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# Detection of Multi-Drug Resistant Food-borne Bacteria in Ready-to-Eat Meat Products in Luxor City, Egypt

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## Abstract

A total of five *Escherichia coli* and eight *Salmonella* isolates were recovered from ready-to-eat meat samples obtained from different street vendors in Luxor city, Egypt. Bacterial isolates were assayed for antimicrobial susceptibility, its virulence and antimicrobial resistance genes. The total number recovered positive *Salmonella* spp and *E. coli* were 8 (6.66%) and 5 (4.16%) respectively. All *E. coli* isolates were exhibited resistance against streptomycin and cephalothin. While all *Salmonella* isolates were resistant to nalidixic acid. PCR screening for virulence genes showed that 2 (40%) of the *E. coli* (O111:H4) serovar were positive for *stx*1, *stx*2, and *eae*A. While *Salmonella enteritidis*, *typhimurium*, and *virchow* hold *inv*A, *hil*A and *stn* genes with percentage of 37.5, 25 and 12.5% respectively. The identified tetracycline resistance gene for *E. coli* isolates were *tet*B (60%), *tet*C (20%) and *tet*D (20%). The *β*-lactamase resistance gene $bla_{CTX}$ was identified in 50% of *Salmonella* isolates represented by *S. enteritidis*, *S. typhimurium* and *S. Virchow*. The $bla_{CMY}$ genes were detected in *S. typhimurium* and *S. infantis* (37.5%). These results highlighted the role of ready-to-eat meat as a potential source for multidrug-resistant strains of *E. coli* and *Salmonella*. The current results indicate the need for applying hygienic practices in food outlets - especially in street vendors - to reduce the incidence of food-borne bacteria and to prevent future food-borne outbreaks in the studied area.

Keywords: Antibiotics, *Escherichia coli*, Meat products, *Salmonella spp*., Virulence genes.

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## Introduction

Currently, increasing the antimicrobial resistance is a community health concern, and the spread of antimicrobial resistance are multipart complications that motivated by plentiful interrelated aspects, such as the misapplication of antibiotics (WHO, 2001). The relationship between severe use of antimicrobial agents and development of resistant bacteria is well documented for foodborne pathogens (Swartz, 2002). Bacterial foodborne pathogens are the most causative agents for human intestinal disease, especially in many countries. Approximately 1.8 million people died due to food-borne diarrheal infections each year in developing countries (Akbar and Anal, 2015**;** Nyabundi et al., 2017). Thus, enhanced research labors and investigation agendas are required from administration agencies with a great consideration and awareness from the food manufacturing and industries (Díaz-Sánchez et al. 2012). Street foods are contaminated with bacteria and other microbes, making them unsafe for consumers' health. In Egypt, street contaminated supplied food especially meat products may represent a risk due to the inadequate quality (Elamary et al., 2018; Noor, 2016). Such using of raw ingredients, insufficient workers hygiene, holding for long period, lead to contamination of food with pathogenic microorganisms (Gundogan et al., 2005). *Escherichia coli* and *Salmonella spp.* are Gram-negative short rods-bacilli bacteria, belong to the family Enterobacteriaceae. It successfully colonizes the gastrointestinal tract of mammals (Hammoudi and Aggad, 2008). Recently, *E. coli* has become recognized as a serious foodborne pathogen that associated with diseases outbreaks including diarrhea, hemorrhagic colitis and the life-threatening hemolytic-uremic syndrome in humans (Hussein, 2007). Poultry meat is one of the frequent vehicles of salmonellosis as infectious diseases and is a major concern of public health (Elamary et al., 2018; Salem et al., 2017). Consumption of contaminated food by *salmonella* causes diarrhea, mild fever, nausea and abdominal pain with the symptoms developing in 12-72 hr. In life-threatening cases, it can also lead to death (Abubakar et al., 2007). Consumption of ready-to-eat products is thought to be the major cause of the *Salmonella* outbreaks (Thai et al., 2012). *Salmonella* was found responsible for outbreaks of food-borne infections in Ghana (Andoh et al. 2017). Three different types of antigens were detected as adhering properties of somatic O, flagellar H, and capsular Vi- antigens and used to differentiate between more than 2500 serologically distinct types of *Salmonella***.** (Madajczak and Szych, 2010). Numerous virulence factors have been associated with the family Enterobacteriaceae and they clarified its ability to cause disease such as toxins, verotoxin (shiga-like toxin), attaching and effecting mechanisms (*eae*A) (Pass et al., 2000). The most common ready-to-eat meat products sold by street vendors in Egypt are Burger, Kofta, Kibda, and Shawirma which are usually sold in the form of sandwiches. Therefore, this study was conducted to evaluate the bacterial contamination of such street supplied meat products in Luxor city, Egypt. And to highlight the antimicrobial susceptibility of *Escherichia coli* and *Salmonella* isolates from ready to eat meat products with further molecular analysis of its virulence and antibiotic- resistant genes.

