in treatment pigeon's diseases [6- 9]. Cautious regarding the development of bacterial resistant against different antibiotic necessitated frequent updated studies on antibiotic sensitivity assay [10, 11]. Multidrug resistant (MDR) was recognized as resistance to at least one agent in three or more antimicrobial families. Extensively drug resistant (XDR) was recognized as resistance to at least one agent in all but two or fewer antimicrobial families (i.e., bacterial isolates remain susceptible to only one or two antimicrobial families [12].

Recently, rapid detection of avian pathogens by PCR allows reliable and faster results through detection of its important virulence genes. Virulence factors are encoded by several genes located on the bacterium own chromosome (housekeeping genes), which give specific and basic characteristics to bacteria from the same family. These genes are clustered

within *Salmonella* pathogenicity islands (SPIs)-1 and participate in the adhesion and invasion of the pathogen to the host as *inv* gene / or in mobile genetic elements such as transposons, plasmids, and bacteriophages. The presence of plasmid encoded fimbriae (*pefA*) potentiates the ability of salmonella to adhere to the epithelial cell and the invasion ability of salmonella isolates. The enterotoxin (*stn*) gene was demonstrated as a suitable PCR target for detection of salmonella strains [13, 14]. Many important virulence genes as *invA*, *stn*, *sopE1*, *pefA* and *fimH* accompanied with pathogenic salmonella serotypes were identified by PCR that allows accurate diagnostic tool of many pathogenic causes of diseases and can employ in molecular mapping, virulence genotyping and detection of antimicrobial resistant gene [15, 16].

the current study intended to investigate salmonella species infected fancy pigeons and identify its resistance pattern to different antibiotics and study its pathogenicity through detection of *invA*, *stn*, *sopE1*, *pefA* and *fimH* virulence genes among four chosen Salmonella serotypes (*S. Paratyphi*, *S. Typhimurium*, *S. Virchow*, *S. Montevideo*) using advanced molecular technology.

Material and Methods

Examined pigeons

Two hundred diseased pigeons suffered from general signs of illness and diarrhea were obtained from different private pigeon houses (150 pet stores and 50 lofts) located in Port-said Governorate, Egypt between December 2016 and November 2017. These birds were clinically examined and then, cloacal swabs were collected aseptically for bacteriological examination.

Bacteriological examination

Each cloacal swab was inoculated into 10 mL buffer peptone water (Oxoid) and incubated at 37°C for 18 hrs. Then, sub-cultured into tubes containing 10 mL Rappaport-Vassiliadis soy broth (RVS) (Oxoid) and incubated at 41.5°C for 24 hrs. A loopful from inoculated RVS broth was streaked onto MacConkey agar (Oxoid);

Xylose lysine-deoxycholate (XLD) agar (Oxoid) and incubated at 37°C for 24 h under aerobic condition. A pure culture of suspected salmonella colonies was then obtained through three successive subcultures. Biochemical and morphological characterizations were applied to identify salmonella isolates according to procedures described by Macfadian [17] and Brenner [18]. Sub-culture of the pure isolates onto semi-solid nutrient agar slant for preservation and further identification were carried out according to Wilson and Miles [19].

Serological identification of Salmonella isolates

Salmonella suspected isolates were subjected to serological identification according to Kauffmann [20] and was carried out in Reference Laboratory for Veterinary Quality Control on poultry production, Dokki, Giza, using diagnostic salmonella antisera polyvalent somatic (O) and monovalent (O) factors antisera, flagellar (H) antisera through slide agglutination test. The Kauffmann–White serotyping scheme used to identify serotypes.

Antimicrobial sensitivity Test

All Salmonella serotypes were subjected to sensitivity test using available commercial antibiotic discs. In brief, the overnight bacterial isolate was inoculated onto a Muller–Hinton agar plates (MHA), (Oxoid) and subsequently left for 10-15 min to dry. Then, commercial antibiotic discs (Oxoid Ltd., U.K.) were placed on MHA plates and incubated under aerobic condition at 37°C for 24 h [21]. Finally, the interpretation of inhibition zone was estimated according to the limits given by Bauer [22].

