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Original Research Article

INCIDENCE AND CHARACTERIZATION OF ANTIMICROBIAL RESISTANCE OF *Escherichia coli* IN FAST FOOD WITH SPECIAL REFERENCE TO THE ANTIBACTERIAL EFFECTS OF CINNAMON AND OREGANO ESSENTIAL OILS AGAINST *E. coli* O157:H7 IN MINCED MEAT

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Abstract: This study aimed to investigate the incidence and phenotypic and genotypic resistance of *Escherichia coli* isolates recovered from fast food in Sharkia Governorate, Egypt, as well as to evaluate the cinnamon and oregano essential oils treatments for inoculated minced meat with *E. coli* serotype O157:H7. The highest incidence of *E. coli* was found to be 44% in smoked herring, followed by kofta (40%), shawarma (36%), sausage (32%) and fried shrimp (24%). *E. coli* isolates were serotyped into O157:H7 (12.5%) and O128:H2 (12.5%) from kofta samples, O125:H21(25%) from both of sausage and fried shrimp, O119:H6 (12.5%) and O86a:H3 (12.5%) from shawarma; and O158:H7 (25%) from smoked herring. Six isolates belonging to *E. coli* serotypes O157, O158, O119, O128, O125 and O86a showed maximum resistance (100%) to erythromycin, gentamicin, cefepime, streptomycin, ampicillin, chloramphenicol and tetracycline. Moreover, 4 *E. coli* strains belonging to O157, O128, O125 and O86a serotypes showed moderate resistance (66.7%) to ciprofloxacin, sulfamethoxazole trimethoprim. The resistance genes including *bla*TEM, *aad*A1 and *mph*A were detected with a percentage of 100% in the six investigated isolates phenotypically resistant to ampicillin, gentamicin and erythromycin, respectively. The average count of *E. coli* serotype O157:H7 after 0.5-hour of dipping in essential oils (EOs) from oregano 2% (3.52 ±0.16) showed a significant decrease in comparison with that of the control. The average counts of *E. coli* serotype O157:H7 and reduction percentages after one hour of dipping in EOs from oregano 2% (3.41± 0.99, 48.4%); and in EOs from cinnamon 2% (3.74 ±0.13, 43.4%) showed a significant decrease when compared with that of the control. Our study clarified that the average counts of *E. coli* O157:H7 after one hour of dipping in EOs from oregano 2% and cinnamon 2% were significantly reduced when compared with those counts after dipping in EOs from oregano 1% and cinnamon 1%, respectively. The present study confirmed that cinnamon 2% and oregano 2% had powerful antibacterial activities against *E. coli* O175: H7 recovered from ready-to-eat (RTE) meat products and prevent food poisoning.

Key words: *Escherichia coli*; incidence; resistance; essential oils; fast food

Introduction

Ready to eat (RTE), food such as meat and fish products, is considered a necessary source of valuable proteins, however it is implicated in foodborne outbreaks in humans. Some pathogenic bacteria including *Escherichia coli (E. coli)*, *Salmonella serovars*, *Listeria monocytogenes* and *Staphylococcus aureus* are recurrently recovered from RTE food from Africa (1). The dissemination of pathogenic *E. coli* in fast food is an indicator of fecal contamination

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and unhygienic conditions during the preparation of food (2).

Although, *E. coli* was inactivated by heat treatment of processed food, the cross contamination might occur post-processing from the equipment and the environment owing to persistence of the pathogen (3). Meat products could be easily contaminated with pathogens due to improper handling and preservation, resulting in food spoilage, loss of quality and public health hazards (4). *Escherichia coli* serotype O157 can induce hemorrhage in colon and hemolytic uremic syndrome in humans (5). Moreover, *E. coli* is recognized as a marker of faecal contamination of water and seafood (6). Recently, different serotypes of *E. coli* (O111, O26, O128, O125, and O119) were detected in fresh water fish in Egypt (7). Also, an incidence of shigatoxigenic serotypes of *E. coli* in RTE chicken sandwiches was investigated in Egypt (8).

An exposure of *E. coli* isolates to β-lactams has induced excess invention besides mutations in β-lactamases (9). So, resistant *E. coli* can contaminate carcasses during slaughter and processes of marketing resulting in dissemination of antimicrobial resistance. Multidrug-resistant strains in RTE food can be passed to humans through food, posing a health risk (10). In Egypt, the rise of antibiotic-resistant bacteria in food, particularly contaminated meat and its products, as well as their transmission to humans, is a major source of concern (11). The intestinal transport of antimicrobial-resistant *E. coli* in cattle for future consumption as food items will increase the incidence of antimicrobial-resistant *E. coli* infections in humans (12). The distribution of antimicrobial resistance (AMR) in *E. coli* and AMR genes are a major challenge for the treatment of human infections. ESBL-producing *E. coli*, in particular, are rising all over the world (13). The treatment of infections by ESBL-producing *E. coli* is fairly challenging owing to the phenotypic resistance to the most beta-lactam antibiotics (14). Therefore, it is very crucial to find out alternatives as antibacterial compounds to control MDR *E. coli* O157:H7 recovered from fast food.