## Materials and Methods

*I. Sample collection*:

One hundred twenty food samples (sandwiches) were randomly collected

from the different street vendors in Luxor city. The samples in two different times were grouped into four categories: (Shawirma, Kofta, Burgers, and Liver/Kibda). All samples were collected aseptically and placed in sterile containers, stored at 4ºC, then transferred to the laboratory for bacterial isolation.

*I.1. Isolation of Salmonella spp. and Escherichia coli*:

The isolation of *Salmonella* and *E. coli* were performed according to Snyder and Atlas (2006). Briefly, 25 grams of each sample were transferred into a stomacher bag with 225 ml of buffer peptone water (Sigma-Aldrich® 70179), the samples were incubated at 37ºC ± 1ºC / 18 ± 2 h. For *Salmonella* isolation, serial dilution was made up and 0.1ml of each dilution was spread on Rappaport-Vassiliadis Soy Peptone (Oxoid® CM0866) Broth and incubated at 41.5ºC ± 1 ºC / 24 hr. The samples were plated on a selective medium such as XLD agar (HiMedia® Laboratories, Mumbai, India) for 18-24 hours at 37°C. To detect *E. coli*, a loop-full of the incubated broth culture was then streaked onto eosin methylene blue agar (BioWorld, USA 30620025-1) plates as a selective medium then incubated at 37ºC for 24 h. Suspected colonies of *E. coli* and *Salmonella sp*. were picked up and identified by classical biochemical methods (Gram staining, citrate, indole, urease, triple sugar and lysine). *E. coli* isolates that were mainly identified by biochemical tests were then serologically identified according to Hammoudi and Aggad, 2008 by using rapid diagnosis *E. coli* antisera sets (Denka Seiken Co., Japan) for diagnosis of enteropathogenic types (Mirzaie et al., 2010) as follows:

***Set 1: O- antisera:***

Polyvalent antisera 1: O1, O26, O86a, O111, O119, O127a and O128.

Polyvalent antisera 2: O44, O55, O125, O126, O146 and O166.

Polyvalent antisera 3: O18, O114, O142, O151, O157 and O158.

Polyvalent antisera 4: O2, O6, O27, O78, O148, O159 and O168.

Polyvalent antisera 5: O20, O25, O63, O153 and O167.

Polyvalent antisera 6: O8, O15, O115 and O169.

Polyvalent antisera 7: O28ac, O112ac, O124, O136 and O144.

Polyvalent antisera 8: O29, O143, O152 and O164.

***Set 2: H- sera:***

H2, H4, H6, H7, H11, H18 and H21.

*Salmonella* isolates were primarily identified by biochemical tests, then subjected to serological identification that was carried out according to the White-Kauffmann-Le Minor scheme (Guibourdenche et al. 2010) using *Salmonella* antiserum **(**Denka Sei ken Co., Japan).

*I. 2. Detection of virulence and antibiotic resistant genes of isolates:*

Molecular characterization of the recovered *E. coli* and *Salmonella spp* were carried out by PCR, except the *tet* genes that were carried out by multiplex PCR. The encoding enterotoxins and antibiotic resistant genes (six for *E. coli* and five for *Salmonella*) were performed using twenty-two primers sets including forward and reverse. The extraction of DNA was carried according to QIAamp DNA mini kit instructions. All primer sequences and corresponding references are listed in Table (1).