Molecular characterization of salmonella virulence determinants

Eight isolates from four Salmonella serotypes [S. Paratyphi (n=2), S. Typhimurium (n=2), S. Virchow (n=3) and S. Montevideo (n=1)] Four chosen isolates out of 12 salmonella serotypes [S. Paratyphi, S. Typhimurium, S. Virchow and S. Montevideo] were subjected to conventional PCR for detection of 5 virulence genes of *Salmonella*. Five pairs of primers have specific sequences targeting *invA*, *stn*, *sopE1*, *pefA* and *fimH* genes of salmonella were supplied from Metabion (Germany) as exhibited in Table 1 and used.

Table 1: Oligonucleotide primers sequences for detection of Salmonella virulence genes

Genes	Sequence	Cycling conditions	Amplified product (bp)	Reference
<i>invA</i>	F: GTGAAATTATCGCCACGTTTCGGGCAA R: TCATCGCACCGTCAAAGGAACC	Denaturation: 94°C/30 sec., Annealing:55°C/30 sec; Extension:72°C/30 sec., final extension: 72 °C/7min.	284	[24]
<i>stn</i>	F: TTG TGT CGC TAT CAC TGG CAA CC R: ATT CGT AAC CCG CTC TCG TCC	Denaturation:94°C/30sec., Annealing:59°C/40sec., Extension:72°C/45sec., final extension: 72 °C/10min.	617	[25]
<i>pefA</i>	F: TGT TTC CGG GCT TGT GCT R:CAG GGC ATT TGC TGA TTCTTC C	Denaturation: 94°C/30sec., Annealing:55°C/40sec., Extension:72°C/45sec., final extension: 72 °C/10min.	700	
<i>sopE1</i>	F: ACT CCT TGCACA ACC AAA R: TGC GGA TGT CTTCTG CAT TTC GCC ACC	Denaturation: 94°C/30sec., Annealing:58°C/40sec. Extension:72°C/45sec., final extension: 72 °C/10min.	422	[26]
<i>fimH</i>	F: GTGCCAATTCCTCTTACCGTT R: TGGAATAATCGTACCGTTGCG	Denaturation:94°C/30sec., Annealing:64°C/5min Extension:72°C/30sec., final extension: 72 °C/7min.	164	[27]

fimH: Type 1 fibrin D- Mannose specific adhesion; *stn*: Enterotoxin; *invA*: Invasion protein; *sopE1*: Guanine nucleotide exchange factor; *pefA*: plasmid encoded fimbriae. All genes involved initial denaturation at 94°C for 5 min. followed by 35cycles

DNA extraction was achieved by following QIAamp DNA mini kit manufacturer's instructions using specific cycling conditions as shown in (Table 1). The volume of PCR reaction was 25 μ L, in which, 12.5 μ L Taq master mix (Emerald Amp, Takara, Japan Code No. RR310A kit), 1 μ L each of forward and reverse primers, 4.5 μ L sterile distilled water and 6 μ L of template DNA were used. Positive and negative controls were provided by Animal health research institute, Dokki, Giza, Egypt. Using Gel casting apparatus (Biometra), PCR products were electrophoresed using 1.5 percent agarose gel. A gel documentation system was used to photograph the gel and the data were then analyzed through computer software according to Sambrook [23].

Statistical analysis

The Chi-square test and P value were performed to analyze the obtained results (SPSS software, version 22) (significance level; $P < 0.05$).

Results

Clinical findings

Clinically examined pigeons showed general signs of illness including excessive thirst, anorexia, weight loss and diarrhea with basted vent. The predominant sign in some fancy pigeons was a swollen joint or lameness with loss of ability to fly.

Bacterial isolation and identification

Bacteriological analysis revealed isolation of salmonella species by 6% (12/200) in which (5.3%) was from pet stores and (8%) was from pigeon lofts. Biochemical identification revealed red slant, yellow butt with H₂S and gas production on TSI agar, purple colored on Lysine Iron agar test, slant and butt with H₂S at the middle of tube, urease test negative tube still yellow, Simmon's citrate positive media become blue and fermentation tests negative in Sucrose and Dulcitol.

