

Some Pathogenic Bacteria In Laying Chicken And Its Eggs With Reference To Zoonotic Importance

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ABSTRACT

A total of 150 cloacal swabs and two hundred chicken eggs were collected from laying chickens of native breed which were reared in farmer's houses and ISA Brown layers farms at Ismailia province for detection of some pathogenic bacteria and its zoonotic importance. In addition fifteen worker's hand swabs were collected from workers in contact with these birds.

The obtained results revealed out that the isolation of *Campylobacter jejuni* from cloacal swabs, egg shell and content of native breed and ISA Brown laying chickens were at the rates of (33.3% & 13.3%), (45% & 20%) and (30% & 0%), respectively.

E. coli was isolated at the rates of 93.3%, 60% and 40% from cloacal swabs, egg shell and content of native breed, and 93.3%, 55%, 50% from ISA Brown chickens, respectively. These isolates were serotyped as O26:NM, O86:H34, O119:H6, O164:NM, and O15:H11.

Staphylococcus aureus was isolated from egg shell and egg content of native and ISA Brown laying chicken at the rates of (55% & 45%) and (20% & 15%), respectively.

On the other hand, *Campylobacter jejuni*, *E. coli* and *Staphylococcus aureus* were isolated from worker's hand swabs at the rates of 53.3%, 73.3% and 86.7%, respectively.

Salmonella spp. could not be isolated from any of the examined samples.

Sensitivity tests showed that different *E. coli* isolates were more susceptible to Ciprofloxacin, Spectinomycin, Gentamycin and Neomycin. They were resistant to Ampicillin, Colistin and Spiramycin. *Campylobacter jejuni* isolates were highly sensitive to Enrofloxacin, Ciprofloxacin, Gentamycin, Spiramycin, Norfloxacin and Amoxicillin. On the other hand, it showed moderate sensitivity to Streptomycin, Neomycin and were resistant to Lincomycin, Colistin and Ampicillin.

These results reflect that laying hens could have a role in foodborne zoonotic diseases, epidemiology and necessitate the need to improve hygienic conditions in these farms.

INTRODUCTION

Chickens and chicken products usually harbor pathogenic microorganisms. Chicken products have been reported as vectors of food poisoning outbreaks (1). Eggs may be responsible for several food borne outbreaks and act as a vehicle for transmission of pathogens to consumers (2, 3).

The egg may be infected through trans-ovarian transmission during development of eggs or through the shell contamination by fecal matter from the bird, contact with dirty surfaces, washing water and during handling

(4, 5). Moreover, the rate of penetration of these organisms from the intact shell is influenced by farm biosecurity, humidity and storage temperature at which the eggs are produced and stored (6).

Campylobacter is a major bacterial cause of infectious diarrheal illness in many countries all over the world. Handling and consumption of chicken meat or eggs have been considered as important risk factors (1, 7). The intestinal content of poultry has been shown to be the main container of *Campylobacter jejuni* colonization. Bird-to-bird transmission within flock was extremely

rapid once flock colonization was detected and the majority (up to 100%) of birds in positive flock were colonized within only a few days (8, 9). Birds infection was generally not associated with clinical disease, even if large number of bacteria were expelled with the feces (10).

Some pathogenic bacteria such as, *E. coli*, *Staphylococcus aureus* and *Salmonella* spp. were isolated from the shell and contents of marketable eggs at variable percentages (11). Furthermore, numerous cases of food-poisoning outbreaks were traced to these pathogenic bacteria (3, 12). The presence of such pathogenic bacteria in the eggs causes serious diseases for human beings through consumption of such contaminated eggs and the main symptoms of these diseases are septicemia, meningitis, gastroenteritis, epidemic diarrhea in infants, sporadic summer diarrhea in children, urinary tract infection (13).

The aim of this study was to determine the occurrence and health hazard of *Campylobacter* spp., *E. coli*, *Staphylococcus aureus* and *Salmonella* in chickens and their eggs, as well as to detect the antimicrobial sensitivity of these isolates against different common available antimicrobial agents.