**Table 1:** Primers sequences, target genes, amplicon sizes and cycling conditions.[a]

| Target gene | Sequence | Amplified segment (bp) | Primary denaturation | Amplification (35 cycles) | | | | References |
|---|---|---|---|---|---|---|---|---|
| | | | | Secondary denaturation | Annealing | Extension | Final Extension | |
| **Genes used for *E. coli* isolates** | | | | | | | | |
| ***stx*1** | 5′ACACTGGATGATC TCAGTGG ′3<br>5′CTGAATCCCCCTC CATTATG ′3 | 614 | 95°C / 3 min. | 95°C / 20 sec. | 58°C / 40 sec. | 72°C / 90 sec. | 72°C / 5 min. | Dhanashree and Mallya, 2008 |
| ***stx2*** | 5′CCATGACAACGG ACAGCAGTT ′3<br>5′CCTGTCAACTGAG CAGCACTTTG ′3 | 779 | 95°C / 3 min. | 95°C / 20 sec. | 58°C / 40 sec. | 72°C / 90 sec. | 72°C / 5 min. | |
| ***eae*A** | 5′GTGGCGAATACT GGCGAGACT ′3<br>5′CCCCATTCTTTTT CACCGTCG ′3 | 890 | 95°C / 3 min. | 95°C / 20 sec. | 58°C / 40 sec. | 72°C / 90 sec. | 72°C / 5 min. | Mazaheri et al., 2014 |
| ***tet*B** | 5′TTGGTTAGGGGCA AGTTTTG ′3<br>5′GTAATGGGCCAA TAACACCG ′3 | 695 | 94°C / 1 min. | 60°C / 1 min. | 95°C / 3 min. | 72°C / 2 min. | 72°C / 7 min. | Ng et al., 2001 |
| ***tet*C** | 5′CTTGAGAGCCTTC AACCCAG ′3<br>5′ATGGTCGTCATCT ACCTGCC ′3 | 418 | 94°C / 1 min. | 60°C / 1 min. | 95°C / 3 min. | 72°C / 2 min. | 72°C / 7 min. | |
| ***tet*D** | 5′AAACCATTACGG CATTCTGC ′3<br>5′AAACCATTACGG CATTCTGC ′3 | 787 | 94°C / 1 min. | 60°C / 1 min. | 95°C / 3 min. | 72°C / 2 min. | 72°C / 7 min. | |
| **Genes used for *Salmonella* isolates** | | | | | | | | |
| ***inv*A** | 5′GTGAAATTATCGCC ACGTTCGGGCA ′3<br>5′TCATCGCACCGTCA AAGGAACC ′3 | 284 | 94°C / 5 min. | 94°C / 30 sec. | 55°C / 30 sec. | 72°C / 90 sec. | 72°C / 7 min. | Shanmugasamy et al., 2011 |
| ***stn*** | 5′CTTTGGTCGTAAA ATAAGGCG ′3<br>5′TGCCCAAAGCAG AGAGATTC ′3 | 260 | 94°C / 5 min. | 94°C / 30 sec. | 55°C / 30 sec. | 72°C / 90 sec. | 72°C / 7 min. | Makino et al., 1999 |
| ***hil*A** | 5′CTGCCGCAGTGTT AAGGATA ′3<br>5′CTGTCGCCTTAAT CGCATGT ′3 | 297 | 94°C / 5 min. | 94°C / 30 sec. | 55°C / 30 sec. | 72°C / 90 sec. | 72°C / 7 min. | Guo et al., 2000 |
| $bla_{CTX}$ | 5′CGCTTTGCGATGT GCAG ′3<br>5′ACCGCGATATGCT TGGT ′3 | 550 | 94°C / 5 min. | 94°C / 30 sec. | 60°C / 45 sec. | 72°C / 45 sec. | 72°C / 10 min. | Ahmed et al., 2009 |
| $bla_{CMY}$ | 5′GACGCCTCTTTCT CCACA ′3<br>5′TGGAACGAAGGC TACGTA ′3 | 1007 | 94°C / 5 min. | 94°C / 30 sec. | 60°C / 45 sec. | 72°C / 45 sec. | 72°C / 10 min. | |

[a] The specific amplified sequences for each primers were (Metabion, Germany).