Serological confirmation of salmonella serotypes

Salmonella isolates was distributed in eight serotypes, in which *S. Virchow* was more frequently isolated (25%) followed by *S. Typhimurium* and *S. Paratyphi* (16.6% each). Meanwhile, *S. Akay*, *S. Salamae*, *S. Anderlecht*, *S. Magherafelt* and *S. Montevideo* was 8.3% each. Statistically, there is a significant difference in the prevalence of different salmonella serotypes ($p < 0.001$).

Antimicrobial susceptibility and resistance pattern

Antimicrobial sensitivity and resistance pattern of all salmonella isolates (n=12) are shown in Tables (2,3), in which, all salmonella isolates showed 100 % sensitivity towards norfloxacin followed by ciprofloxacin (83.3%), doxycycline and gentamicin by (66.7%) and then to nalidixic acid and oxytetracycline (58.3%). In contrast, ten salmonella serotypes showed high resistance to erythromycin and rifampicin by 91.7%. The recovered isolates exhibited MDR by 41.7% to 4 antibiotics family and 16.6% of salmonella serovars exhibited XDR to 7 antibiotics family.

Molecular determination of virulence profile of salmonella serotypes

PCR amplification results detected genes *invA* at 284 bp, *stn* at 617 bp and *fimH* at 164 bp among all examined salmonella serotypes (*S. Paratyphi*, *S. Typhimurium*, *S. Virchow* and *S. Montevideo*). Meanwhile, *SopE1* gene was detected at 422 bp only in 25% of *S. Virchow* and *pefA* gene was detected at 700 bp in 25% of *S. Typhimurium* (Figure 1 and Table 4).

Table 2: Susceptibility of 12 Salmonella isolates from fancy pigeon in Port-Said, Governorate, Egypt to 12 tested antibiotics

Antibiotic group	Antibiotics	Disk concentration	Susceptibility of all 12 Salmonella isolates to 12 tested antibiotics								
			Resistant			Intermediate			Sensitive		
			Salmonella spp (No.)	No. of isolates	%	Salmonella spp	No. of isolates	%	Salmonella spp. (No.)	Total No. of isolates	%
Fluroquinolon	NA	30 µg	S. Magherafelt S. Virchow	1 2	25	S. Typhimurium S. Salamae	1 1	16.7	S. Anderlecht S. Montevideo S. Paratyphi S. Typhimurium S. Akay S. Virchow	1 1 2 1 1 1	58.3
	NOR	10µg	-	-	-	-	-	-	All tested isolates	12	100
	CIP	5 µg	-	-	-	S. Montevideo S. Anderlecht	1 1	16.7	All except S. Montevideo S. Anderlecht	10 1 1	83.3
Tetracycline	DO	30 µg	S. Magherafelt S. Virchow S. Paratyphi	1 2 1	33.3	-	-	-	All except S. Magherafelt S. Virchow S. Paratyphi	8 1 2 1	66.7
	OT	30 µg	S. Magherafelt S. Virchow S. Paratyphi	1 3 1	41.7	-	-	-	All except S. Magherafelt S. Virchow S. Paratyphi	7 1 3 1	58.3
Macrolide	E	15 µg	All except S. Typhimurium S. Akay	10 1 1	83.4	S. Akay	1	8.3	S. Typhimurium	1	8.3
Penicillin	AMP	10µg	All except S. Paratyphi S. Montevideo S. Anderlecht S. Virchow S. Salamae	7 1 1 1 1 1	58.3	S. Salamae	1	-	S. Paratyphi S. Montevideo S. Anderlecht S. Virchow	1 1 1 1	33.4
	AMC	30 µg	-	-	-	S. Typhimurium S. Paratyphi S. Virchow	2 1 3	50	S. Salamae S. Akay S. Paratyphi S. Montevideo S. Anderlecht Magherafelt	1 1 1 1 1 1	50
Aminoglycoside	CN	10µg	S. Typhimurium S. Magherafelt S. Virchow	1 1 2	33.3	-	-	-	All except S. Typhimurium S. Magherafelt S. Virchow	8 1 1 2	66.7
Cephalosporin	CRO	30 µg	S. Typhimurium S. Paratyphi S. Anderlecht S. Virchow	1 1 1 2	41.7	S. Typhimurium S. Salamae S. Paratyphi S. Montevideo S. Magherafelt	1 1 1 1 1	41.7	S. Akay S. Virchow	1 1	16.6
Phenicols	C	30 µg	All except S. Anderlecht S. Typhimurium S. Virchow	7 1 1 3	58.4	-	-	-	S. Anderlecht S. Typhimurium S. Virchow	1 1 3	41.6
Ansamycin	RD	5µg	All except S. Virchow	11 1	91.7	-	-	-	S. Virchow	1	8.3
Chi square				31.857			33.118			22.465	
P value				0.000804			0.0005036			0.02101	