MATERIALS AND METHODS

1. Collection of samples

A total of 150 cloacal swabs and 200 eggs were collected from different laying chickens of native breed reared in farmers' houses (75 cloacal swabs and 100 eggs) and ISA- Brown farms (75 cloacal swabs and 100 eggs) in Ismailia province. On the other hand, 15 hand swabs were collected from workers who were in contact with these birds. The swabs were placed in sterile test tubes containing peptone water. All samples were immediately transported in an ice-box to the laboratory for bacteriological examination.

2. Bacteriologic examination

Preparation of egg samples: Egg samples (shells and contents) were prepared for bacteriological examination according to (14).

The samples were inoculated into different media according to the type of bacteria as follows:

Isolation and identification of *Campylobacter* spp.: cloacal swabs, egg shell and content and hand swabs were inoculated into semisolid thioglycollate and Brain Heart Infusion (BHI) agar media supplemented with *Campylobacter* selective supplement Skirrow (SR069E, Oxoid), then incubated under microaerophilic condition using anaerobic pack system (BR056A, Oxoid) at 42°C for 48 hours. The isolates were identified by using Gram stain, motility under phase contrast microscope, catalase, oxidase, hippurate hydrolysis, aerobic growth, growth in 1% glycine and 3.5% NaCl and sensitivity to Nalidixic acid and Cephalothin (15).

Isolation and identification of *E. coli*: cloacal swabs, egg shell and content and hand swabs were inoculated into peptone water, Brilliant green broth and Eosin Methylene Blue agar media and incubated at 37 °C for 24 hours (14). The isolates were identified by indole, methyl red, Voges-Proskauer and citrate (IMViC), urease and TSI tests. Moreover, serotyping of the suspected isolates was performed at the Central Laboratory of Ministry of Health, Cairo.

Isolation and identification of *Staphylococcus aureus*: Serial dilutions using sterile 0.1% peptone water were made from samples of egg shell and content and hand swabs. One hundred microliters from the selected dilutions were plated on Baird Parker agar medium (Merk, Art. Nr., 5406) using a sterile smooth bent glass rod. Then, incubated at 37°C for 48 hours and identified by Gram stain, catalase, DNase, mannitol fermentation and coagulase activity (16).

Isolation and identification of *Salmonella* spp.: Cloacal swabs, egg shell and content and hand swabs were inoculated into peptone water, Rappaport-Vassiliadis broth and Xylose Lysine Desoxycholate agar medium (XLD) and identified by urease test, TSI, indole, methyl red, Voges Proskauer test,

utilization of citrate and lysine decarboxylase (17).

Antibiogram determination:

Different available commercial anti-bacterial discs were used for detection the sensitivity of the bacterial isolates (18).

Statistical analysis:

Chi square test was performed to analyze the obtained data (19).

RESULTS

The isolation of different bacteria from eggs, cloacal and hand swabs were summarized in (Tables 1-5)

Table 1. Occurrence of *Campylobacter jejuni* in chicken's eggs and their cloacal swabs

<div>Samples</div> <div>Source</div>	Eggs (N =100 for each)				Cloacal swabs (N =75 for each)	
	Shell		Content		Positive	
	positive		positive			
	No.	%	No.	%	No.	%
Native breed	45	45	30	30	25	33.3
ISA-Brown breed	20	20	-	-	10	13.3
Total	65	32.5	30	15	35	23.3

Table 2. Occurrence of different *E. coli* serotypes in native breed chicken's eggs and their cloacal swabs.

<i>E. coli</i> serotypes	Eggs (N =100)				Cloacal swabs (N =75)	
	Shell		Content		Positive	
	positive		positive			
	No.	%	No.	%	No.	%
STEC O 26:NM	11	11	-	-	13	17.3
EPEC O 86:H34	8	8	12	12	15	20
O119:H6	7	7	8	8	4	5.3
EIEC O164:NM	24	24	14	14	26	34.7
Untypable	10	10	6	6	12	16
Total	60	60	40	40	70	93.3

STEC: Shiga toxin producing *E.coli*. EPEC: Enteropathogenic *E.coli*. EIEC: Enteroinvasive *E.coli*.

