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SQUAB AND QUAIL MEATS: MICROBIAL STATUS AND PREVALENCE OF MULTIDRUG-RESISTANT SHIGA TOXIN-PRODUCING *E. coli*

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Abstract: The consumption of exotic meats, such as squab and quail meats, is common in many parts of the world. However, little is known about the safety of these foods. This study aimed to evaluate the microbial characteristics of squab and quail meats, with a particular interest in the prevalence of Shiga toxin-producing *Escherichia coli* (STEC). Meat samples were examined for total bacterial counts, total mold counts, most probable number of coliforms, total *E. coli* counts, and the prevalence of STEC. The presence of virulence genes *(stx1, stx2, and eae)* in STEC isolates was also investigated. Results from microbial analyses revealed poor hygienic status of squab and quail meats. *E. coli* was isolated from 16% and 10% of squab and quail meats, respectively. Six *E. coli* serotypes were recovered, including *O26, O78, O111, O114, O119,* and *O127*, and STEC genes were detected in all these isolates. Squab liver had the highest *E. coli* prevalence rates, followed by gizzard, heart, spleen, and breast muscles. The prevalence of *E. coli* in quail meat samples was similar across all tissues. STEC serotypes showed notable multidrug resistance profiles. We then used ascorbic and rosmarinic acids to increase the safety of breast muscle. Treatment of breast muscles with these acids significantly improved their microbial safety. These findings highlight the potential role of squab and quail meats as a vehicle for STEC transmission to humans, and the beneficial effect of treatment with ascorbic and rosmarinic acids on enhancing the safety of exotic meats.

Key words: food safety; shiga toxin; *E. coli*; squab; quails; meat

Introduction

The consumption of pigeon squab and quail meats is common in many countries, such as Egypt, Italy, and China. meat is characterized by its higher degree of tenderness and juiciness compared with other poultry meats. Squab meat is similar to that of the duck in terms of color and fatty skin with higher vitamin, minerals and protein content (1, 2). Similarly, quail meat is rich in biodigestible proteins, vitamins, and minerals, and its composition is similar to broiler meat. (3).

There are many sources in which microbial contamination may occur in poultry meat, including the bird itself (e.g., feathers and excreta), the operator (e.g., hands, hair, and clothes), the use of contaminated raw materials, washing water, collecting containers, and equipment (4). Contamination of poultry meat with foodborne pathogens is a particularly a major public health concern. Human infection with multidrug-resistant pathogens may result in severe health problems (5, 6).

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Shiga toxin-producing *Escherichia coli* (STEC) is a major foodborne pathogen responsible for significant losses worldwide. Diarrheagenic *E. coli* mainly causes enteric infections. Currently, diarrheagenic *E. coli* are categorized based on their dominant virulence attributes into enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enteroaggregative (EAEC), diffusively adherent (DAEC) and enterohemorrhagic (EHEC)/ Shiga toxin-producing *E. coli* (STEC) (6, 7). The latter is classified into two broad categories: O157 STEC and non-O157 STEC. *E. coli* O157 is responsible for about 90% of human cases, while the remaining cases are associated with other serotypes such as *O26, O45, O103, O111, O121* and *O145* (8, 9).

STEC serotypes can be transmitted to humans mainly through contaminated food, including contaminated poultry meat. However, the potential role of squab and quail meats as a vehicle for STEC transmission to humans remains unknown.

Rosmarinic acid (RMA) is one of the phytochemicals which represents the major component of rosemary, a plant that is cultivated worldwide and used as a flavoring agent in many dishes. Rosmarinic acid possesses significant antioxidant and antimicrobial properties (10). Likely, ascorbic acid (ASA) also has positive effects in wound healing, as an antioxidant, and with significant antimicrobial activities as reported against *Campylobacter spp.*, *Listeria monocytogenes, Staphylococcus aureus*, and *Mycobacterium tuberculosis* (11). Therefore, the first objective of this study was to evaluate the microbial status and the prevalence of STEC in squab and quail meats and giblets. The second objective was to characterize STEC isolates and to assess the antimicrobial effect of treating breast muscle with rosmarinic (RMA) and ascorbic (ASA) acids.

