Occurrence And Molecular Identification Of Some Zoonotic Bacterial Pathogens In Quails And Their Eggs

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ABSTRACT

A quail farm with a problem of depression and severe diarrhea was examined for the prevalence of *Campylobacter* spp. and some other important bacterial pathogens with reference to their hazards and zoonotic importance. Eighty cloacal swabs as well as 67 (liver, gall bladder and cecum) samples, 116 edible eggs and 20 workers' hand swabs were collected from this farm. *Campylobacter* spp. were isolated and identified in 156 (62.4%) quail samples for which *C. jejuni* and *C. coli* represented 96.2% and 3.8% of the total *Campylobacter* spp., respectively. *C. jejuni* was recovered from cloacal swabs, egg shell, egg content, ceca, bile content, liver and hand swabs (52.5%, 44.8%, 37.9%, 24%, 16.7%, 12.5% and 45%, respectively. But *C. coli* was isolated only from bile (22.2%) and liver (8.3%).

Campylobacter isolates were identified by using 23S rRNA-PCR. The amplicon (650 bp) for the *Campylobacter* 23S rRNA primers was present in all tested *Campylobacter jejuni* isolates. The present PCR assay offers an effective alternative to traditional biochemical typing methods for the identification of *Campylobacter* isolates from humans and poultry.

Arcobacter spp. were recovered from liver, egg shell, ceca, cloacal swabs, bile and hand swabs at the rates of 16.7%, 13.8%, 8 %, 7.5%, 5.6% and 10%, respectively.

Other important bacterial pathogens were incriminated in the problem. *E. coli* was isolated at high rates from cloacal swabs, egg shell, egg content and hand swabs (80%, 55.1%, 37.9% and 75%), respectively. *E. coli* isolates were serotyped as $O_{26:H11}$, $O_{118:H2}$, $O_{86:H34}$, $O_{119:H6}$, $O_{115:H-}$, $O_{158:H-}$ and $O_{164:HNM}$. Human *E. coli* isolates were grouped as $O_{26:H11}$, $O_{128:H-}$, $O_{158:H-}$, and $O_{164:HNM}$.

Salmonell species could not be detected in any of the examined samples. *Staphylococcus aureus* was isolated from egg shell, content and hand swabs with a percentage of 58.6, 55.2 and 90%, respectively.

Antibiotic sensitivity of all bacterial isolates showed that Ciprofloxacin, Enrofloxacin, Spectinomycin and Gentamycin were effective drugs. All the bacterial isolates showed high resistance to different drugs with 100% resistance observed against Ampicillin and Colistin. Treatment of the flock with Enrofloxacin controlled the problem.

INTRODUCTION

Poultry meat is considered one of the most important sources of animal protein. Nowadays, great attention was gone toward quail and their eggs which represent a comparatively economic and highly palatable source of animal protein. Quail eggs as a food product were examined by a number of authors to determine bacterial contamination and to assess them as a risk factor for the consumer's health *(1-5)*. On the other hand, eggs are perishable just like raw meat and fish, which makes them a suitable medium for microbial growth unless they are properly refrigerated and cooked. The main sources of egg contamination are through transovarian transmission, oviduct, contamination of shell from bird's fecal matter during laying and/or from nest material, litter, dust and egg's cartons in which eggs were packed *(6)*.

Quail and other migratory birds play a considerable role in harboring and

dissemination of many pathogens of public health significance *(7)*. *Campylobacter* spp. can colonize the mucus overlying the epithelial cells primarily in the ceca and the small intestine of quails causing severe diarrhea with excretion of a large number of the microorganism in dropping for a long time *(8)*. Once colonization was detected, fecal shedding was presumably an important factor in transmission of organism around large bird flocks *(9)*. However, the favored environment appears to be the intestines of all avian species including quails *(8,10)*. *C. jejuni* can cause necrotizing hepatitis in Japanese quail *(11)*. It was isolated at high rates from ceca, cloacal swabs and liver of quails *(2,5,12)*.

In human, *Campylobacter* bacteria have been incriminated as a significant cause of enterocolitis. The correlation between specific *C. jejuni* and *C. coli* serotypes in poultry and in humans diarrhea had been documented *(13)*. The infection was mainly associated with handling of infected poultry and consumption of under cooked poultry meat or eggs *(14)*. The number of human cases of campylobacteriosis had increased dramatically in recent years in many countries *(15)*. While *Campylobacter* is recognized as a major foodborne pathogen, only within the last decade has it become evident that *Arcobacter* may also be pathogenic to humans *(16)*. The disease caused by *Arcobacter* was clinically similar to that caused by *Campylobacter (17)*. *Arcobacter* has been isolated from poultry suggesting that poultry may be a significant reservoir *(18)*.