Essential oils (Eos) that are generally recognized as safe (GRAS) have been utilized as food preservatives due to their strong antibacterial effects (15). The principal antibacterial components of essential oils are eugenol in cloves, cinnamic aldehyde and eugenol in cinnamon, carvacrol and thymol in oregano. Enteric bacteria such as *E. coli* serotype O157:H7 and *Salmonella* Typhimurium were found to be inhibited by thymol and carvacrol. In Egypt, thyme oil is identified as a natural preservative in minced meat against vancomycin resistant (VRE) and *E. coli* O157:H7 contamination (16). Synergism of EOs united with antimicrobials could avoid AMR transmission (17). In Egypt, there is a little literature available on the antimicrobial effects of EOs against MDR *E. coli* serotype O157:H7 recovered from RTE meat products. Therefore, the current study was designed to investigate the incidence and phenotypic and genotypic resistance of *E. coli* strains isolated from RTE meat and fish products in Sharkia Governorate, Egypt as well as to assess the effect of treatments with cinnamon and oregano EOs for minced meat artificially inoculated with *E. coli* O157:H7.

Materials and Methods

Collection of RTE food samples

One hundred and twenty-five samples from RTE meat and fish products including kofta, shawarma, sausage, fried shrimp and smoked herring (25, each) were purchased from different restaurants and supermarkets from different cities at Sharkia Governorate, Egypt during the period from October, 2018 to April, 2019. The collected samples were packed into sterile boxes. The RTE meat and fish samples were aseptically transferred in a cooler box to the Department of Food Control, Faculty of Veterinary Medicine, Zagazig University and bacteriologically analyzed within four hours of purchase.

Isolation and biochemical identification of Escherichia coli isolates

Twenty-five grams of each RTE food sample were deposited in sterile Whirl-Pak bags (Thomas Scientific Inc, USA) containing a sterile buffered peptone water (225 mL), (Oxoid, CM0509, UK). Briefly, the instruction sheet of Whirl-Pak sample bag included tearing off bag top along perforation, opening bag by use of pull tabs on each side, putting buffered peptone broth into the bag, placing RTE sample in the bag, and finally pulling the ends of the wire to close the bag. All bags were sealed then subjected to homogenization by

using a stomacher (Merc, Darmastadt, Germany) for 2 minutes; and finally, the food samples were pre-enriched via incubation at 37°C for 24 hours (18). The pre-enriched food samples (1mL) were subjected to inoculation into 9 mL of MacConkey broth (Oxoid, CM0005, UK) then subjected to incubation at 37°C for 18 hours for enrichment as previously explained (19). Loopfuls of MacConkey enriched broth were subjected to direct streaking on Eosin Methylene Blue (EMB, Oxoid, CM0069, UK) agar plates, then incubated at 37°C for 18-24 hours as described before (20). The metallic green colonies of presumptive *E. coli* isolates on EMB agar plates were biochemically confirmed using IMViC tests (Indole, methyl Red, Voges-Proskauer and citrate utilization), H_2S production and urease test and triple sugar iron (TSI) agar media according to the standard techniques (21).

To isolate and identify *E. coli* serotype O157:H7, other loopfuls from MacConkey enriched broth were streaked on the Sorbitol MacConkey (SMAC) agar (Oxoid Ltd, Hampshire, UK) provided with cefixime (0.5 mg/L) and potassium tellurite (2.5 mg/L) (CT-SMAC) and subjected to incubation at 37°C for 24 hours (22). After incubation, the colorless non -sorbitol fermenting colonies were picked up and further streaked onto sorbitol MacConkey agar plates to obtain typical colorless *E. coli* serotype O157. For further purification, *E. coli* serotype O157 isolates were streaked on Tryptone Soy Agar (Merc, Germany). For subsequent examination, pure cultures of O157 and non-O157 *E. coli* isolates were kept at -20°C in sterile brain heart infusion broth (CM0225, Oxoid, Hampshire, UK) containing glycerol 20% (Sigma-Aldrich).

Serotyping of Escherichia coli isolates

At the Animal Health Research Institute in Doki, Egypt, biochemically verified *E. coli* isolates (n=8) were basically selected to be representative for various RTE food sources and serotyped using quick diagnostic *E. coli* antisera sets (DENKA SEIKEN Co., Japan) (23).

Antimicrobial susceptibility testing

E. coli isolates representing all identified serotypes (n=6) were selected for testing their antimicrobial susceptibility against 9 antimicrobials onto plates of Mueller-Hinton agar (Oxoid, England) by utilizing the disk diffusion technique (24). The panels of antimicrobials (Thermoscientific, Oxoid, USA) comprising erythromycin (E,15μg), gentamicin (CN,10μg) ciprofloxacin (CIP, 5μg) sulfamethoxazole + trimethoprim (SXT,25 μg), cefepime (FEP,30 μg), streptomycin (S, 10 μg) ampicillin (AMP,10 μg), chloramphenicol (C, 30 μg) and tetracycline (TE, 30 μg) were used in this study. The diameters of inhibition zones were measured and interpreted in accordance with the Clinical Laboratory Standard Institute's criteria (25). *E. coli* isolates revealing resistance to three or more classes of antibiotics were recognized multidrug-resistant (10).