Materials and methods

Sample collection and preparation

Two hundred samples of squab and quails (100 each) were collected from farmed birds that had been slaughtered and distributed in local markets in Sharkia Governorate, Egypt.

Samples were prepared according to APHA (12). Briefly, 90 ml of 0.1% sterile buffered peptone water (LAB104, LAB M, UK) were used to homogenize 10 g of each sample using a sterile meat homogenizer (M-p3-302, mechanic, Precyzina, Poland) for 1-2 minutes at 2000 rpm. Such homogenate represents the dilution of 10^{-1} and further ten-fold decimal serial dilutions were prepared.

Determination of total bacterial count (TBC)

The total number of bacteria was calculated according to the APHA protocol. Aliquots (1 mL) of each dilution were transferred to a sterile Petri dish plate. For each plate, add 12–15 mL of plate count agar (Difco, Detroit, Michigan, USA) cooled to 45 ± 1 °C, mix well, and then allow to harden before incubating inverted for 48 hours at 35 ± 2 °C. Record all colonies including pinpoint size colony forming units as TBC in plates with 25-250 colonies per dish.

TBC/g = average No. of colonies × reciprocal of the dilution

Counted colonies expressed as log 10 cfu/g.

Determination of total mold count (TMC):

Total mold counts were determined by the pour plate technique using Sabouraud's dextrose agar media (Oxoid, UK) supplemented with chloramphenicol 100 mg/L. Plates were incubated in the dark at 25 °C for 5-7 days. and the plates were checked daily for mold growth. Estimation of total mold count was obtained by direct counting of the inoculated plates (12).

TMC/g = average No. of colonies × reciprocal of the dilution

Determination of the most probable number (MPN) of Coliform:

Three tubes most probable number (MPN) method was used (12). Briefly, Aliquots (1 mL) were inoculated into three separate test tubes containing MacConkey broth on inverted Durham's tubes. Tubes were then incubated at 37 °C for 24-48 h. Tubes with visual acid and gas production were considered positive and MPN of coliforms was calculated according to the recommended tables. To determine the MPN of *E. coli*, positive tubes were inoculated into tubes containing 7 ml of *E. coli* (EC) broth (Himedia, Mumbai) using a 10 µL loop (12). After inoculation at 44.5 °C for 24-48 hrs, positive tubes (showing acid and gas

production) were used to calculate the MPN of *E. coli* according to the recommended tables.

Isolation of Escherichia coli

Eosin Methylene blue (EMB) agar was used for the isolation of *E. coli* according to APHA protocol (12). From each positive tube of EC broth, a loopful of culture was streaked onto EMB agar. The inoculated plates were incubated at 37 °C for 24 h. Typical colonies of *E. coli* (metallic greenish colonies with dark purple center) were purified and sub-cultured onto a nutrient agar slopes and incubated for further confirmation by Gram's staining and biochemical tests (12).Biochemically identified isolated of *E. coli* were then subjected to serological examination using antisera sets specific for *E. coli* (Difco, USA) as previously described (13).

DNA extraction and gene detection

Enriched *E. coli* isolates in nutrient broth were centrifuged at 1500 rpm for 10 min. The pellet was lysed with TE buffer, then frozen for 10 min, and incubated at 98°C for 10 min. The concentration of the released DNA was measured using Nanodrop (ND-1000, Nanodrop Technologies, USA). Tested *E. coli* were examined by PCR for harboring Shiga toxin-coding genes, including *stx1, stx2,* and *eae.* The previously published protocol (14) was followed during all PCR procedures and the used primers were stated in Table 1.