NA: nalidixic acid; DO: doxycycline; OT: oxytetracycline; E: erythromycin; NOR: norfloxacin; CIP: ciprofloxacin; CN: gentamycin; AMP: ampicillin; CRO: ceftriaxone; C: chloramphenicol; RD: rifampicin; AMC: amoxicillin and clavulanic acid.

Table 3: Distribution of antimicrobial resistance phenotypes and virulence genes among 12 Salmonella isolates from fancy pigeons in Port-Said, Governorate, Egypt

Serotypes	No. of recovered serotypes	Virulence determinant combinations	Phenotypic resistance	MAR index = (a/b)
S. Typhimurium	1	<i>invA, stn, fimH</i> and <i>pefA</i>	RD, E, AMP, CN and CRO	0.41
S. Paratyphi	1	<i>invA, stn</i> and <i>fimH</i>	E, DO, OT, AMP, RD and C	0.5
S. Virchow	1	<i>invA, stn, fimH</i> and <i>sopE1</i>	NA, OT, AMP, CN, CRO, C and RD	0.58
S. Montevideo	1	<i>invA, stn</i> and <i>fimH</i>	NA, DO, OT, NOR, AMP, CN, C and AMC	0.66
S. Virchow	1	NT	NA, DO, OT, AMP, E, RD and CN	0.58
S. Virchow	1	NT	DO, OT, E and CRO	0.33
S. Typhimurium	1	NT	AMP, RD and C	0.25
S. Paratyphi	1	NT	E, CRO, RD and C	0.33
S. Akay	1	NT	AMP, RD and C	0.25
S. Anderlecht	1	NT	E, RD and CRO	0.25
S. Magherafelt	1	NT	NA, DO, OT, E, AMP, RD and CN	0.58
S. Salamae	1	NT	E, RD and C	0.25

NA: nalidixic acid; DO: doxycycline; OT: oxytetracycline; E: erythromycin; NOR: norfloxacin; CIP: ciprofloxacin; CN: gentamycin; AMP: ampicillin; CRO: ceftriaxone; C: chloramphenicol; RD: rifampicin; AMC: amoxicillin and clavulanic acid

fimH: Type 1 fibrin D- Mannose specific adhesion; *stn*: Enterotoxin; *invA*: Invasion protein; *sopE1*: Guanine nucleotide exchange factor; *pefA*: plasmid encoded fimbriae; NT: not tested.

MAR: multiple antibiotic resistance = (a) The number of antibiotics to which the isolates are resistant/ (b) The total number of tested antibiotics (n=12)

Table 4: Seroprevalence of Salmonella species isolates from fancy pigeons in Port-Said Governorate, Egypt

Pigeon locality	Pigeon examined No.	Salmonella isolation		Serologically Identified Salmonella serotypes		
		No. of Positive isolates	Percent of positive isolates (No. of positive / No. of examined)	Species	No.	% = Spp. No. /Total isolates
Pet stores	150	8	5.3 % (8/150) ^a	S. Typhimurium	2	16.6%
				S. Paratyphi	1	16.6%
				S. Virchow	2	25%
				S. Montevideo	1	8.3%
				S. Anderlecht	1	8.3%
				S. Salamae	1	8.3%
Lofts	50	4	8 % (4/50) ^b	S. Paratyphi	1	16.6%
				S. Virchow	1	25%
				S. Akay	1	8.3%
				S. Magherafelt	1	8.3%
Total	200	12	6%	12	12	6%

^a The percentage of Salmonella spp isolation with respect to the total positive samples was 8/12 (66.6%) in pet stores.

^b The percentage of Salmonella spp isolation with respect to the total positive samples was 4/12 (33.3%) in lofts.