Poultry bacterial pathogens such as *E. coli* and *Salmonella* species were the most common bacterial infections that cause economic losses in poultry industry *(19)*. *E. coli* caused an outbreak of septicaemic colibacillosis in Japanese quails associated with high mortality *(20,21)* and it was recovered from quail egg shells as a result of recent fecal contamination. Moreover, this organism can penetrate the shell which leads to contamination of the egg content *(22,23)*. *E. coli* strains can be divided into at least 6 different categories with corresponding pathogenic scheme. The infection ranges from watery diarrhea, persistent diarrhea to hemorrhagic colitis and hemolytic uraemic syndrome *(24)*.

Concerning *Staphylococcus aureus*, it's considered as an important foodborne pathogen that produce thermostable enterotoxin of serous public health significant. In chicken, it causes arthritis with low mortality rate (0-15%) as a result of septicemia *(25)*.

Salmonella was isolated from cecal contents of quails with enteritis and diarrhea *(20)*. Several researches detected this microorganism in quail's eggs *(26)*. *Salmonella* is one of the leading causes of human gastroenteritis all over the world.

In the current study, an investigation was undertaken to find out the existing genus *Campylobacter*, *Arcobacter*, *E. coli*, *Salmonella* and *Stapylococcus aureus* as a probable cause of severe diarrhea and mortalities in quail farms emphasizing its role as a potential source for human infection through handling or consumption of it and its product. Also, determination the in vitro susceptibility of these bacterial isolates against available commercial antimicrobial agents to overcome the problem.

MATERIAL AND METHODS

Sampling

Eggs

A total of 116 quail edible eggs were collected from the Faculty of Agriculture quail farm, Suez Canal University in clean cartons during the period of May, 2008 to August 2008. This farm had a history of mortalities in baby chicks, depression and severe diarrhea.

Swabs

Eighty cloacal swabs from these birds as well as 20 hand swabs were collected from workers at the same farm using sterile cotton tipped swabs and put into sterile tubes containing 0.1% peptone water.

Organs

Tissue organs from diseased and freshly dead quail birds (24 livers, 18 gall bladder "bile content" and 25 cecum) samples were taken from the farm and submitted for bacteriological investigation.

Preparation of samples

Preparation of shell eggs for microbiologic examination of shell and content was done *(27)*.

Microbiolgical examination

Samples and swabs were placed into thioglycollate broth enrichment medium, one part was incubated at 37°C for *Arcobacter* spp. and the other one at 42°C for *Campylobacter* spp. for 24 hours. Skirrow's agar media were used for initial isolation of *Campylobacter* spp. supplemented with Skirrow supplement (SR069E) under microaerophilic conditions using *Campy.* Gaspak system (BR056A Oxoid) at 37°C and 42°C for 48 hours. Presumptive colonies were identified as *Campylobacter* spp. or *Arcobacter* by phase-contrast microscopy and Gram stain, and they were confirmed and differentiated biochemically by using catalase, oxidase, hippurate hydrolysis; aerobic growth, growth in 1% glycine and 3.5% NaCl and sensitivity to Nalidixic acid and Cephalothin *(27,28)*.

Isolation of *E. coli, Salmonella* and *Staphylococcus aureus* were performed using peptone water, brilliant green broth, Rappaport-Vassiliadis broth, Eosin methylene blue, XLD and Baird Parker's agars medium, incubated at 37°C for 24-48 hours. Characterization and biochemical identification of suspected colonies were confirmed *(29,30)*.

Serotyping of suspected *E. coli* isolates was done at The Central Lab. of The Ministry of Health, Cairo.

Molecular typing of *Campylobacter* isolates by PCR assay

The PCR was carried out at the PCR Unit, Dept. Of Infectious Diseases, Fac. of Vet. Med., Suez Canal Univesity.

DNA extraction: Bacterial cells were collected from 1 mL of overnight thioglycollate broth culture by centrifugation at 3000 rpm for 10 minutes. Bacterial pellet were washed twice with 500 µl of ice cold phosphate buffered saline (pH 7.3) and bacterial cells were collected by centrifugation between washes, then bacterial pellet was resuspended in 500 µl of phosphate buffered saline. Suspended pellet was treated by lysozymes 10mg / mL and incubated for at least one hour at 37°C. Then, 50 µl of 10% sodium dodecyl sulphate (final concentration of 1 %) and 8µl of proteinase K (250µg/ml) were added and mixed gently. The mixture was incubated at 56°C for three hours in a water bath, then mixed with an equal volume of equilibrated phenol and centrifuged at 14,000 rpm for 15 min. The aqueous fraction was subjected to one more cycle of phenol: chloroform: isoamyl-alcohol (25: 24: 1) treatment followed by one cycle of chloroform: isoamyl-alcohol (24:1) treatment. The DNA was precipitated with 1/10 th volume of 3M sodium acetate and equal volume of ice cold absolute ethyl alcohol, for proper precipitation of DNA overnight incubation at –20°C was occurred. DNA was collected by centrifugation at 14,000 rpm for 15 min. The DNA pellet was washed twice with 500µl of ice cold 70% ethanol by centrifugation at 14,000 rpm for 15 min, then air-dried and resuspended in 80µl of sterile distilled water. Quantity of DNA was calculated by spectrophotometric method *(31,32)*.