Antimicrobial sensitivity testing,

Antimicrobial sensitivity testing was carried out according to the guidelines of Clinical and Laboratory Standards Institute (CLSI) (15). The tested antimicrobials were trimethoprim/ sulfamethoxazole (SXT), ampicillin (AM), enrofloxacin (En), cephalothin (CN), ciprofloxacin (CP), chloramphenicol (C), erythromycin (E), kanamycin (K), gentamicin (G), nalidixic acid (NA), oxacillin (OX), oxytetracycline (T), penicillin (P), and neomycin (N). Multiple antibiotic resistance index was calculated according to the following equation Singh *et al.* (16):

MAR index= Number of resistance (Isolates classified as intermediate were calculated as sensitive for MAR index) antibiotics/ Total number of antibiotics tested.

Improvement of the microbial status of the quail's breast muscle

The effect of RMA and ASA acids in reducing the microbial load of quail's breast muscle was investigated (11). Quail's breast samples (n = 20) were first examined for their microbial quality. Samples were then grouped into groups (n = 4). Samples were immersed in 1% RMA (Group 1), 2% RMA (Group 2), 1% ASA (group 3), 2% ASA (Group 4), or a combination of the two acids (Group 5; 2% each) for 30 min. The microbiological examination was then conducted as described above.

Statistical analysis

All values are expressed as means ± SE, and measurements were carried out in duplicates. Microbial counts were converted into base logarithms, 10 of colony-forming units (cfu) per g (log 10 cfu/g). Statistical analysis was performed using One-way analysis of variance (ANOVA), followed by the Tukey–Kramer HSD post-hock test. Significance was considered when $P < 0.05$.

Results

The obtained results of the present study revealed higher microbial loads in quails than those in squab samples. TBC counts in quails were (log 10 cfu/g): gizzard 6.5 ± 0.19 and 6.2 ± 0.55; liver 6.1 ± 0.24, and 5.4 ± 0.23; heart 5.5 ± 0.18, and 4.2 ± 0.18; spleen 5.2 ± 0.32, and 4.5 ± 0.20; breast muscle 3.2 ± 0.25, and 2.8 ± 0.14 of quail, and squab respectively.

The average TMC in the quail's samples was 5.5 ± 0.22, 5.2 ± 0.18, 4.8 ± 0.18, 4.2 ± 0.16, and 2.8 ± 0.16 in the examined liver, gizzard, heart, spleen, and breast muscle, respectively. These counts were 4.3 ± 0.18, 4.4 ± 0.25, 3.7 ± 0.28, 3.5 ± 0.22, and 2.5 ± 0.33 in the same samples of squab. MPN count of coliforms (log 10 cfu/g) were 5.2 ± 0.18, 4.2 ± 0.20, 3.5 ± 0.18, 3.2 ± 0.15, and 2.8 ± 0.18 in the examined gizzard, liver, heart, spleen, and breast muscle of quails, respectively. These values were 4.5 ± 0.11, 3.6 ± 0.14, 3.2 ± 0.33, 3.0 ± 0.22, and 2.5 ± 0.13 in the same samples of squab. MPN counts of *E. coli* (log 10 cfu/g) were 4.2 ± 0.18, 3.6 ± 0.21, 3.2 ± 0.18, 3.1 ± 0.20, and 2.4 ± 0.33 in the examined

gizzard, liver, heart, spleen, and breast muscle of quails, respectively, and 2.8 ± 0.33, 3.2 ± 0.15, 2.8 ± 0.11, 2.6 ± 0.10, and 2.1 ± 0.22 in the same samples of squab (Fig. 1).

E. coli was recovered from 16% and 10% of squab and quail samples, respectively. Six *E. coli* serotypes were isolated from the samples, including *E. coli O127: H6, O114: H32, O78: H–, O26: H11, O119: H6* and *O111: H–*. *E. coli* O26 was the most prevalent serotype in squab samples (50%), followed by *O127, O78*, and *O119*. In quail samples, *E. coli O127* was the most prevalent serotype (40%), followed by *E. coli O26* (20%), *O78* (20%), *O111*(10%) and *O114* (10%) (Fig. 2).