Genotypic detection of resistant Escherichia coli

Genomic DNA from *E. coli* isolates was extracted using QIAamp DNA mini kit (Takara Kit, Catalogue no. 51304, Japan) according to the manufacturer's guidelines. According to the available primers at Biotechnology Unit, Animal Health Research Institute, Doki, Egypt; the primer sequences were used to detect beta-lactamase, aminoglycoside and macrolide resistance through amplification of *bla*TEM, *aad*A1 and *mph*A resistance genes in serotyped *E. coli* strains (Table 1) as previously described (26,27,28). For each PCR reaction, PCR reaction volume of 25 μL comprising 12.5 μL of the Emerald Amp GT PCR master mix (2x premix, Takara, Japan); 1 μL of forward primer (20 pmol); 1 μL of reverse primer (20 pmol); 6 μL of bacterial DNA and 4.5 μL of PCR grade water were placed in T3 Thermal cycler (Biometra, USA).

The PCR cycling conditions of *bla*TEM gene in *E. coli* isolates included primary denaturation at temperature 94°C for 5 minutes followed by 35 cycles of secondary denaturation at a temperature of 94°C for 30 seconds, annealing at 54°C for 40 seconds and an extension step at 72°C for 45 seconds, followed by a final extension step at 72°C for 10 minutes (29). For amplification of *mph*A gene, PCR conditions included primary denaturation (94°C, 5 minutes), followed by 35 cycles of secondary denaturation (94°C, 30 seconds), annealing (54°C, 40 seconds) and an extension step (72°C, 45 seconds); and a final extension at 72°C for 10 minutes (27). While, the PCR conditions of *aad*A1 gene included primary denaturation at

94°C for 5 minutes, 35 cycles of secondary denaturation at 94°C for 30 seconds, annealing at 54°C for 40 seconds, an extension step at 72°C for 45 seconds; and a final extension at 72°C for 10 minutes (30). The PCR products of each resistance gene were run on 1.5% of agarose gel stained with 0.5 µg/mL of ethidium bromide with the use of gel pilot 100 bp ladder (QIAGEN, USA). Positive and negative controls were included in each PCR reaction. The positive control *E. coli* strains showing resistance for *bla*TEM, *mph*A and *aad*A1 genes were recovered from kofta, milk and sausage, respectively. The negative control of *E. coli* isolate was obtained from fish. Those positive and negative isolates were kindly obtained from Biotechnology Unit at Animal Health Research Institute, Doki, Egypt. All PCR assays were repeated to confirm the results.

Preparation of essential oils

The pure cinnamon and oregano oils were purchased from National Research Center, Doki, Egypt. Each of the essential oil was diluted to 1% and 2% by mixing with propylene glycol in sterilized distilled water as an inert substance, which acts as dissolving agent for oil without any harmful effects on the food (31). The EOs were kept at 4 °C in sealed vials of dark glass until they were used.

Preparation of Escherichia coli O157:H7 inoculum

A loopful of *E. coli* serotype O157: H7, previously thawed and frozen, was streaked on Sorbitol MacConkey agar plates and then subjected to incubation at a temperature of 37 °C / 24 hours. After that, the strain's colony was transferred to the brain heart infusion (BHI) broth, then incubated at 37 °C for 24 hours. Before the experiment, a consecutive transfer on BHI broth was conducted for revitalization of the bacterial culture; and reaching bacterial stationary phase; then followed by a last transfer to the broth of BHI. After cultivating *E. coli* serotype O157:H7 in the broth of BHI, the bacterial cells were centrifuged at 3000 rpm /15 minutes. After removing the supernatant, the bacterial pellet was recovered and mixed with 10 ml of sterile peptone water (0.1%). The suspension was diluted to 1.0 x 10^6 CFU/ mL in 0.1% peptone water. The count of *E. coli* serotype O157:H7 in the suspension of bacteria was achieved by serial dilutions of the suspension and then streaking of each dilution onto Sorbitol MacConkey agar (32).

Minced meat inoculation with bacterial inoculum and treatment with essential oils

A total of 300 grams from minced meat was aseptically divided into 5 subgroups including control subgroup and other four subgroups for treatment with EOs (60 gm each). Each subgroup was sub-divided into 2 categories for testing after 0.5 and one hour of EOs treatment (30 gm each)

These 30 grams were distributed into 3 polyethylene bags containing 10 gm of minced meat for the test triplicate.