Primers

Oligonucleotides primers (Bio Basic Inc., Canada) were selected from the published DNA sequences of *Campylobacter* species using Oligo software (version 3.4). A 23-bp forward primer termed (23SF) and a reverse complementary sequence (23-bp) primer termed (23SR) were thereby derived and their sequences were 23SF, 5'TATACCGGT-AAGGAGTGCTGGAG3' and 23SR, 5'A-TCAATTAACCTTCGAGCACCG 3'. When tested at an annealing temperature of 59°C,

these primers amplified a 650-bp amplicon from *Campylobacter* spp. *(33).*

Polymerase chain reaction

Three µl of DNA samples (100 ng per reaction) were amplified in a 25 µl reaction mixture consisting of 1.5 unit *Taq* polymerase (Sibenzyme, Russia), 2.5 µl of 10 X PCR buffer, 0.5 µl of 200 µM of dNTPs mixture, 2 µl of 20 pmole of each primers and sterile distilled water up to 25 µl. Amplification was performed in thermal cycler (Techne Progene, UK). Parameters for amplification included an initial denaturation at 95°C for 5 min., followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 59°C for 30 seconds and extension at 72°C for 30 seconds, followed by a final extension at 72°C for 5 min. Amplified products were separated by electrophoresis in a 1.7 % agarose gel (Biobasic) stained by ethidium bromide (0.5 µg / ml), in 1 X TAE buffer at constant voltage of 4 V/cm , and photographed with Sony digital camera. A 100 bp DNA marker (Axygen) was used as a DNA molecular size standard *(33).*

Visualization of PCR products by agarose gel electrophoresis

To confirm the targeted PCR amplification, five µl of the PCR product from each tube was mixed with two µl of 6X gel loading buffer and electrophoresed along with 100 bp DNA Ladder (SibEnzyme Ltd., Russia.) on 1.7% agarose gel containing ethidium bromide (at the rate of 0.5µg/ml) at constant 80V for 30 min. in 1X TAE buffer. The amplified product was visualized as a single compact band of expected size under UV light and photographed by using Sony digital camera.

Antimicrobial Sensitivity

Antimicrobial susceptibility testing of bacteria was carried out by the disk diffusion method using commercial disks, according to the guidelines of the National Committee for Clinical Laboratory Standards *(34).*

RESULTS

Bacterial isolation results were represented in Tables (1-5).

Table 1. *Campylobacter* spp. isolates obtained from Quail eggs, swabs and tissues

Number of samples	*Campylobacter* positive	%	C. *jejuni*	%	C. *coli*	%
263	156	59.3	150	96.2	6	3.8

Table 2. Prevalence of *Campylobacer* (*jejuni & coli*) and *Arcobacter* species in Quail samples.

Samples / Isolates		Eggs				Cloacal swabs		Organs							
		Shell		Content				Liver		Bile		Cecum		Total	
Campylobacter spp.	Spp.	No	%	No	%	No	%	No	%	No	%	No	%	No	%
	jejuni	52	44.8	44	37.9	42	52.5	3	12.5	3	16.7	6	24	12/67	17.9
	coli	-	-	-	-	-	-	2	8.3	4	22.2	-	-	6/67	8.9
Arcobacter spp.	-	16	13.8	-	-	6	7.5	4	16.7	1	5.6	2	8	7/67	10.4

N.B.: Total number of (Egg samples= 116, Cloacal swabs = 80, Livers = 24, Biles= 18, Ceca = 25) No.: number of positive

Table 3. Prevalence of *E. coli* serotypes in different quail samples.

E. coli serotypes	Egg samples (N =116)				Cloacal swabs (N =80)	
	Shell		Content			
	No.	%	No.	%	No.	%
$O_{26:H11}$ EHEC	-	-	11	9.5	-	-
$O_{118:H2}$ EHEC	11	9.5	-	-	13	16.3
$O_{86:H34}$ EPEC	15	12.9	14	12.1	19	23.8
$O_{119:H6}$ EPEC	9	7.8	-	-	8	10
$O_{115:HNM}$ EHEC	5	4.3	-	-	8	10
$O_{158:H}$ EPEC	6	5.2	-	-	5	6.3
$O_{164:HNM.}$ EIEC	12	10.3	10	8.6	7	8.8
Untypable	6	5.2	9	7.8	4	5
Total	64	55.2	44	37.9	64	80

N: Total samples number. No. = number of positive.