In squab samples, livers had the highest prevalence rate of *E. coli* (37.5%), followed by gizzard (25%), heart (12.5%), spleen (12.5%), and muscle (12.5%). By contrast, the recovery of *E. coli* in quail samples was similar across all tissue samples (20%) (Fig. 3).

The detection of Shiga-toxin genes varied among STEC isolates. In addition, all isolated *E. coli*

Table 1: PCR primers used in the present study

Shiga toxin coding genes	Primer sequence (5‘-3‘)	Size (bp)	Gene Bank (Acc. No)	Tm◦
stx1	F-ACACTGGATGATCTCAGTGG R-CTGAATCCCCCTCCATTATG	614	MF039301	58
stx2	F-CCATGACAACGGACAGCAGTT R-CCTGTCAACTGAGCAGCACTTTG	779	MF039302	58
eae	F-GTGGCGAATACTGGCGAGACT R-CCCCATTCTTTTTCACCGTCG	890	AJ875041	58

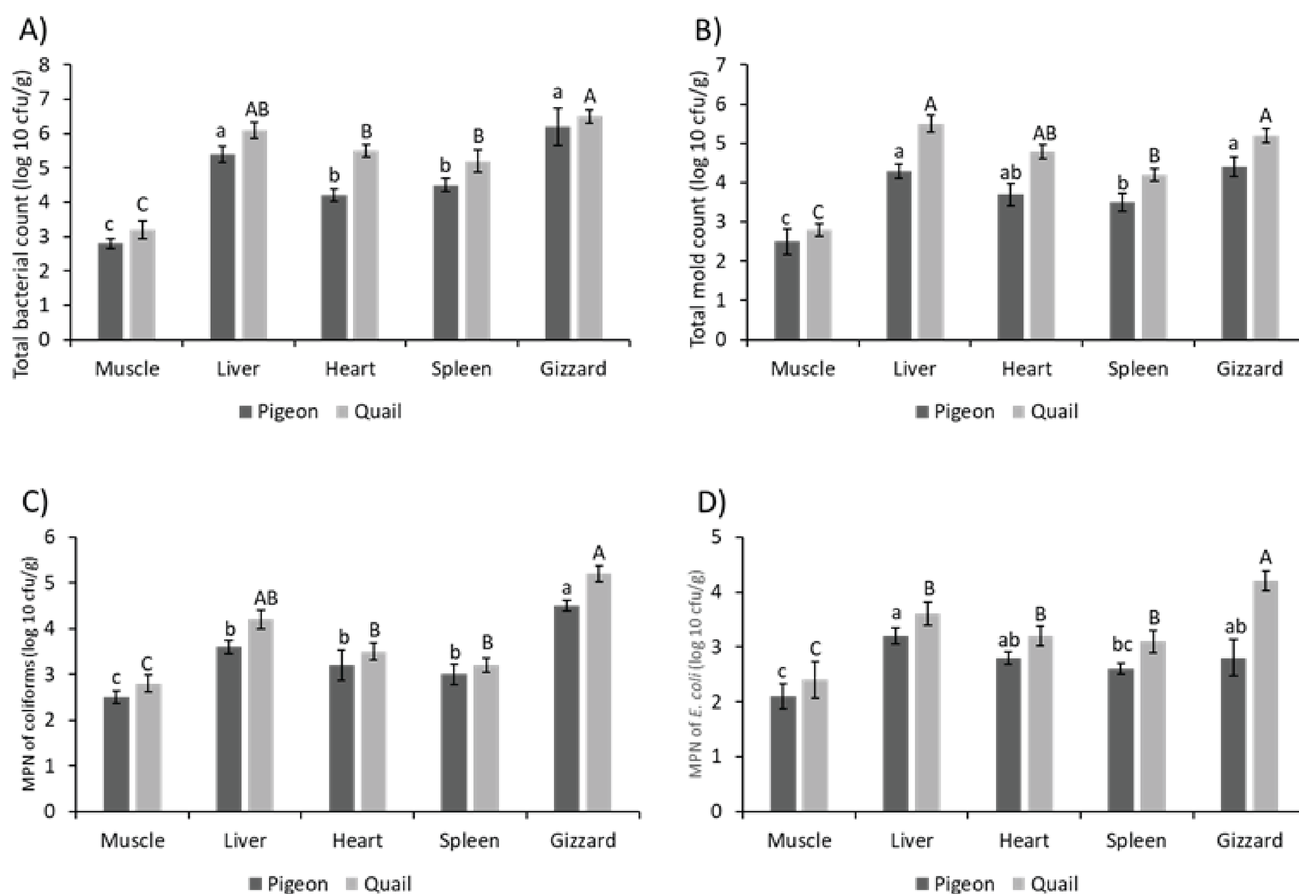

Figure 1: Indicators of the microbial quality of the examined squab and quail samples

A) Total bacterial count (TBC); B) Total mold count (TMC); C) Most probable number (MPN) of coliforms; D) Most probable number (MPN) of *E. coli*. Data represent means ± SD where n = 20 samples for each tissue. Columns carrying different letter a, b, c in case of squab, or A, B, C in case of quail are significantly different at *P*< 0.05. Microbial counts were changed into its log 10 cfu/g.

serotypes had notable multidrug resistance profiles. For example, the MAR index of *O26* ranged between 0.428 and 1, whereas that of *O127* was between 0.642 and 1 . The lowest MAR index was observed in *O114* at 0.357 (Table 2).

The antimicrobial resistance of the recovered *E. coli O26* isolates showed pan-resistance (100%) to AM, CN, CP, En, E, and P, 90% resistance to C, 80% to G, 70% to K, 60% to NA, 50% to N, 40% to OX, 30% to T, and 205 to STX. *O78* isolates showed 100% resistance to AM, G, K, NA, T, and P, 66.66% resistance to CN, C, CP, En, E, N, OX, and 33.33% resistance to SXT. *O127* isolates showed100% resistance to AM, CN, C, CP, En, K, NA, N, and P, 90% resistance to E, 80% resistance to G, 70% resistance to OX, 40% resistance to T, and 20% resistance to SXT. Isolates of *O111* were 100% resistant