Table 3. Occurrence of different *E. coli* serotypes in ISA Brown chicken's eggs and their cloacal swabs

<i>E. coli</i> serotypes	Eggs (N =100)				Cloacal swabs (N =75)	
	Shell		Content		Positive	
	positive		positive		Positive	
	No.	%	No.	%	No.	%
EPEC						
O 86:H34	9	9	8	8	17	22.7
O119:H6	11	11	12	12	13	17.3
EIEC						
O164:NM	10	10	11	11	15	20
ETEC						
O15:H11	15	15	9	9	20	26.7
Untypable	10	10	10	10	5	6.7
Total	55	55	50	50	70	93.3

EPEC: Enteropathogenic *E. coli* EIEC: Enteroinvasive *E. coli* ETEC: Enterotoxigenic *E. coli*

Table 4. Prevalence of *Staphylococcus aureus* isolates in chickens' eggs

Sample Source	Egg shell (N =100 for each)					Egg content (N =100 for each)				
	Positive		Count (cfu /mL)			Positive		Count (cfu /mL)		
	No.	%	Min.	Max.	Mean	No.	%	Min.	Max.	Mean
Native breed	55	55	3x10 ³	1x10 ⁵	1.99x10 ⁴	45	45	1x10 ³	9.8x10 ⁴	1.46x10 ⁴
ISA-Brown breed	20	20	2x10 ³	8x10 ⁴	2.77x10 ⁴	15	15	4x10 ³	7.4x10 ⁴	2.08x10 ⁴
Total	75	37.5	-	-	-	60	30	-	-	-

Table 5. Pathogenic bacteria in hand swabs collected from poultry farms' workers who were in contact with the examined chickens

Number Examined	Positive		Bacteria recovered					
			<i>C. jejuni</i>		<i>E. coli</i>		<i>S. aureus</i>	
	No.	%	No.	%	No.	%	No.	%
15	13	86.7	8	53.3	11	73.3	13	86.7

Sensitivity of the isolated bacteria against different antimicrobial discs was summarized in (Tables 6-7).

Table 6. Antimicrobial sensitivity of different *E. coli* serotypes by using disc diffusion method

Antibiotic disc	<i>E.coli</i> serotypes						
	O26	O86	O115	O118	O119	O158	O164
Ciprofloxacin	19.5±1.12	15.7±0.5	22±0.49	20±0.39	23±0.44	19±0.91	25.5±0.41
Spectinomycin	12±1.41	18.2±0.41	15.6±0.6	14±0.5	23.5±0.38	24±1.8	20±0.34
Amoxicillin	11.5±0.7	20±0.61	22.5±0.55	10±0.45	-ve	18.5±0.5	12.5±0.3
Gentamycin	8.5±0.35	9±0.62	8.5±0.36	11±1.0	15.5±0.41	15.7±0.8	10±0.37
Neomycin	9.5±0.7	10±0.44	8±0.41	12±0.5	7.5±0.37	13.5±0.6	9.8±0.42
Streptomycin	-ve	10.5±0.53	10±0.4	11.5±0.39	12±0.5	15.5±0.4	11.5±0.33
Ampicillin	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Colistin	11±1.0	-ve	-ve	-ve	-ve	11±0.9	-ve
Spiramycin	-ve	-ve	-ve	-ve	-ve	13.5±1.4	-ve

-ve means resistant.

Table 7. Antimicrobial sensitivity of *Campylobacter jejuni* isolates.