Table 4. Prevalence of *Staphylococcus aureus* in Quail eggs

Total samples number	Egg shell					Egg content				
	No	%	Count/ CFU/mL			No	%	Count/ CFU/mL		
			Min.	Max.	Mean			Min.	Max.	Mean
116	68	58.6	$3x10^2$	$1.5x10^4$	$3.5x10^3$	64	55.2	$2x10^3$	$10x10^3$	$1.5x10^3$

No. = number of positive samples. Min = minimum Max = maximum

Table 5. Prevalence of pathogenic bacteria from workers'hand swabs at the quail farms.

Examined samples	Recovered bacteria							
	Campylobacter jejuni		*Arcobacter* spp.		*E. coli*		*S. aureus*	
	No.	%	No.	%	No.	%	No.	%
20	9	45	2	10	15	75	18	90

Antimicrobial sensitivity results of isolated *Campylobacter* and *E.coli species* were summarized in Tables (6, 7 & 8).

Table 6. Antimicrobial sensitivity of *C. (jejuni & coli)* and *Arcobacter* spp. from quail samples.

Antibiotic disc	C. *jejuni*	C. *coli*	Arcobacter spp.
Ciprofloxacin	29± 0.25	24.5 ± 0.28	22.5 ± 0.31
Norofloxacin	27 ± 0.23	17± 0.58	15±0.75
Enrofloxacin	23.5 ± 0.13	21±0.62	14±0.47
Spiramycin	22 ± 0.51	20 ± 0.41	21.8 ± 0.33
Lincomycin	17.5 ± 0.14	16.8 ± 0.48	-ve
Gentamycin	16.5 ± 0.13	15.2±0.34	14.5 ± 0.24
Spectinomycin	13 ± 0.77	9.3±0.32	5 ± 0.38
Amoxicillin	8.5 ± 0.12	9.6 ± 0.42	11 ± 0.38
Neomycin	10±0.29	8 ± 0.69	12 ± 0.36
Streptomycin	6.4 ± 0.32	5 ± 0.65	13 ± 0.23
Colistin	-ve	-ve	-ve
Ampicillin	-ve	-ve	-ve

N.B: -ve = resistant.

Table 7 . Antimicrobial sensitivity of different *E.coli* serotypes from quail samples

Antibiotic disc	*E.coli* serotypes						
	O26	*O86*	*O115*	*O118*	*O119*	*O158*	*O164*
Enrofloxacin	27±0.9	23±0.38	20±0.8	20±0.78	18±0.67	17±0.71	21±0.86
Spectinomycin	26±1.9	25.1±0.3	10.7±1.1	26.5±0.45	23.5±0.6	19±0.91	19.5±0.3
Ciprofloxacin	23±0.94	27±0.4	14±1.7	24.5±0.44	25.5±0.7	24±1.8	18±1.0
Norofloxacin	20±1.2	15±0.71	19±0.83	16±1.0	14±0.8	16±1.3	16±0.65
Gentamycin	13±0.9	24.5±0.3	20.3±0.94	13.6±0.88	15.7±0.8	15±0.8	12±1.1
Amoxicillin	15.5±1.4	16.7±0.58	16.3±1.2	11.3±0.78	9.5±0.5	17.5±0.5	18.6±0.6
Neomycin	12.5±0.66	25±0.4	6.3±1.2	16.5±0.64	13.5±0.6	11±0.9	10±1.2
Streptomycin	12±0.63	28±0.23	4±0.87	21.5±1.2	10.5±0.8	15.5±0.4	13±1.9
Ampicillin	-ve	-ve	-ve	-ve	-ve	12.5±0.5	11.8±0.7
Colistin	-ve	8±0.2	-ve	6.7±0.9	-ve	11±0.9	10.5±0.8
Spiramycin	-ve	-ve	-ve	-ve	-ve	13.5±1.4	-ve

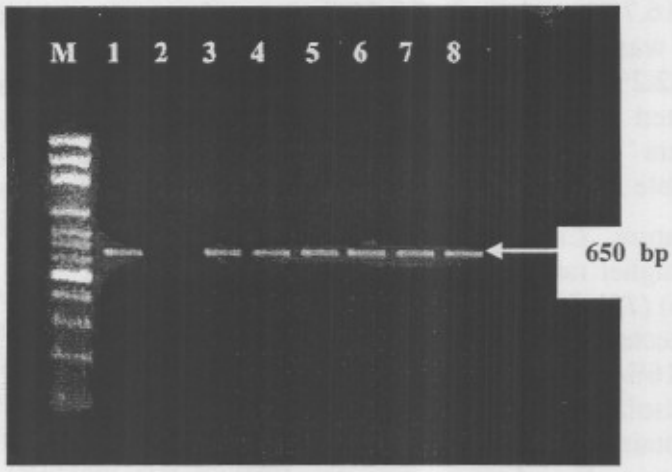
N.B: -ve = resistant.