to AM, CN, CP, En, E, and P, but were sensitive to the other tested antimicrobials. The *O114* isolates showed 100% resistance to AM, CP, En, E, and P, while sensitive to the other tested antimicrobials. Isolates of *O119* were 100% resistant to AM, CP, En, K, NA, N, and P, but were sensitive to the other tested antimicrobials (Table 3).

Treatment of breast muscle with a combination of ASA and RMA at a concentration of 2% was the most effective in reducing TBC counts by 62.5%, TMC by 71.43%, MPN of coliforms by 82.14%, and MPN of *E. coli* by 91.67% (Table 4).

Discussion

Exotic meats, such as those of squab and quails, are becoming popular as an alternative source of meat in many countries around the world. In this study, the hygienic status of squab and quail meats and giblets was investigated. Total bacterial and coliform counts are regarded as acceptable indicators for hygienic measures and fecal contamination of foods (18). In Egypt, the upper allowable limit of TBC in poultry meat is 5 log 10 cfu/g and of coliforms is 2 log 10 cfu/g but must be free from mold spores (17). Results showed higher counts for the tested hygienic indicators, including TBC, TMC, MPN of coliforms, and MPN of *E. coli*. These high levels reflect the poor hygienic measures adopted during the preparation and processing of such meat sources. Similarly, Javadi and Safarmashaei (19) found the bacterial contamination of broiler meat sold in Iran had average values of ~ 5 log cfu/g for TBC and ~ 4 log cfu/g for coliforms, respectively. However, higher values (6.6 to 7.1 log 10 cfu/g for TBC) were found in poultry slaughtered in traditional shops in Morocco Amara *et al.* (20). It should be noted that, in Egypt, squab and quails are slaughtered and processed in conventional poultry slaughterhouses located near chicken and other birds. In many cases, the scalding tanks and water used in the wash process are the same. Therefore, there is a high risk of microbial cross-contamination between different birds processed at the same slaughterhouse (21).