*I.2.1. DNA amplification for the selected virulence and antibiotic resistance genes of Salmonella:*

The amplification was performed on a Thermal Cycler (Master cycler, Eppendorf, Hamburg, Germany). The reaction mixes invariably consisted of 5 µl of the bacterial lysate, 5 µl of 10x assay buffer for Taq polymerase containing 1.5 mM $MgCl_2$, 2 µl of 10mM dNTP mix and 1.25 U of Taq DNA polymerase. Then 1µl of different primers specific for *Salmonella* virulence genes (*inv*A, *hil*A and *stn*) each of forward

and reverse primer (10 pmol) were added as previously described (Table 1). Distilled water was added to bring the final volume to 50µL. For antibiotic resistance genes, PCR amplifications were performed in a total volume of 50µL reactions containing 25µL DreamTaq Green PCR Master Mix (Thermo Scientific, St. Leon Roth, Germany), 2µL of each forward and reverse primer (*β*-lactams- encoding genes; *bla*$_{CTX}$ and/or *bla*$_{CMY}$) (10 pmol), 20µL of sterile water and 1µL of DNA-mixture.

*I.2.2. Amplification reaction of E. coli:*

The amplification was performed on a Thermal Cycler (Master cycler, Eppendorf, Hamburg, Germany). PCR assays were carried out in a 50µl volume containing 1 ml of nucleic acid template prepared by using reference EHE isolates (approximately 30 ng of DNA), 10 mM Tris-HCl (pH 8.4), 10 mM KCl, 3 mM $MgCl_2$; 2 mM concentrations of each primer (shiga toxins encoded by *stx*1 and *stx*2 genes, intimin surface protein encoded by *eae*A gene), 0.2 mM concentrations of each 2´-deoxynucleoside 5´-triphosphate, and 4 U of AmpliTaq DNA polymerase (Perkin-Elmer).

*I.2.3. Multiplex PCR detection of tet genes*:

All isolates of tetracycline-resistant *E. coli* strains were assessed for carriage of the tetracycline resistance genes *tet*B, *tet*C and *tet*D. The method was optimized using reference *E. coli* strains with known *tet* genes as follows: a small amount of biomass from a bacterial colony was added to a sterile thin-walled reaction tube containing Taq Master Mix, primers, $MgCl_2$, and distilled water. The multiplex PCR reaction **mix** (total 50µl) included primers for *tet*B, *tet*C (0.25 µM each) and *tet*D (3 µM) and 1.5 mM $MgCl_2$.

Finally, 5 µl of each amplicon was electrophoresed in 1.5 % agarose gel (Sigma –USA, stained with ethidium bromide, visualized and captured on UV transilluminator. A 100 bp DNA ladder was used as a marker for PCR products.

*II. Antimicrobial Susceptibility test:*

The antibiograms for all the recovered isolates were determined as described earlier by using disk diffusion test (Rodríguez-Tudela et al., 2003). The susceptibility of *E. coli* and *Salmonella* isolates was tested for 14 antibiotics from *B*-lactams group (Bioanalyze®). The used antibiotics were Nalidixic acid; Oxacillin; Neomycin; Streptomycin; Penicillin; Erythromycin; Cephalothin; Kanamycin; sulfamethoxazole; Ampicillin; Chloramphenicol; Oxytetracycline; gentamycin and Ciprofloxacin. Interpretation of the results was performed according to clinical and laboratory standard institute guidelines (CLSI, 2007) to determine if the isolate is resistant, intermediate, or susceptible to the tested antibiotics.