Each of the control and treatment subgroups were inoculated with the prepared *E. coli* serotype O157:H7 strain (1.0 x 10^6 CFU/ mL in peptone water. Each subgroup was treated as follow: the 1st subgroup was treated with cinnamon oil 1%; the 2nd subgroup was treated with cinnamon oil 2%, the 3rd subgroup was treated with oregano oil 1%, the 4th subgroup was treated with oregano oil 2%, and the 5th subgroup was stored without treatment as a positive control. Afterwards, all control and treated subgroups were kept in refrigerator at 4±1°C to determine the antimicrobial effects of EOs on the inoculated pathogens, at different periods of cold storage (0.5 and one hour) as previously described (32).

Table 1: Primer sequences utilized for amplification of antimicrobial resistance genes in *E. coli* serotypes recovered from fast food

Antimicrobial resistance gene	Primer	Sequence (5' to 3')	PCR Products (bp)	Reference
β-lactamase (*bla*TEM)	BlaTEM	ATCAGCAATAAACCAGC CCCCGAAGAACGTTTTC	516	(26)
Aminoglycoside(*aad*A1)	aadA1	TATCAGAGGTAGTTGGCGTCAT GTTCCATAGCGTTAAGGTTTCATT	484	(27)
Macrolide (*mph*A)	MphA	GTGAGGAGGAGCTTCGCGAG TGCCGCAGGACTCGGAGGTC	403	(28)

Microbiological counting

Each minced meat sample (10 gm previously weighed) was subjected to aseptic homogenization with 0.1% concentration of sterile peptone water (90 mL) for 2 minutes at 3000 rpm using a sterile homogenizer. The original dilution (1 mL) was transported to a sterile 9 mL of peptone water solution to be diluted in sub-sequential manner by tenfold serial dilution to cover the expected range of sample contamination (7). A volume of 0.1 mL from each prepared dilution was evenly streaked on the plates of Sorbitol MacConkey agar and Trypticase Soya agar to enable the recovery of damaged cells (33). The plates were subjected for an aerobic incubation for 24 hours at 37 °C.

Statistical analysis

The Chi-square analysis was run to investigate the variations in the incidence of *E. coli* among different meat and fish products. SPSS version 24.0 was used to carry out the analysis (IBM. Corp., Armonk, NY) as previously described (34). The data regarding the treatment of inoculated minced meat with essential oils was statistically evaluated using the analysis of variance (ANOVA) test, and the Duncan Multiple Range test was used to compare the means using SPSS version 14 as formerly reported (35). Results were recorded as mean ± standard errors (SE), and calculations were also made for minimum (Min) and maximum (Max). A statistical significance is deemed when the P value was < 0.05.

Results and discussion

Incidence of Escherichia coli in fast food

Escherichia coli is a fecal contamination indicator in food and its existence is frequently associated with poor hygiene, inadequate foodstuff handling or cross contamination and inadequate food preservation (36). Moreover, *E. coli* enters through the gut during slaughter, and readily contaminate animal carcasses due to the contamination of raw or undercooked meat with *E. coli* if they are not properly handled (28). Our result revealed that the total incidence of *E. coli* was 35.2% (44 out of 125) in RTE food samples. However, higher incidence of *E. coli* was detected in raw beef (78%) and in RTE beef products (53%) were recorded previously (11). This study revealed higher incidence of *E. coli* in fast food due to unhygienic environments and inappropriate handling (37).

Concerning isolation percentages of *E. coli* from RTE meat, the incidence was 40% (10 out of 25) in kofta, 36% (9 out of 25) in shawarma and 32% (8 out of 25) in sausage (Figure 1A). Chi square results revealed that there was no significant association between infection of *E. coli* and the source of sample $\chi 2(4)=2.43$, $P= 0.66$. In Kaliobia Governorate, nearly similar finding was detected in kofta (32%) and in sausage (40%) (38). In Egypt, nealy similar incidence of 34% was detected in luncheon (8). Another study in Egypt declared that *E. coli* was found in 20% of minced beef samples, 30% in sausage, 40% in kofta and 10% in Shawarma (39). The current study showed higher isolation percentage of *E. coli* from shawarma when compared with the results of El-Dosoky *et al.* (40) who recorded 8% from shawarma samples. In Menofia Governorate, Egypt, percentages of *E. coli* isolation were 25% from kofta and 37.5% from sausage (41). However, *E. coli* was recovered with higher percentage of 66.6% from each of kofta and sausage in Egypt (11). Although, *E. coli* is readily inactivated above 55°C, the post cooking cross contamination may occur as a result of contact of raw ingredient with cooked meat (42).The high prevalence of *E. coli* in the investigated RTE meat products refers to inadequate processing or post processing contaminations (most from the workers, dirty instruments, machinery and other contact surfaces), or from raw ingredients before the processing that drive their contamination from human contact, the polluted water, soil and manure (43). On the contrary, lower prevalence of *E. coli* was 13.3% in minced meat and 10% in roasted beef (44). Therefore, higher isolation percentages of *E. coli* from fast food in this study could be accounted for using conventional methods of slaughtering and evisceration (45).

The distribution percentage of *E. coli* isolates in the examined fast food samples in relation to the total recovered *E. coli* isolates was 20% (9 out of 44) from shawarma, 18% (8/44 from sausage), 23% (10/44) from kofta, 25% (11/44) from smoked herring and 14% (6/44) from fried shrimp (Figure 1B).