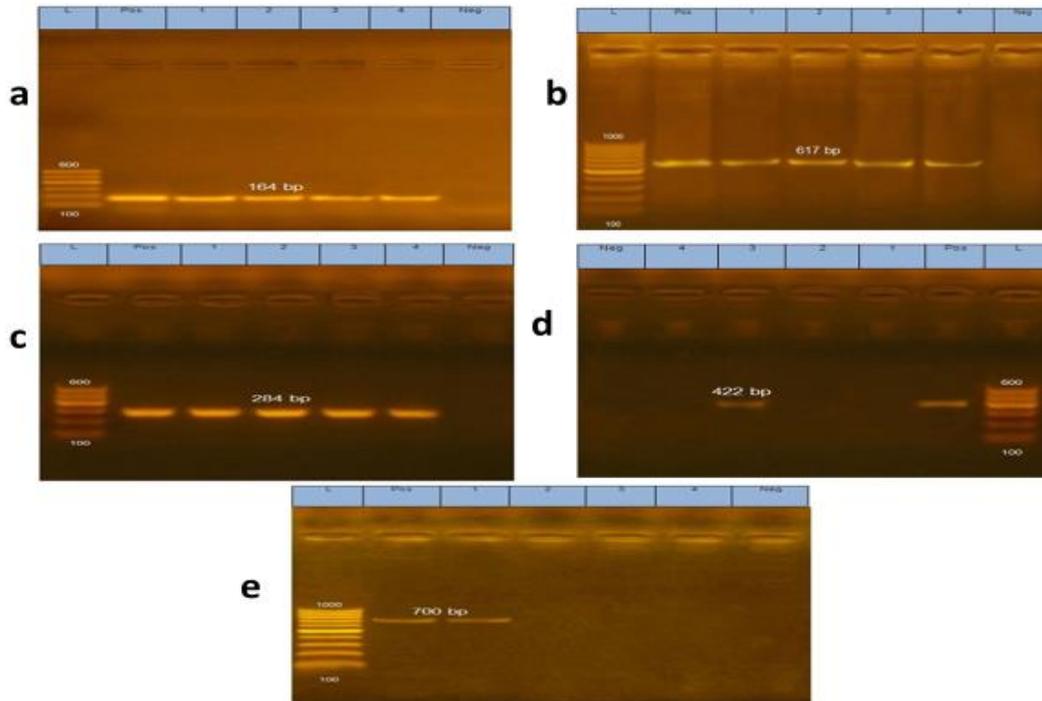


Figure 1: Agarose gel electrophoresis showing amplification of (a) *fimH* gene at 164 bp, (b) *stn* gene at 617 bp, (c) *invA* gene at 284 bp, (d) *sopE1* gene at 422 bp and (e) *pefA* gene at 700 bp among four chosen *salmonella* serotypes (*S. Typhimurium*, *S. Paratyphi*, *S. Virchow* and *S. Montevideo*) isolated from examined pigeons. Lane L: ladder (100 bp), Lane pos: Positive control, Lane 1: *S. Typhimurium*, Lane 2: *S. Paratyphi*, Lane 3: *S. Virchow*, Lane 4: *S. Montevideo* and Lane Neg: Negative control.

Discussion

Salmonellosis is one of the most frequent bacterial diseases in racing and fancy pigeons. The main problem with salmonellosis in pigeon lofts that the fanciers have carriers of paratyphoid in their lofts without knowing or suspecting it until clear symptoms appeared, so, they do essential treatment and vaccination. In this study, particular attention was paid to the clinical analysis, isolation and identification of the most significant salmonellae involved in pigeon problems, as well as its sensitivity pattern to specific antibiotics. In addition, the obtained isolates were mapped for the existence of certain genes associated with bacterial virulence (*invA*, *stn*, *sopE1*, *pefA* and *fimH*) using PCR technology.