STEC is a major foodborne pathogen that causes fatal complications, especially in vulnerable populations such as young children and the elderly. Between October 2002 and February 2003, a cluster of *E. coli O157:H7* hemorrhagic colitis was discovered in Canada.

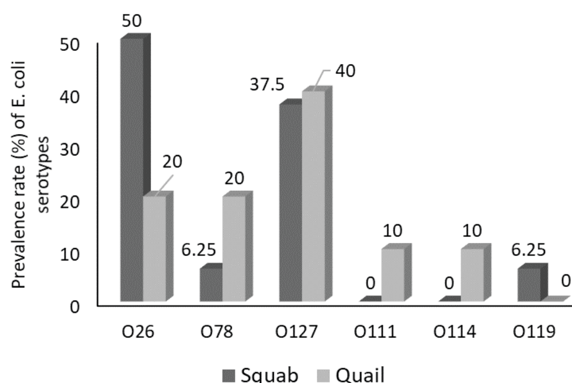

Figure 2: Prevalence (%) of different *E. coli* serotypes isolated from the examined squab and quail samples (n = 100/each) based on serodiagnosis

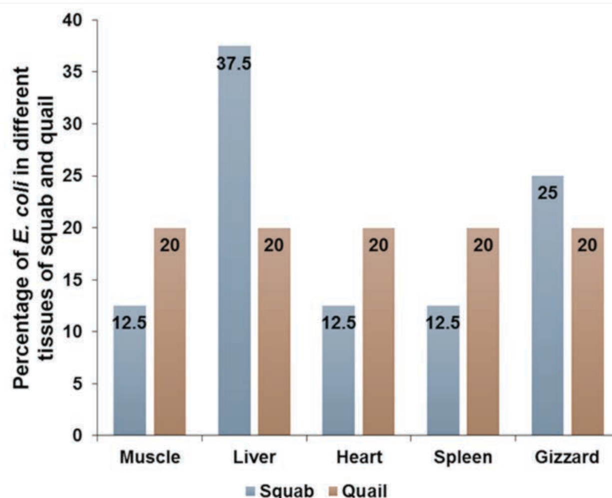

Figure 3: Prevalence (%) of *E. coli* in the examined tissue samples of squab and quails

Table 2: Characterization of the recovered *E. coli* serotypes in the present study