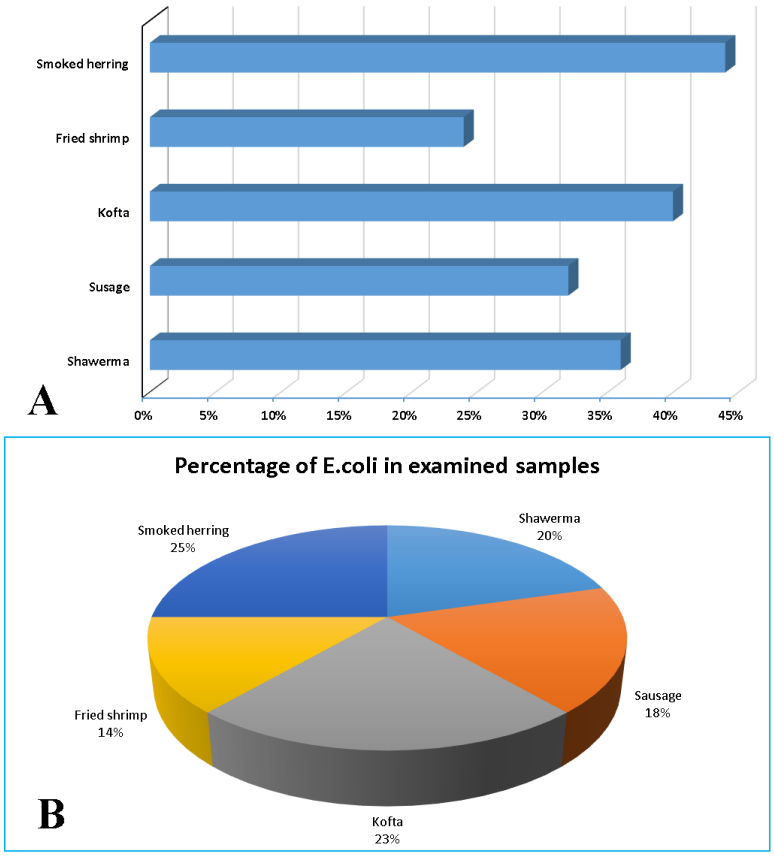

Figure 1: Pie chart and incidence of *E. coli* in RTE food. A. Incidence of *E. coli* in RTE meat and fish. B. The percentage of recovered *E. coli* isolates in the examined samples in relation to the total number of the recovered isolates

Regarding isolation percentages of *E. coli* from RTE fish, the highest incidence was found to be 44% (11 out of 25) in smoked herring followed by lower isolation rate (24%, 6 out of 25) as illustrated in Figure (1A). In Egypt, lower isolation percentage (36%) was detected in smoked herring (46). The present study recovered a higher percentage of *E. coli* isolation from smoked herring, which was contrary to the finding of Gupta *et al.* (47), who reported lower incidence (12.96%) from RTE fish products. In Egypt, Hassanien *et al.* (48) isolated *E. coli* with different percentages of 27% in smoked herring and 33% in breaded shrimp. However, another study revealed lower incidence (10%) of *E. coli* from oyster, while the peeled shrimp was free from *E. coli* infection (49). Lower isolation rate (2.8%) was detected in stuffed mussel in Turkey (50). The present study exhibited higher incidence of *E. coli* in smoked herring which might be owing to the mishandling during production and distribution process as previously explained (51). The differences in isolation percentages of *E. coli* in the present study comparing with previous studies might be due to the variations in production practices, handling from manufacturers to the consumers and the efficacy of hygienic procedures applied during the production (52).

Escherichia coli serotypes in RTE food

The variety of *E. coli* serotypes, the infective dosage, and the microorganism's capability to persist in food, and in the host gut have an influence on the pathogenicity of STEC (53,54). Therefore, the serotyping of *E. coli* isolates in RTE foodstuffs and environment is very important to recognize the epidemiology of pathogenic *E. coli* (55). In this study, eight *E. coli* isolates were basically selected to be representative for RTE food sources as the following :2 isolates/ kofta, 2/shawarma, 2/smoked herring, 1/fried shrimp and 1 /sausage. The isolates were serologically identified into *E. coli* serotype O157:H7 with a percentage of 12.5% (1 out of 8 *E. coli* isolates) and the serotype O128:H2 (12.5%, 1/8) from kofta. *E. coli* O125:H21(25%, 2/8) were serotyped from both of sausage and fried shrimp. *E. coli* serotypes O119:H6 (12.5%, 1/8) and O86a:H3 (12.5%, 1/8) were identified from shawarma samples. *E. coli* O158:H7 (25%, 2/8)

were serotyped from smoked herring. Our findings pointed out that all recovered *E. coli* serotypes from RTE food represented a hazard to the global health. Previously, the most common *E. coli* serotype was O26: H11 (31.6%) which was isolated from shawarma and grilled chicken, and followed by five O91: H21 (26.3%) from hawawshi and chicken shawarma in Sharkia Governorate, Egypt (37). EPEC strains were obtained from RTE meat products representing O119:H6 from shawarma, O55:H7 isolates from burger and luncheon, O113:H4 from shawarma and luncheon in Sharkia Governorate, Egypt (8). Also, EHEC strains representing *E. coli* O157:H7 were 5% in cooked shawarma in Aswan Governorate, Egypt (39).