Clinical investigation of 200 pigeon samples showed general signs of illness including excessive thirst, anorexia, weight loss and diarrhea, in addition, signs of lameness and swollen joints with loss of ability to fly were commonly observed in some cases as those recorded in paratyphoid

infection in racing pigeons and wild birds by many authors [5,28,29]. No

The results revealed recovery of twelve salmonella isolates (6%) from fancy pigeon cloacal swabs which was belonged to eight serotypes in which the highest rate of isolation was from pet stores than that from pigeon lofts (66.7 % & 33.3% respectively) which may referred to microbial contamination between mixed breeds of multispecies in pet stores than that in pigeon lofts. Our results run parallel with those recorded in; Dakahlia Governorate (6.7%, 2/30) by Abd El-Tawab et al. [30], in Giza governorate (5%, 10/200) by Ahmed [28]. Moreover, Adesiyun et al. [31], Dovč et al. [32] and Methner and Lauterbach [33] isolated salmonella from pigeon cloacal swabs with percentages of 5, 5.7 and 7.04 respectively and less than that obtained by Abdeen *et al.* [29] and Karim *et al.* [34] who isolated salmonella from pigeons as 20% in Menoufiya Governorate. Also, Nabil and Younis [35] detected salmonella spp in 17% of pigeons collected from different governorates, where the isolation rate was (17.24%, 5/29) in

Dakahlia, (17.39%, 4/23) in Damietta, (15%, 3/20) in Gharbia, (15.38%, 2/13) in Kafr ElSheik and (20%, 3/15) in Sharika Governorates But, lower rate of salmonella isolation was recorded as 2% only in the examined free-living pigeons in Makkah region, Saudi Arabia by Abulreesh [7] and as 1.5% in north England in the examined pigeon samples by Hughes *et al.* [36]. This variation of isolation rates may be attributed to the geographical differences, environmental stressor, system of management, concurrent infections, and others stress factors [37].

Serogrouping analysis clarified that the obtained isolates were belonged to eight serovars, in which *S. Virchow* was the highest frequent one (25%), followed by *S. Typhimurium* and *S. Paratyphi* (16.6% each). Meanwhile, *S. Akay*, *S. Salamae*, *S. Anderlecht*, *S. Magherafelt* and *S. Montevideo* were 8.3% each. The obtained results agreed with that previously reported by Gong *et al.* [38] who isolated *S. Typhimurium* with 15.5% (50/ 323). Meanwhile, it was recorded by different authors as 7.3% (3/41) [39]; 28.13% [40] and 22.8% [41]. In contrast, it was detected with lower rate (1.38%) in examined pigeons by Hughes *et al.* [36] and 1.4% by Gargiulo *et al.* [42]. Gong *et al.* [38] documented that, *S. Typhimurium* was the most detected serovar among pigeons (10/17 isolates) by 58.8%, in contrast, *S. Virchow* was the predominant (25%, 3/12) isolated serotypes in the current work. As well as *S. Salamae* was detected with 17.19% in pigeons by Krawiec *et al.* [40] and was detected with high rate (66.7% (4/6) by Abdeen, *et al.* [29]. *S. Montevideo* isolation rate agreed with that previously reported (25%, 1/4) by Abd El-Tawab *et al.* [30], while Hughes *et al.* [36] found all salmonella isolates were negative to *S. Montevideo*. This variation may be correlated to species or region differences. According to our knowledge, no available documented reports about detection or isolation record of *S. Salamae*, *S. Magherafelt*, *S. Akay*, *S. Anderlecht* and *S. Paratyphi* in free living, fancy and/or domestic pigeons in Egypt and this may be the first detection record in pigeons in Egypt. At the same time, several

previous reports by Elgohary *et al.* [43] and Abd El-Tawab *et al.* [30] declared the presence of these serotypes in commercial poultry farms in Egypt which may impute to that salmonella serotypes may be disseminated through chicken farms to pigeons by direct or indirect contact with contaminated food, water, feather, dust, and feces through live bird market or contact human. The variations in serotype prevalence and its association with the developing disease condition depends on the health state of birds, climatic conditions, variation in water supply and food along with geographical situation and management strategies [43- 44].