Table 8. Antimicrobial sensitivity of pathogenic bacteria from worker hand swabs at the quail farms.

Antibiotic disc	*E.coli* serotypes			*C.jejuni*
	O26	*O158*	*O164*	
Ciprofloxacin	26±0.8	8±0.43	30±1.5	23±1.4
Spectinomycin	21±1.1	6±0.3	24±0.9	9±0.61
Enrofloxacin	20±0.5	15±1.7	11±0.81	19±0.24
Gentamycin	14±1.4	17±1.3	10±0.78	21±0.61
Amoxicillin	16±1.9	-ve	-ve	12±0-1.3
Neomycin	12±0.7	-ve	16±1.4	14±0.8
Streptomycin	18±0.65	8±1.2	20±0.34	10±0.72
Ampicillin	-ve	-ve	-ve	-ve
Colistin	-ve	18±0.7	6±0.81	-ve

N.B: -ve = resistant.

Photograph 1 illustrates the PCR-amplified product (650 bp) of 23S rRNA gene which occurred in *Campylobacter* spp. following 1.7% agarose gel electrophoresis. The amplicon for the *Campylobacter* 23S rRNA primers was present in all tested *Campylobacter jejuni* isolates but failed to amplify *A. hydrophila* isolate (negative control, lane 2).



Photograph 1: Agarose gel electrophesis pattern of 23S rRNA gene of *Campylobacter* spp. 650-bp fragment specific PCR product amplified with the primers.
M: DNA molecular weight ladder of 100 bp.
Lane 1: Positive control (*Campylobacter jejuni* strain).
Lane 2: Negative control (*Aeromonas hydrophila* strain)
Lanes 3-8: *Campylobacter jejuni* strains

DISCUSSION

The current study revealed that, *Campylobacter* spp. were successfully isolated at the rate of 59.3% of the whole examined quail samples. Whereas, *C. jejuni* and *C. coli* represented 96.2% and 3.8% of the total *Campylobacter* spp. isolates, respectively (Table 1). The prevalence of *C. jejuni* in cloacal swabs (52.5%) and egg shell (44.8%) were higher than in egg content (37.9%) (Table 2). The presence of *C. jejuni* in intestine may lead to contamination of quail carcasses *(35)*. Little available literatures dealt with the microbial examination of fresh quail eggs. But experimentally, *C. jejuni* was isolated at the rate of (28% and 4.3%) from quail egg shells and content respectively *(1)*. Also, C. *jejuni* was detected in 0.5% and 8.33% of the examined chicken egg shells by ***Jones et al.,(4) and Bastawrows et al. (36)*** respectively. On the other hand, ***Vashin et al.*** *(5)* recorded the presence of *Campylobacter* spp. in the cloacal swabs (76.7%) and in the excrements (63.3%) of laying quail but failed to detect it in egg samples.

At the same time, *C. coli* couldn't be recovered from both quail eggs and their cloacal swabs. These results go hand to hand with that detected by ***Ibrahim et al. (2) and Vashin et al. (5).***

Beard (37) and Callicott et al. (38) reported that horizontal transmission was the primary route for *Campylobacter* infection and vertical transmission is not a natural route. ***Doyle (39)*** suggested that the natural infection of egg contents, if it occurs, it was primarily due to fecal contamination of the external surface and penetration via shell cracks. Moreover, chicks could become orally infected during hatching from such egg shell contamination. These results supported our finding that we didn't detect *Campylobacter*

spp. in egg content alone which reflects that vertical transmission is a remote possibility.

Concerning the prevalence of *Campylobacter* spp. in the visceral organs, *C. jejuni* was recovered at the rate of (17.9%), the highest isolation was recorded in ceca (24%), followed by bile (16.7%) and liver (12.5%). Meanwhile, *C. coli* was detected at the rate of 8.9% only in bile (22.2%) and liver (8.3%) and could not be detected in ceca. *C. jejuni* was isolated from workers ' hand swabs at the farm at a rate of 45% (Table 2 & 5).

In quail's literature, *Campylobacter* spp. were detected at a higher rate in cecum (80%) than in liver (16.7%) ***(12)***. In the same manner, *C. jejuni* were detected at a higher rate in intestinal samples (16%) than in liver (4%) ***(2)***. Meanwhile, it was isolated from the intestinal samples of quails obtained from slaughter shop at the rate of 18% as well as from apparently healthy poultry workers (8.3%) and from workers with bowl incontinence (17.9%) ***(2)***.