Phenotypic and genotypic resistance of Escherichia coli from RTE food

The antibiotic resistance of *E. coli* is recovered globally in fast food such as meat and meat products (56). From the results mentioned in Table 2, the six investigated isolates belonging to *E. coli* serotypes O157, O158, O119, O128, O125and O86a were completely resistant to erythromycin, gentamicin, cefepime, ampicillin, streptomycin, tetracycline and chloramphenicol. Moreover, *E. coli* strains belonging to O157, O128, O125 and O86a serotypes showed moderate resistance (66.7%, 4 out of 6 isolates) to ciprofloxacin, sulfamethoxazole trimethoprim. *E. coli* serotypes O158 and O119 exhibited lower intermediate resistance (33.3%, 2 out of 6) to ciprofloxacin. Also, *E. coli* serotype O119 showed the lowest resistance (16.7%, 1out of 6) to sulfamethoxazole+trimethoprim (Table 2). Our study was in agreement with the finding of Younis *et al.* (57), where a maximum resistance (100%) was found to streptomycin followed by penicillin (80%). In Ghana, forty-five *E. coli* strains derived from RTE meat showed a resistance percentage (≥ 50%) to trimethoprim, amoxicillin and tetracycline, but their sensitivity percentages were 87.1% for azithromycin, 81.3% for chloramphenicol, 74.8% for imipenem, 72.0% for gentamicin and 69.5% for ciprofloxacin (58).Ceftriaxone has a high intermediate resistance for *E. coli* isolates with a percentage of 33% in Ghana (59). Also, *E. coli* strains from retail meat in the United States were resistant to tetracycline and gentamicin with the percentages of 50.3 %and 18.6%, respectively (60). On the contrary, *E. coli* isolates from fast food showed lower resistance rates 29% for amoxycillin/clavulanic acid,14% for gentamicin and 29% for ciprofloxacin (50).

This study clarified that RTE meat could be a source of antibiotic-resistant *E. coli* posing a threat of resistant bacteria to enter into food chain, ecosystem and humans. In Egypt, the ampicillin, gentamicin and erythromycin are common antibiotics used in the veterinary medicine and livestock production. Therefore, the high prevalence of antimicrobial resistance in *E. coli* serotypes of RTE food in this study might be associated to the unrestricted use of these antibiotics in food animals and animal farms as previously described (10).

Livestock is a predictable major reservoir for numerous *E. coli* pathotypes, which is epidemiologically linked to various meat-related foodborne illness and outbreaks (61). As a result of its presence in the gastrointestinal tracts of animals and its capability to gain antimicrobial resistance, *E. coli* has been elected as a monitor microorganism in the antimicrobial resistance surveillance programs all over the world (62). Concerning the recognition of antimicrobial resistance genes in *E. coli* isolates of RTE meat and fish products, the six serotypes conferring resistance to ampicillin carried *bla*TEM genes (100%) with a molecular weight of 516 bp (Figure 2A). Also, six resistant *E. coli* strains to gentamicin harbored *aadA1* gene (100%) with PCR product of 484 bp (Figure 2B). Moreover, all isolates of *E. coli* conferring resistance to erythromycin possessed *mph*A resistance genes (100%) with a molecular weight of 403 bp (Figure 2C).

The *bla*TEM gene (encoded for β-lactamase-resistance) was detected in all the examined *E. coli* isolates by 100%, as mentioned before by Altalhi *et al.* (63), who showed that all examined multiresistant *E. coli* isolates, for ampicillin, nalidixic acid, streptomycin, chloramphenicol and gentamicin, harbored *bla*TEM gene (100%). Moreover, Hemeg (64) detected *bla*TEM gene in *E. coli* strains from all resistant strains to ampicillin and amoxicillin-clavulanic acid, meanwhile, lower incidence rates were reported by Vuthy *et al.* (65), where they detected *bla*TEM gene in 62% of the amoxicillin resistant *E. coli* isolates. Nearly identical finding was cited by Hussein *et al.* (37), where *E. coli* strains derived from RTE meat sandwiches, and conferring resistance to amoxicillin-clavulanic acid and erythromycin had similar percentage (100%) of *bla*TEM and *mph*A resistance genes, respectively.