Antibiotic disc	<i>C. jejuni</i>
Enrofloxacin	28.6± 0.47
Ciprofloxacin	27 ± 0.23
Spiramycin	19.5 ± 0.13
Gentamycin	21.6 ± 0.51
Norofloxacin	16.4 ± 0.14
Amoxicillin	12.1 ± 0.15
Spectinomycin	9.5 ± 0.75
Streptomycin	10.2 ± 0.65
Neomycin	9.5 ± 0.13
Lincomycin	-ve
Colistin	-ve
Ampicillin	-ve

-ve means resistant.

DISCUSSION

Our results illustrate that *Campylobacter jejuni* could be detected with percentages of 45 and 30 from native breed eggs shell and content, respectively. On the other hand, in ISA-Brown eggs, *Campylobacter jejuni* couldn't be isolated from content, it was found only in egg shells at a rate of 20%. This may be attributed to prophylactic antibiotic routinely used in ISA Brown poultry farms. These results were supported by Sahin *et al.*

(20). The variation in the occurrence rates between the two examined types of eggs may be attributed to the unhygienic condition under which native breed chickens are raised at farmers' houses and the egg shells can become contaminated as a result of intestinal carriage of this pathogen. The same result was confirmed by Zaki and Reda (21) and Bastawrows *et al.* (22). On the other hand, eggs in poultry farms are produced under good strict hygienic measures during collection, handling and distribution as *Campylobacter*

organism is very liable to chilling and drying (23, 24). Lower rates of *Campylobacter* isolation from egg shell (4%) and content (8.33%) were recorded by Moustafa (25) and Bastawrows et al. (22), respectively. *Campylobacter jejuni* was detected from egg shell (4.3%) and from content (1.4%) (26).

The prevalence of *Campylobacter jejuni* in cloacal swabs was 33.3% and 13.3% for native and ISA Brown breeds, respectively (Table 1). There was a highly significant difference in the isolation rates between native breed and ISA Brown chickens. Chi square and p values were ($X^2 = 13.128$, $p < 0.0005$), ($X^2 = 32.98$, $p < 0.0001$) and ($X^2 = 7.304$, $p < 0.0069$) for egg shell, egg content and cloacal swabs, respectively.

In other studies, *Campylobacter jejuni* was previously recovered at variable rates, 15%, 32.4%, 73%, 31.4% and 17.8% from cloacal swabs by Ahmed and Ahmed (27); Draz et al. (28); Modugno et al. (29); Ibrahim and Hebat-Allah (30) and Fonseca et al. (31), respectively. During slaughtering, the damage of intestinal tract integrity can lead to direct contamination and subsequent human infection (32).

Tables (2 and 3) pointed out that *E. coli* was isolated at the rates of 93.3%, 60% and 40% from cloacal swabs, egg shell and content of native breed, respectively. Moreover, it was recovered at a rate of 93.3%, 55% and 50% from cloacal swabs, egg shell and content of ISA Brown breed, respectively. The isolated *E. coli* serotypes belonged to STEC (O26:NM), EPEC group (O86:H34 & O119:H6), EIEC group (O164:NM) and ETEC group (O15:H11). The difference between different chicken breeds was non-significant ($p > 0.05$).

These results were confirmed by others different studies where, *E. coli* was isolated from native breed chickens' eggs shell and content at rates of 46.7% and 6.7% and at a lower rate from farm eggs (13.3% and 6.7%), respectively (2). Also, *E. coli* was isolated from cloacal swabs (20%, 15.6%), egg shell (11.1%, 8.9%) and egg content (3.3%, 2.2%)

of native breed and farm breed, respectively (33).

The highest rate of *E. coli* isolation from chickens eggs and swabs supports the fact that poultry may act as a transmission vehicle for human *E. coli* infection (34).