In an outbreak reported by ***Finch and Blake (40),*** 26 out of 81 people contracted campylobacteriosis after consuming undercooked eggs. Also an outbreak of *C. jejuni* enteritis amongst workers at chicken processing plant in Sweden was detected which did emphasize the occupational hazard of handling contaminated chickens ***(41)***. The high existence of *Campylobacter* microorganism in quail's digestive tract should be noted indication for risk of its spread through the eggs as well as during slaughtering, and consequently contamination of processed carcasses ***(42)***. Our result confirmed the conclusion of ***Carvalho et al. (43)*** that the liver might be the organ of choice for isolation of *Campylobacter* spp. in presence of diarrhea in chickens.

Some studies recorded the higher rate of *Campylobacter* isolation may be seasonally related which was higher in summer than in winter and the timing of this peak also appears to vary with latitude ***(44)***. The reason for these seasonal variations is unknown but may reflect levels of environmental contamination. Certainly, poultry houses have more ventilation in the summer, increasing the contact of the birds with the outside environment ***(39)***. These observations supported that our high isolation rate may be attributed to the summer.

Photograph 1 illustrates the PCR-amplified product (650 bp) of 23S rRNA gene which occurred in *Campylobacter* spp. following 1.7% agarose gel electrophoresis. In the assay, *Campylobacter* isolates were identified by using biochemical assays and 23S rRNA-PCR. Complete agreement was obtained with the specific primers used in the present assay for all isolates examined. The amplicon for the *Campylobacter* 23S rRNA primers was present in all tested *Campylobacter jejuni* isolates but failed to amplify *A. hydrophila* isolate (negative control, lane 2). In addition to clinical use, the method has potential as a diagnostic kit for detecting *Campylobacters* in complex samples, such as foods in which low pathogen numbers are frequently present. The present PCR assay offers an effective alternative to traditional biochemical typing methods for the identification of *Campylobacter* isolates from humans and poultry.

PCR will be useful for addressing important issues in epidemiology. They include rapid detection which is important in food hygiene and in the prevention of foodborne diseases. This should greatly speed up identification by replacing the current biochemical phenotypic schemes, which are subjective in interpretation and time-consuming.

Table 2 and 5 cleared out that *Arcobacter* spp. were isolated from quails in descending order as follows: liver (16.7%), egg shell (13.8%), cloacal swabs (7.5%), bile (5.6%), cecum (8%) and worker's swabs (10%). To our knowledge, no researches study the prevalence of this pathogen in quails. ***Vindigni et al. (45)*** detected *Arcobacter butzelri* in 4% of chicken eggs with intact shells and in 38% of chicken meat. On the other hand, ***Zanetti et al. (46)*** could not find *Arcobacter butzleri* in egg samples and detected it in poultry meat only. *Arcobacter* was detected in cloacal swabs and

chicken meat samples ***(18,47). Vandenberg et al. (17)*** reported that *Arcobacter* spp. have been associated with enteritis and occasionally bacteremia in man. ***Prouzet-Mauléon et al. (48)*** reported that *Arcobacter* spp. ranks fourth for *Campylobactereace* isolation in human clinical samples and appears to have the same pathogenic potentials as the other species in the genus. The prevalence of *Arcobacter* may be underestimated because of false identification as *Campylobacter* The differentiation of *Campylobacter* and *Arcobacter* species by phenotypic analysis was difficult due to several reasons:(i) the lack of standardized procedures, (ii) partial biochemical inertness, (iii) their numerous phenotypic similarities, and (iv) the prevalence of atypical strains. These difficulties have increased interest in the development of molecular identification approaches ***(49).***

E. coli was isolated at a higher rate from cloacal swabs and egg shell (80% & 55.2%, respectively) than in egg contents (37.9%). *E. coli* was not detected in egg content alone which indicated horizontal transmission. The recovered *E. coli* serotypes from quail samples belonged to serotypes $O_{26:H11}$, $O_{118:H2}$, $O_{86:H34}$, $O_{119:H6}$, $O_{115:HNM}$, $O_{158:H-}$ and $O_{164:HNM}$ and from worker's hand swabs (75%), Human *E. coli* isolates were grouped as $O_{26:H11}$, $O_{128:H}$, $O_{158:H}$ and $O_{164:HNM}$, which were classified according to ***Nataro and Kaper (24)*** to four distinct groups: enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC), and enterohemorrhagic *E. coli* (EHEC) (Table 3).