Table 2: Antimicrobial susceptibility test of *E. coli* serotypes (n=6) recovered from fast food against different antimicrobial agents (ND: not detected)

Antimicrobial agents (µg/Disc)	Resistant (R)			Intermediate (I)			Sensitive (S)		
	E. coli serotypes	No	%	*E. coli* serotypes	No	%	*E. coli* serotypes	No	%
Erythromycin (15)	O157, O158, O119, O128, O125, O86a	6	100%	ND	0	0	ND	0	0
Gentamicin (10)	O157, O158, O119, O128, O125, O86a	6	100%	ND	0	0	ND	0	0
Ciprofloxacin (5)	O157, O128, O125, O86a	4	66.7	O158, O119	2	33.3	ND	0	0
Sulfamethoxazole - Trimethoprim (25)	O157, O128, O125, O86a	4	66.7	O119	1	16.7	O158	1	16.7
Cefepime (30)	O157, O158, O119, O128, O125, O86a	6	100%	ND	0	0	ND	0	0
Streptomycin (10)	O157, O158, O119, O128, O125, O86a	6	100%	ND	0	0	ND	0	0
Ampicillin (10)	O157, O158, O119, O128, O125, O86a	6	100%	ND	0	0	ND	0	0
Chloramphenicol (30)	O157, O158, O119, O128, O125, O86a	6	100%	ND	0	0	ND	0	0
Tetracycline (30)	O157, O158, O119, O128, O125, O86a	6	100%	ND	0	0	ND	0	0

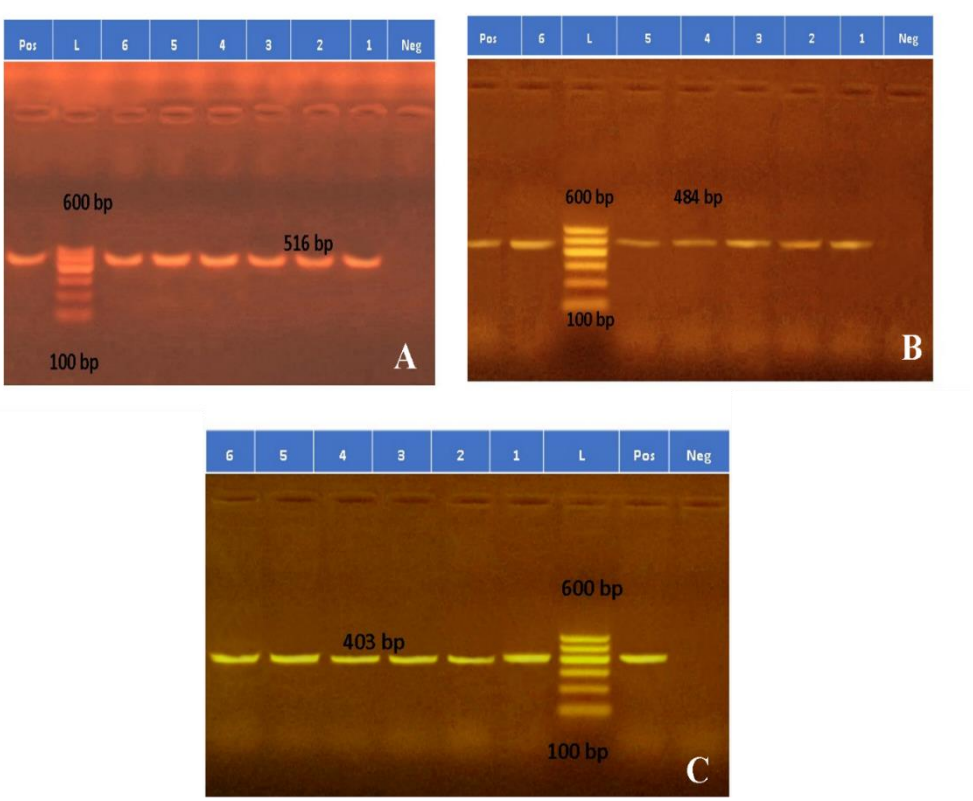

Figure 2: Agarose gel electrophoresis of ß-lactam, aminoglycoside and macrolide resistant genes in MDR *E. coli* isolates from RTE food. A- PCR products of *bla*TEM gene among 6 *E. coli* isolates showing phenotypic resistance to ampicillin. Lane (L): 100 bp DNA ladder, Lanes (1:6): positive isolates at 516 bp, Lane negative control (-ve C): negative control *E. coli* isolate of fish source, Lane positive control (+ve C): positive control *E. coli* isolate for *bla*TEM from kofta source. B- PCR products of *aad*A1 gene among *E. coli* isolates showing phenotypic resistance to gentamicin. Lane (L): 100 bp DNA ladder ; Lanes (1:6): positive isolates at 484 bp; Lane negative control (-ve C): negative control *E. coli* isolate; Lane positive control (+ve C): positive control *E. coli* isolate for *aad*A1gene of sausage source. C- PCR products of *mph*A gene among 6 *E. coli* isolates showing phenotypic resistance to erythromycin. Lane (L): 100 bp DNA ladder; Lanes (1:6): positive isolates at 403 bp; Lane negative control (-ve Control): negative control *E. coli* isolate; Lane positive control (+ve Control): positive control *E. coli* isolate for *mph*A gene of milk source

Table 3: The mean counts of *E. coli* O157:H7 in the inoculated minced meat after treatment with cinnamon and oregano essential oils