Species	Isolate number	Sero-type	Origin	*Shiga-toxin coding genes*			Antimicrobial resistance profiling	MAR index
				stx1	*stx2*	*eae*		
Squab (Prevalence rate = 16%)	1	O26	liver	+	+	+	AM, CN, C, CP, En, E, G, K, NA, N, OX, T, P, SXT	1
	2	O26	gizzard	-	-	-	AM, CN, C, CP, En, E, G, K, NA, N, OX, T, P	0.928
	3	O26	gizzard	-	-	-	AM, CN, C, CP, En, E, G, K, NA, N, OX, P	0.857
	4	O26	heart	+	-	+	AM, CN, C, CP, En, E, G, K, NA, N, P	0.785
	5	O26	spleen	+	-	+	AM, CN, C, CP, En, E, G, K, NA, P	0.714
	6	O26	muscle	-	-	-	AM, CN, C, CP, En, E, G, K, P	0.642
	7	O26	muscle	+	-	-	AM, CN, C, CP, En, E, G, P	0.571
	8	O26	liver	+	-	-	AM, CN, C, CP, En, E, P	0.5
	9	O78	liver	+	+	-	AM, G, K, NA, T, P	0.428
	10	O127	liver	+	+	+	AM, CN, C, CP, En, E, G, K, NA, N, OX, T, P, SXT	1
	11	O127	gizzard	-	-	-	AM, CN, C, CP, En, E, G, K, NA, N, OX, T, P	0.928
	12	O127	gizzard	+	+	-	AM, CN, C, CP, En, E, G, K, NA, N, OX, P	0.857
	13	O127	heart	-	-	-	AM, CN, C, CP, En, E, G, K, NA, N, P	0.785
	14	O127	spleen	-	-	-	AM, CN, C, CP, En, E, K, NA, N, OX, P	0.785
	15	O127	liver	+	+	+	AM, CN, C, CP, En, K, NA, N, P	0.642
	16	O119	liver	+	+	-	AM, CP, En, K, NA, N, P	0.5
Quail (Prevalence rate = 10%)	17	O26	muscle	-	-	-	AM, CN, CP, En, E, P	0.428
	18	O26	liver	+	-	+	AM, CN, C, CP, En, E, G, K, NA, N, OX, T, P	0.928
	19	O78	muscle	+	-	-	AM, CN, C, CP, En, E, G, K, NA, N, OX, T, P, SXT	1
	20	O78	heart	-	-	-	AM, CN, C, CP, En, E, G, K, NA, N, OX, T, P	0.928
	21	O127	heart	-	+	-	AM, CN, C, CP, En, E, G, K, NA, N, OX, T, P, SXT	1
	22	O127	gizzard	+	+	+	AM, CN, C, CP, En, E, G, K, NA, N, OX, T, P	0.928
	23	O127	gizzard	-	-	+	AM, CN, C, CP, En, E, G, K, NA, N, OX, P	0.857
	24	O127	spleen	-	-	-	AM, CN, C, CP, En, E, G, K, NA, N, P	0.785
	25	O111	spleen	-	-	-	AM, CN, CP, En, E, P	0.428
	26	O114	liver	+	+	-	AM, CP, En, E, P	0.357

Table 3: Antimicrobial resistance rates among the recovered *E. coli* serotypes in the present study

	O26		O78		O127		O111		O114		O119	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
AM	10	100	3	100	10	100	1	100	1	100	1	100
CN	10	100	2	66.66	10	100	1	100	0	0	0	0
C	9	90	2	66.66	10	100	0	0	0	0	0	0
CP	10	100	2	66.66	10	100	1	100	1	100	1	100
En	10	100	2	66.66	10	100	1	100	1	100	1	100
E	10	100	2	66.66	9	90	1	100	1	100	0	0
G	8	80	3	100	8	80	0	0	0	0	0	0
K	7	70	3	100	10	100	0	0	0	0	1	100
NA	6	60	3	100	10	100	0	0	0	0	1	100
N	5	50	2	66.66	10	100	0	0	0	0	1	100
OX	4	40	2	66.66	7	70	0	0	0	0	0	0
T	3	30	3	100	4	40	0	0	0	0	0	0
P	10	100	3	100	10	100	1	100	1	100	1	100
SXT	2	20	1	33.33	2	20	0	0	0	0	0	0

(No.) indicates the number of isolates in this serotype, (%) indicates the percent of the positive isolates to the total, number. AM: ampicillin, CN: cephalothin, C: chloramphenicol, CP: ciprofloxacin, En: enrofloxacin, E: erythromycin, G: gentamicin, K: kanamycin, NA: nalidixic acid, OX: oxacillin, T: oxytetracycline, P: penicillin, N: neomycin, SXT; trimethoprim/sulfamethoxazole

Table 4: Reduction rates of microbial counts of the quail's breast muscle by the use of RMA, ASA, or their mixture

	Control	RMA 1%	RMA2%	ASA 1%	ASA 2%	RMA + ASA
TBC	0	12.50	43.75	21.88	53.13	62.50
TMC	0	14.28	42.86	35.71	57.14	71.43
MPN coliforms	0	21.43	57.14	28.57	71.43	82.14
MPN *E. coli*	0	16.67	66.67	37.50	79.17	91.67