Some studies recorded the presence of *E. coli* in quail's egg shell at a rate of 30% ***(23)*** and 11.8% ***(20)*** and in 4.88% of quail's egg content ***(22).*** In quail flocks, *E. coli* was detected in cases of diarrhea, septicemia, and high mortalities ***(20,21).*** *E. coli* was detected in quail carcasses ***(50).*** On the other hand, Shiga Toxin 2f producing ($O_{115:HNM}$) (EHEC) *E. coli* was isolated from a symptomatic patient who had eaten raw chicken meat and raw chicken eggs prior to onset of symptoms ***(51).*** *E. coli* was taken as an indication of bad hygienic measures and recent fecal contamination. It can grow and penetrate the egg shell leading to contamination of the contents. Serotype O_{26} has emerged as the most important non-$O_{157:H7}$ human pathogen with respect to its ability to cause diarrhea and the haemolytic uraemic syndrome ***(52).*** An outbreak of acute diarrhoea occurred amongst babies in a special care baby unit caused by *E. coli O* group 158, thirteen babies were at risk, six suffered diarrhoea and three of them died ***(53).***

These finding supported that both *Campylobacter* and *E. coli* were incriminated as main causes of severe diarrhea and mortalities in our quail flock at the same time it can pose an occupational hazard to human in contact and consumers.

Salmonella could not be detected in any of the examined samples. This finding supported the finding of ***Jehan and shabana, (23)*** who didn't detect *Salmonella* in fresh and canned quail eggs. Our result contradicted with the results obtained by ***Hedawy and Wassel (20), Erdoğrul (26), Sander et al. (54) and Azanza (55)*** who isolated this microorganism from different quail samples.

The prevalence rates of *Staphylococcus aureus* were higher in egg shell (58.6%) than egg contents (55.2%) (Table 4). Comparing our results in quails with other researches in chickens' eggs, the current study agreed to some extent with that recorded by ***Bastawrows et al. (36)*** **and** ***Sabreen (56).*** Meanwhile, ***Azanza (55)*** confirmed the presence of *S. aureus* (10^4 MPN/g) in a Philippine emerging street food based on quail eggs. The current study diverged from the results of ***(23)*** who couldn't detect *S. aureus* in neither fresh nor canned quail eggs at all. *S. aureus* was isolated from quail (5.9%) ***(20).*** In the present study, *S. aureus* was isolated from 75% of workers' hand swabs.

Staphylococcus aureus is an important foodborne pathogen and the thermostable enterotoxin elaborated by this microorganism is of serious concern to public health aspects ***(57).*** The comparatively low level of *S. aureus* in egg contents than egg shell may be due to

the presence of lysozyme in the inner shell membrane which acts as an effective agent against gram positive microorganisms. Bird's eggs contamination may be originated from ova, accidental contamination of shell from dust, and from skin, nose and throat of poultry workers *(58).*

All isolates of *Campylobacter and Arcobacter* spp. showed high susceptibility against Ciprofloxacin, Norofloxacin, Enrofloxacin, Spiramycin and Lincomycin except *Arcobacter* spp. showed resistance for Lincomycin (Table 6). The moderate inhibition was performed for Neomycin, Streptomycin and Amoxicillin. Most strains tested were resistant against Colistin and Ampicillin. These results were similar to some extent with ***Sagara et al. (59)*** who found all of the *C. jejuni* isolates were susceptible to, Ciprofloxacin, Erythromycin, Kanamycin, and Nalidixic acid. ***Senok et al. (60)*** found that, Human and chicken *Campylobacter* isolates were sensitive to Ciprofloxacin, Erythromycin, Tetracycline and Trimethoprim-sulfamethoxazole. ***Mazi et al. (61)*** determined that *Campylobacter jejuni* isolates were susceptible to Erythromycin, Ciprofloxacin, and Tetracycline. *Campylobacter* isolates were resistant to Ampicillin and Colistin *(62,63)*.

The sensitivity of *E. coli* isolates to different antibiotics showed that Enrofloxacin, Ciprofloxacin, Norofloxacin, and Spectinomycin were the most effective drugs (Table 7) The highest sensitivity was recorded against Nitrofurantoin by ***Roy et al. (64)*** to all *E.coli* isolates from Japanese quail and with *(20)*. Our results recorded complete resistant to Ampicillin, Colistin and Spiramycin which where similar to some extent with that reported by ***Roy et al. (64).*** Variation of antimicrobial sensitivity results might be attributed to development of drug resistant bacterial strains. Treatment of quail flock with Enrofloxacin through drinking water for 5 days controlled the problem.

C. jejuni, *Arcobacter* spp., *E. coli* and *S. aureus* were isolated from hand swabs of workers (during their work time) at quail farms. These findings support the possibility linking the occurrence of *C. jejuni* enteritis to the consumption of eggs or handling of birds *(41,65).*

Ciprofloxacin, Gentamycin, Streptomycin and Neomycin were more effective for most human bacterial isolates while all of them were resistant to Ampicillin. These finding agree with that detected by ***Amal and Nagla (66).*** The development of antimicrobial resistance in bacteria has become a global problem leading to variation in antimicrobial sensitivity results which may be due to intensive haphazard antibiotics therapy given in most cases of bacterial infections in chicken farms.