Staphylococcus aureus is an important food borne pathogen which can grow in a large number in food with production of thermostable enterotoxins (the usual level found in food about 0.5-10 µg / 100g food (24). Some food poisoning outbreaks have been traced to consumption of egg and egg-based dish contaminated with *S. aureus* (35). It was clear in Table (4) that the prevalence rate of *S. aureus* in native breed eggs was 55% and 45% from egg shell and content with mean count values of 1.99×10^4 and 1.46×10^4 cfu / mL, respectively. While in Brown eggs the prevalence rates were 20% and 15% with mean count values of 2.77×10^4 and 2.08×10^4 cfu / mL from egg shell and content, respectively. The difference between different chicken breeds was highly significant. Chi square and p values were ($X^2 = 24.661$, $p < 0.0001$) and ($X^2 = 20.024$, $p < 0.001$) for egg shell and egg content samples, respectively.

These results were nearly similar to many previous studies who isolated this microorganism at a rate of 36.7% and 23.3% from egg shell and content, respectively (22). Also, *Staphylococcus aureus* was recorded with lower rate of isolation 26.7% and 13.3% from egg shell and content of native breed, respectively and 26.7% from egg shell of farm breed, and it couldn't isolated from egg content of farm breed (2). *S. aureus* isolated from egg shell (3.3% and 0%) and from egg content (1.1% and 1.1%) of native breeds and poultry farms, respectively (33). In contrast, it was isolated with higher rates from egg shell and content of native breed eggs (96% & 88%) and farm eggs (84% & 56%), respectively (36).

In this study, it was noteworthy to mention that all the recovered pathogenic bacteria could not be recovered from egg content alone. This supported the possibility of horizontal

transmission through dropping, dirt, egg cartons and workers (24, 29, 37).

On the other hand, *Salmonella* could not be recovered from all examined samples. On the contrary, *Salmonella* could be isolated with a variable percentage from native and farm eggs (2). Also, it isolated from cloacal swabs (8.9% & 4.4%), egg shell (6.7% & 2.2%) and egg content (2.2% & 0.0%) of native breed and poultry farms, respectively (33).

From the mentioned above, the variations in isolation of different types of bacteria may be attributed to the pathogenicity of these isolates for chickens which had been correlated with numerous extrinsic and intrinsic factors related to birds. These extrinsic factors includes environmental conditions, exposure to other infections agents, virulence of bacteria, levels and duration of exposure, while the intrinsic factors affecting susceptibility of the bird include age, route of exposure, active and passive immune status and breed of chickens (38).

Results illustrated in Table (5) showed that *Campylobacter jejuni* was recovered from poultry farms' workers who were in contact with the examined chickens at the rate of 53.3%. This high isolation rate predisposes these workers to infection as previously recorded by Corry and Atabay (32). Epidemiological investigations have demonstrated a significant correlation between the handling and consumption of poultry meat, eggs and the occurrence of *Campylobacter* enteritis (7, 39).

Data shown in Table (5) pointed out that *E. coli* was isolated from workers' hand swabs at the rate of 73.3%. The recovered serotypes were (O26:NM, O86:H34 and O164:NM). Most cases of bloody diarrhea are caused by several strains of *E. coli* which are major causes of childhood mortality among children in developing countries (40, 41, 42).

Moreover, *S. aureus* was isolated from 86.7% of workers' hand swabs (Table, 5).

The obtained data of antimicrobial susceptibility tests of *E. coli* serotypes revealed that these isolates were more susceptible to Ciprofloxacin, Spectinomycin, Gentamycin and Neomycin, at the same time; they were resistant to Ampicillin, Spiramycin and Colistin. These results were in agreement to some extent with (43) who found that *E. coli* isolates were sensitive to Gentamycin and Erythromycin while they were resistant to Ampicillin, Penicillin and Tetracycline. Similar results were detected by Amal and Nagla, (42) and Zhao *et al.*, (44) whose isolates were sensitive to Gentamycin. Our results contradicted with those recorded by Moawad *et al.* (45) whose isolates were sensitive to Colistin. This variation in results may be due to intensive haphazard antibiotics therapy usually given by owners in most cases of bacterial infections in chicken farms which results in development of antimicrobial resistance.