A big serious problem and a major challenge for poultry industry is the antimicrobial resistance among different pathogens which also threat human health and its resistance to diseases. Our results showed higher sensitivity of Salmonella serovars to Norfloxacin and Ciprofloxacin by 100% and 83.3% respectively, which was documented by many authors as Jahantigh and Nili [6] who detected its sensitivity to Norfloxacin and Ciprofloxacin as 100% for both. Dutta *et al.* [45] detected 91.67% and 100% sensitivity to Norfloxacin and Ciprofloxacin respectively, also, Firouzi, *et al.* [8] recorded the effectiveness of Norfloxacin by 82.8%. In contrast, our results disagreed with Sharma and Das [46], who found a moderate sensitivity of their salmonella isolates to norfloxacin by 57.5% and also, a resistance was detected to norfloxacin by AS and Shalaby [47]. At the same time, Dutta *et al.* [45] detected all salmonella isolates exhibited sensitivity to ciprofloxacin and high resistance to ampicillin and amoxicillin by 71.43% and 61.90%, respectively. Our results cleared that both ciprofloxacin and norfloxacin was suitable choices to control salmonellosis as both antimicrobials are the primary agents used against invasive salmonella in humans and livestock's [48]. Salmonellae isolates showed moderate susceptibility to doxycycline, gentamicin, and oxytetracycline (66.7%, 66.7 % and 58.3% respectively). Saifullah *et al.* [49] and Rahman *et al.* [50] recorded susceptibility of Salmonella isolates

to gentamicin with percentages of 76.47% and 78.57%, respectively. At the same time, Elgohary *et al.* [43] recorded a resistant of salmonella isolates to doxycycline. The current Salmonella isolates classified as multidrug resistant (MDR) with highest resistance to rifampicin and erythromycin (91.7% and 83.4%) followed by chloramphenicol and ampicillin (58.4% and 58.3%); which agreed with that previously reported by Abulreesh [7] and Hosain, *et al.* [51] and disagreed with Karim *et al.* [34] who reported resistant of salmonella isolates to erythromycin (19.05%). In contrast, results by Yousef and Mamdouh [52] and AS and Shalaby [47] revealed high sensitivity of salmonella isolates from pigeons to ampicillin, which disagreed with our antibiogram assay to ampicillin. Antibiotic resistance genes may consider a virulence factor for MDR bacteria as the antimicrobial resistance mechanisms consider pandemic threat and create an enormous clinical and financial burden on health care systems worldwide [53]. These variations may be associated with the empirical misuse of antibiotic by owners. Besides that, detecting the resistance of Salmonella strains towards various antibiotics helps the veterinarians to give a more effective treatment to diseased livestock and poultry with Salmonellosis.

Many studies have identified genes encodes chromosomal and plasmid factors that associated with the virulence of salmonella spp. The ability of pathogen to adhere to host's epithelial cells is considered a prerequisite for successful infection. In addition, fimbriae, the proteinaceous hair-like appendages on the outer membrane of bacteria have been implicated in such adherence. Also, *Salmonella* established various strategies to adhere to host tissues through expressing an enormous number of both fimbrial and non-fimbrial adhesins, which sometimes directly linked with the outcome of bacterial infection [54].

The PCR results confirmed the presence of *invA*, *stn* and *fimH* genes in all selected and examined four salmonella serotypes (*S. Typhimurium*, *S. Paratyphi*, *S. Virchow* and *S. Montevideo*), which might reflect and confirm

its important roles in the bacterial invasion and survival in the host and producing clinical disease symptoms. Involvement and detection of *invA* gene in all four salmonella serotypes confirm its pathogenic role, as it has been proved to be essential for entry of the bacteria into epithelial cells; a suitable target for PCR with a potential diagnostic application and recognized as an international standard for detection of salmonella genus [55- 57]. In addition, Everest, *et al.* [58] worked on *S. Typhimurium* strains harboring independent defined mutations of *invA* gene found that these strains were unable to induce fluid accumulation, tissue damage, and local inflammation in ileal loops, so, the presence of *invA* gene in all examined salmonella isolates support its virulence, invasiveness and ability of the pathogen to induce infection. This data agreed with previous report regarding *invA* gene in salmonella isolates from pigeons, as it detected in 100% of the examined isolates [47, 59- 60]. Detection of *fimH* and *stn* gene by 100% in all examined Salmonella isolates from pigeon agreed with that detected by As and Shalaby [47] and Choudhury *et al.* [61], respectively.