As food safety has become an increasing concern for consumers, there is a growing need for fast and sensitive methods for specific detection and identification of zoonotic microorganisms which can be facilitated by using molecular technology. The improvement of hygiene in quail farms and slaughter houses to reduce bacterial pathogens in the food chain is recommended. Isolation of foodborne bacterial pathogens from quail eggs constitutes a great threat to the health of human which can be controlled by avoid eating of undercooked meat and eggs. The increasing rates of bacterial resistance make advisable a more conservative policy for the use of antibiotics in man and poultry.

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الملخص العربي

التواجد و التعرف الجزيىء لبعض البكتريا الضارة المشتركة بين طيور السمان وبيضها الإنسان

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قسم صحة الحيوان والأمراض المشتركة ، **وقسم طب الطيور و الأرانب
*** و قسم الرقابة الصحية على الأغذية, كلية الطب البيطري جامعة قناة السويس مصر.

تم دراسة مدى انتشار و تواجد ميكروب الكامبيلوباكتر المعوي وبعض الميكروبات البكتيرية الممرضة الأخرى في مزرعة سمان تعانى من انخفاض شديد في النشاط الوظيفي وكذلك إسهال حاد مع أهميتهم من الناحية الصحية وكذلك انتقالهم إلى الإنسان.

تم تجميع و فحص 80 مسحة مجمعية و 67 عينة من الأعضاء الداخلية مثل الكبد والكيس المراري وكذلك الأعورين بالإضافة إلى 116 بيضة سمان من إنتاج القطيع و20 مسحة يد من العمال المشرفين في المزرعة ومحتكين مباشرة بالطيور.

أسفرت الدراسة عن عزل ميكروب الكامبيلوباكتر المعوي في 156 عينة سمان بنسبة 62.4% حيث كانت نسبة الكامبيلوباكتر جوجونای إلى الكامبيلوباكتر كولای 96.2% و 3.8% على التوالي في العينات المعزولة , حيث عزلت الكامبيلوباكتر جوجونای من المسحات المجمعية و قشرة البيض الخارجي وكذلك من زلال البيض و الأعورين والكيس المراري والكبد ومسحات ايدى عمال المزرعة بالنسب 52.5% , 44.8% , 37.9% , 24%, 16.7%, 12.5 و 45% على التوالي ولكن لم يتم عزل الكامبيلوباكتر كولای فقط من الكيس المراري والكبد فقط بنسب 22.2% و 8.3% على التوالي.

تم تأكيد عزل ميكروب الكامبيلوباكتر المعوي من عينات الطيور وكذلك الإنسان باستخدام التحليل البيولوجي (أبى سى أر) في الميكروبات المعزولة والتي أثبتت أهميتها في تأكيد الميكروب مع اقتصاد الوقت والجهد.

كما تم عزل ميكروب الأركوباكتر من الكبد وقشرة البيض و الأعورين والمسحات المجمعية والكيس المراري ومسحات الأيدي البشرية بمعدل 16.7%, 13.8%, 8%, 7.5%, 5.6% و 10% على التوالي.

كما تم عزل من المكروبات الضارة الأخرى المسببة للأعراض بكتريا الاشيريشيا كولای بنسب عالية من كل من المسحات المجمعية و قشر البيض وزلال البيض ومسحات الأيدي للعاملين بالمزرعة 80%, 55.1%, 37.9% و 75% على التوالي. وصنفت المعزولات للميكروب كالأتي $O_{26:H11}$, $O_{118:H2}$, $O_{86:H34}$, $O_{119:H6}$, $O_{115:H-}$, $O_{158:H-}$ $O_{164:HNM}$. من طيور السمان و كالأتي $O_{26:H11}$. $O_{128:H-}$, $O_{158:H-}$, and $O_{164:HNM}$ من مسحات الأيدي البشرية. وكانت النتائج سالبة لعزل نوع السالمونيلا من كل العينات التى تم فحصها.

كما تم عزل ميكروب الأستافيلوكوكس أوريس من على قشرة البيض وكذلك زلال البيض و من مسحات الأيدي البشرية بنسب 58.6%, 55.2% و 90% على التوالي.

أثبت اختبار الحساسية للميكروبات المعزولة حساسيتها الشديدة لكل من السيبروفلوكساسين و الأينروفلوكساسين و الاسبكتينومايسين وكذلك الجينتاميسين. وكانت المعزولات مقاومة بنسبة 100% لكل من الأمبيسلين و الكوليستين. تم علاج القطيع باستخدام الأينروفلوكساسين و الذي سيطر على المشكلة.