Time of dipping and reduction percentage	Control	Cinnamon 1%	Cinnamon 2%	Oregano 1%	Oregano 2%
1/2 hour	6.61±0.073[a]*	4.49± 0.087[b]	4.31± 0.086[b]	4.31± 0.054[b]	3.52 ±0.16[c]
Reduction percentage		33.4%	34.8%	34.8%	46.7%
1 hour	6.61±0.073[a]	4.31 ±0.072[b]	3.74 ±0.13[c]	4.27± 0.072[b]	3.41 ±0.99[c]
Reduction percentage		34.7%	43.4%	35%	48.4%

Means from the same raw having various superscripts displayed a significant difference ($P< 0.05$) based on Duncan's multiple comparisons. Reduction percentage = (Mean count of control– mean of treated samples) ÷ mean of control ×100, *: Mean counts ± S.E.

According to the current findings, RTE foodstuffs such as meat and fish are possible vehicles for antibiotic resistant *E. coli* among other potentially harmful foodborne pathogens, and constitute hazards to the community health. In a study in Kaliobia Governorate, Egypt, PCR results declared that *bla*TEM and *aad*a1 genes were amplified in two studied *E. coli* strains with PCR products of 516 bp and 484 bp, respectively (66). Our study clarified that the six *E. coli* serotypes harbored resistance genes to aminoglycosides (*aad*A1), β- lactamase (*bla*TEM) and macrolides (*mph*A). The discovery of antibiotic resistance genes in RTE meat and fish isolates poses a public health risk because the horizontal transfer of genes via contaminated fast food could hasten more disseminations for resistance genes (67).

Essential oils treatment for inoculated minced meat with E. coli O157:H7

Table (3) shows the effect of the two essential oils chosen in this study to decrease the counts of *E. coli* serotype O157: H7 in artificially inoculated minced meat samples after half and one hour of dipping. The average counts of *E. coli* serotype O157:H7 and reduction percentages after half hour of dipping in oregano 2% (3.52±0.16, 46.7%); and from cinnamon 2% (4.31±0.086, 34.8% %) showed a significant decrease when compared with that of the control one (Table, 3). It was clear that the mean counts of *E. coli* O157:H7 after half hour incubation in oregano 2% was significantly reduced comparing with those average microbial counts after dipping in cinnamon 2% (Table, 3). Also, the average counts of *E. coli* O157:H7 and reduction percentages after one hour of dipping in oregano 2% (3.41± 0.99, 48.4%); and in cinnamon 2% (3.74 ±0.13, 43.3% %) displayed a significant reduction when compared with that of the control one (Table, 3). Our study clarified that the mean counts of *E. coli* O157:H7 after one hour of dipping in oregano 2% and cinnamon 2% showed significant reductions in comparison with those average microbial counts with oregano 1% and cinnamon 1%, respectively (Table, 3).

The counts of *E. coli* O157:H7 in the ground beef patties did not decrease during storage of beef patties; while the cooking of those beef patties after dipping in trans-cinnamaldehyde (TC) showed a significant reduction in the counts of *E. coli* O157:H7 by 5.0 log CFU/g, compared to the control one cooked at the same temperature (68). The *E. coli* O157:H7 counts have dropped by 4.10 and 5.10 log CFU/g and *Salmonella* Typhimurum by 2.7 and 2.8 log CFU/g as a result of cinnamon oil treatment with emulsions of cinnamon bark and leaf oil, respectively (69). Similarly, the dipping of cinnamaldehyde 1 and 2% for the chicken breast chunks with marinade (CBM) samples at 4◦C and at only day 1 displayed a significant reduction in *E. coli* O157:H7 by1.3 and 1.6 log10 CFU/g, respectively (70). Cinnamon essential oil has been shown to be beneficial in preserving the quality and prolonging the shelf life of meat and fish products as a natural antibacterial (71). Cinnamon essential oil's antibacterial actions against various bacteria have also been established due to

its engagement with bacterial cell membranes, which leads to a decline in the enzymatic activities and the biosynthesis of cell wall (72, 73). Concerning oregano EO, similar activities for concentration >0.05 % were found by Lambert *et al.* (74) against pathogenic *E. coli* serotypes. The current study clarified that EOs from each of cinnamon 2% and oregano 2% had powerful antibacterial activities against *E. coli* O175:H7 recovered from RTE meat products and therefore protecting humans from food poisoning.

Conclusion

The highest prevalence rates of *E. coli* in RTE meat and fish products reflect inadequate processing or post processing contaminations or from raw ingredients before the processing and mishandling during production. Moreover, detection of *bla*TEM, *aad*A1 and *mph*A antibiotic resistance genes in *E. coli* strains from RTE meat and fish origins represents a public health hazard due to the horizontal gene transfer through contaminated RTE food. Finally, EOs from each of cinnamon 2% and oregano 2% had powerful antibacterial activities against *E. coli* O175: H7 recovered from RTE meat products, and thereby they are considered new strategies used to prevent food poisoning outbreaks.

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