This outbreak was caused by the ingestion of unpasteurized Gouda cheese (22). Furthermore, *E. coli O104:H4* was responsible for an outbreak in Germany in May 2011 that infected over 3000 people and resulted in 50 deaths (23). In addition, 19 people in six states in the United States were infected with shiga toxin-producing *E. coli O121* (24). In the present study, *E. coli* was isolated from 16% and 10% of squab and quail meat samples, respectively. These results support a previous study showing the presence of *E. coli* in quail carcasses at different percentages (25). Similarly, Darwish et al. (2015) were able to isolate *E. coli* from 16% of duck meat and giblets. *E. coli* was also isolated from feral pigeon feces in India (26). In the present study, the STEC serotypes *O127, O114, O78, O26, O119*, and *O111* were identified. The serotypes *O26, O86, O114, O119*, and *O127* are among the human EPEC (27). Likely, *E. coli O26* was detected in rabbit carcasses and calves from Switzerland and Brazil, respectively (28, 29). The presence of these pathogenic STEC serotypes in squab and quail meats is concerning and warrants attention.

The high levels of *E. coli* in the liver and gizzard may be attributed to the potential migration of *E. coli* from the intestinal tract to the liver and gizzard. Unhygienic practices during slaughtering, de-feathering, and processing of slaughtered squab and quail may also have contributed to these contamination levels. Similar to these findings, *E. coli* was isolated from the liver, heart, and gizzards of processed poultry and from retail poultry in Trinidad (30, 31).

STEC strains are characterized by harboring a group of virulence genes such as *stx1, stx2*, and *eae*. *Stx1* and *stx2* are the principal toxin-coding genes in STEC, whereas *the eae* gene facilitates the adhesion of some STECS to the intestinal cells. Therefore, it is a critical determinant for the pathogenicity of *stx1* and *stx2* (32). Interestingly, *stx1*, *stx 2,* and *eae* genes were detected in the identified *E. coli* serotypes at variable rates. Likely, *stx1* and *stx2* genes were previously detected in STEC isolated from meat samples in Argentina (33), India (34) and meat served at hospitals in Egypt (35). Infection with STEC may result in serious complications such as hemolytic uremic syndrome, hemorrhagic colitis, and bloody diarrhea (36).

Antimicrobials are commonly used in poultry farms for disease treatment and prevention, as well as growth promoters. However, the abuse of antimicrobials have contributed to the development of multidrug-resistant pathogens (37). In this study, all STEC serotypes were resistant to multiple drugs. The obtained results go in agreement with those reported in several previous studies (4, 35, 38).

RMA is the active ingredient found in rosemary, whereas ASA is widely distributed in fruits, tomatoes, green leaves, and broccoli (39). Verghese *et al.* (40) found ASA to be effective against *E. coli* and *Klebsiella pneumonae* in broth. Similarly, ASA had antimicrobial effects against *Salmonella* in a cheese model (41). Both ASA and RMA were found effective against the growth of *Listeria monocytogenes* in soft cheese (11). Therefore, we used these acids to reduce microbial contamination of breast muscle. When combined at 2%, these acids were highly effective in reducing the microbial load in meat samples. The reduction rates for TBC, TMC, MPN of coliforms, and MPN of *E. coli* were 62.50%, 71.43%, 82.14%, and 91.67%. Similarly, the antimicrobial activities of ASA and RMA might be attributed to their prooxidation action against ROS generation in microbial cells leading to leakage of protein and sugar and subsequently microbial cell death Shivaprasad *et al.* (42).

Conclusions

These findings demonstrated unsatisfactory hygienic conditions during the preparation of squab and quail meats. In addition, meat and giblets from squab and quails should be

considered as a vehicle for multidrug-resistant STEC. ASA and RMA are promising antimicrobial candidates that can be used during the processing of poultry meat.

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This study was conducted according to the guidelines of Zagazig University, Egypt, and no living animals were used in this study.

All authors approved to participate in this research work and in the manuscript. All authors approved this manuscript to be published. The authors declare that they have no conflict of interest.

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