While, antimicrobial sensitivity results of *Campylobacter jejuni* showed that they were highly sensitive to Enrofloxacin, Ciprofloxacin, Gentamycin, Spiramycin, Norofloxacin, Amoxicillin and moderately sensitive to Streptomycin, Spectinomycin, Neomycin, while resistant to Lincomycin, Colistin and Ampicillin. Similar finding were reported by Ibrahim and Hebat-Allah (30) and Sulonen *et al.*, (46).

In conclusion, the high prevalence rates of *Campylobacter jejuni*, *E. coli* and *Staphylococcus aureus* in chickens and eggs underscores the importance of adoption of hygienic measures in poultry farms and farmers' houses and public health education of workers and consumers and application of biosecurity to decrease risk of infection. The presence of *C. jejuni* and *E. coli* strains resistant to antibiotics currently used in human medicine urgent need to implement strategies for prudent use of antibiotics in food animal production to prevent further increases in the occurrence of antimicrobial resistance in food-borne human pathogenic bacteria such as *Campylobacter* and *E. coli*.

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الملخص العربي

بعض البكتريا الممرضة فى الدجاج البياض و بيضه و أهميتها كامراض مشتركة

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تم فحص ١٥٠ مسحة من فتحة المجمع و مائتى بيضة من الدجاج البياض بمزارع لانتاج البيض البنى و التربية بمنازل المزارعين فى محافظة الاسماعيلية وكذلك خمسة عشرة مسحة من ايدى العمال المخالطين للطيور فى هذه المزارع. وقد تم عزل ميكروب الكامبيلوباكتريز جو جيناي بالنسب الآتية (٣, ٣٣% و ٤٥% و ٣٠%) و (٣, ١٣% و ٢٠% و ٠%) من مسحات فتحة المجمع و من قشرة البيضة و محتويات البيضة من الدجاج البلدى و البنى على التوالى. وايضا تم عزل ميكروب الأشريشيا كولاي بالنسب الآتية (٣, ٩٣% و ٦٠% و ٤٠%) و (٣, ٩٣% و ٥٥% و ٥٠%) من مسحات فتحة المجمع و من قشرة البيضة و محتويات البيضة من الدجاج البلدى و البنى على التوالى. وايضا تم عزل ميكروب الستافيلوكوكس اوريوس من قشرة البيضة (٥٥% و ٤٥%) و محتويات البيضة (٢٠% و ١٥%) من البيض البلدى و البيض البنى على التوالى.

كما تم عزل ميكروب الكامبيلوباكتريز جو جيناي (٣, ٥٣%) و الأشريشيا كولاي (٣, ٧٣%) و الستافيلوكوكس اوريوس (٧, ٨٦%) من مسحات ايدى العمال المخالطين للطيور. ولم نتمكن من عزل ميكروب السالمونيلا من كل العينات المختبرة.

وباجراء اختبار الحساسية للعترات المعزولة معمليا وجد ان ميكروب الكامبيلوباكتريز جو جيناي شديد ا لحساسية للانثروفلوكساسين و الجنتاميسين و السبياميسين و النوروفلوكساسين و الموكسيسلين وكذلك ميكروب الأشريشيا كولاي ثبت انه شديد الحساسية للمضادات الحيوية الآتية السبروفلوكساسين و السيكتينوميسين و الجنتاميسين و النيو ميسين . عزل الميكروبات المقاومة للمضادات الحيوية يتطلب ترشيد استخدام هذه الأدوية و اجراء اختبار الحساسية قبل العلاج.

هذه الدراسة أوضحت أن الدجاج و بيضه يحتوى على العديد من البكتريا الممرضة للانسان ويمثل خطورة للمستهلكين و للأشخاص المخالطين للطيور. وأفضل طريقة للتحكم فى هذه الأمراض عن طريق تقليل نسبة تواجدتها فى الطيور بالتربية على أسس سليمة و اتباع العادات الغذائية السليمة (مثل الطهى الجيد للبيض و اللحوم و غسل الأيدى جيدا بعد ملامسة الطيور).