Nevertheless, *sopE1* gene was detected in *S. Virchow* only, as well as *pefA* gene was detected in *S. Typhimurium* only which may indicate it is not essential for salmonella invasion, but it may potentiate its ability. These data totally agreed with previous reports by As and Shalaby [47] and Mezal *et al.* [60] who documented presence of *pefA* gene in all *S. Typhimurium* isolated from pigeon. Also, agree for some extent with Ahmed *et al.* [28] and Choudhury *et al.* [61] who found *sopE1* and *pefA* genes with 41.18% and 32.90%, respectively in salmonella isolates. Also, agreed with that previously reported by Dione *et al.* [62] who detected *pefA* in 40% of the examined Salmonella isolates and they showed correlation between the presence of *pefA* and Salmonella virulence and resistant to commonly used antimicrobial agents which was similar to our findings in which *S. Typhimurium* isolate possess *pefA* gene and resist to rifampicin, erythromycin, ampicillin, gentamycin, chloramphenicol and ceftriaxone

used. Significant relations between the expression of certain virulence genes and resistance to widely used antimicrobials was identified in Senegal and Gambia [62]. In the present investigation, *Salmonella* Typhimurium, Paratyphi, Virchow and Montevideo isolates have a central pattern of resistance to many antimicrobials as considered as XDR and MDR. That may explain the development and spread of resistance in pathogenic bacteria with the likelihood of resistant zoonotic bacteria reaching the intestinal tract of humans is a possible consequence of antimicrobial misuse [51, 59].

Conclusion:

In conclusion, this study provides an evidence of the recovery of different *Salmonella* serotypes from fancy pigeon in Port-Said, Egypt with detection of certain virulence genes and resistance to widely used antimicrobials as considered as XDR and MDR. To our Knowledge, this may be the first detection record of *S. Salamae*, *S. Magherafelt*, *S. Akay*, *S. Anderlecht* and *S. Paratyphi* in pigeon in Egypt.

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الملخص العربي

محددات الضراوه ونمط مقاومة المضادات الحيويه لأنواع السالمونيلا المعزوله من حمام الزينه بمحافظة بورسعيد. مصر

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تم اجراء هذه الدراسة لبحث مدى انتشار السالمونيلا بين حمام الزينه الفاخر في محافظة بورسعيد بمصر. تم تجميع عدد 200 عينة من مسحات المجمع (150 عينة من الاسواق و 50 عينة من قطعان ابراج الحمام) من الحمام الذي يعاني من علامات عامة للمرض واصابات بالمفاصل وإسهال. أظهر التحليل البكتيري والسيرولوجي عزل السالمونيلا من 6% من العينات موزعة على ثمانية أنماط مصلية، و كانت السالمونيلا فيرشو أكثر الانماط عزلا (25%)، يليه سالمونيلا تيفيموريم و سالمونيلا باراتفيني بنسبة (16.6%) لكل منهما. في حين بلغت نسبة كل من سالمونيلا اكاوي وسالمونيلا سالامى و سالمونيلا اندركليت و سالمونيلا ميجرفيلت و سالمونيلا مونتيبيديو 8.3% لكل منهم. أظهرت جميع عزلات السالمونيلا حساسيتها تجاه النورفلوكساسين بنسبة 100% يليها عقار السيروفلوكساسين (83.3%). ومن ناحية أخرى، أظهرت العشرة أنماط المصلية للسالمونيلا مقاومة عالية للإريثروميسين والريفاميسين بنسبة 91.7%. وفي الوقت نفسه، أظهرت العزلات المعزولة مقاومة متعددة للمضادات الحيويه (MDR) بنسبة 42.1% و 16.6% مقاومة متزايدة للمضادات الحيويه المختلفة (XDR). كشفت دراسة التحليل الجزيئي باستخدام تفاعل إنزيم البلمرة المتسلسل لخمس جينات ضراوة (*invA* و *stn* و *sopE1* و *pefA* و *fimH*) لأربعة أنماط مصلية مختارة من السالمونيلا هما سالمونيلا فيرشو و سالمونيلا تيفيموريم و سالمونيلا باراتفيني و سالمونيلا مونتيبيديو عن وجود جينات *invA*, *stn* و *fimH* في جميع أنماط السالمونيلا المصلية التي تم فحصها بينما تم الكشف عن جينات *pefA* و *sopE1* فقط في سالمونيلا فيرشو و سالمونيلا تيفيموريم على التوالي.