**Results**

*I. Prevalnce of E. coli and Salmonella spp. in ready-to-eat meat samples:*

The incidence of *E. coli* and *Salmonella* isolated from the examined samples were illustrated in Table (2). Out of 120 ready-to-eat meat (RTE) samples, the total number recovered positive *Salmonella* spp and *E. coli* infections were 8 (6.66%) and 5 (4.16%) respectively. From all the infected samples, Kofta samples showed the highest prevalence of *Salmonella* isolates indicated as 4 (13.3%) compared to the number of Kofta samples examined. While, Kofta and Burger samples showed an incidence of *E. coli* as (6.6%) for both samples separately. Interestingly, *E. coli* was absent in all the tested liver samples (Kibda).

**Table 2:** The incidence of *E. coli* and *Salmonella* isolated from ready-to-eat meat samples.

| Samples | No | Positive isolates [a] | |
|---|---|---|---|
| | | ***Salmonella* sp.** | ***E. coli*** |
| **Kofta** | 30 | 4 (13.3%) | 2 (6.6%) |
| **Shawirma** | 30 | 1 (3.3%) | 1 (3.3%) |
| **Burger** | 30 | 1 (3.3%) | 2 (6.6%) |
| **Kibda** | 30 | 2 (6.6%) | 0 (0%) |
| **Total no.** | 20 | 8 (6.66%) | 5 (4.16%) |

[a] Number and percentage of positive samples contaminated with *Salmonella* spp. and/or *E. coli*.

*II. Serotype and virulence genes of isolates:*

The serotypes of *E. coli* from the examined Kofta samples were Enteropathogenic *E. coli* (EPEC, O125:H21, 20%) and Enterohemorrhagic *E. coli* (EHEC, O26:H11, 20%), while in Shawirma, EPEC (O55:H7**,** 20%). Moreover, in Burger EHEC, (O111:H4**,** 40%) was identified. *E. coli* isolates were screened for virulence genes (*stx*1, *stx*2 and *eae*A). In three *E. coli* serovars (O111:H4, O26:H11 and O125:H21) the gene encoding for *stx*1 was detected. *stx2* gene was detected in (O111:H4 and O55: H7) serovars. *eae*A gene was detected in 3 serovars O111:H4, O55: H7 and O26: H11 (Table 3). The serological examination of identified *Salmonella* isolates showed that two isolates were *S. typhimurium from* Kibda represented (25%) from the total isolates, three isolates were *S. enteritidis* (37.5%) two isolates from Kofta and one isolate from the Burger. Also, *S. virchow* and *S. malade* from Kofta, and *S. infantis* isolated from shawarma all of them represented (12.5%). *Salmonella* isolates were screened for virulence genes encoded *stn*, *inv*A, and *hil*A. The results showed that all serovars were positive for all the examined virulence genes except for *S. malade* (isolated from Kofta) that lacks *stn* gene, one isolate of *S. enteritidis* and *S. infantis* (isolated from Burger and Shawirma respectively) lack *hilA* gene (Table 3).

**Table 3:** Serotype and virulence genes of *E. coli* and *Salmonella* isolates obtained from ready-to-eat meat samples.

| Isolate characterization | | Serotype/ group | No. of isolates | % | Sample | Virulence genes profile | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | ***stx*1** | ***stx*2** | ***eae*A** |
| ***Escherichia coli*** | EPEC | O55: H7 | 1 | 20 | Shawirma | - | + | + |
| | EHEC | O111: H4 | 2 | 40 | Burger | + | + | + |
| | EHEC | O26: H11 | 1 | 20 | Kofta | + | - | + |
| | EPEC | O125: H21 | 1 | 20 | Kofta | + | - | - |
| | | | | | | ***inv*A** | ***hil*A** | ***stn*** |
| *Salmonella* spp. | *typhimurium* | B | 2 | 25 | Kibda | + | + | + |
| | *enteritidis* | D1 | 3 | 37.5 | 2Kofta/1 Burger | + | +/- | + |
| | *virchow* | C1 | 1 | 12.5 | Kofta | + | + | + |
| | *infantis* | C1 | 1 | 12.5 | Shawirma | + | - | + |
| | *molade* | C2 | 1 | 12.5 | Kofta | + | + | - |

(+) Virulence genes detected in current study, (-) not detected.

*III. Detection of antibiotic-resistant genes by multiplex PCR:*

*E. coli* isolates were screened for three antibiotic resistant genes (*tet*B, *tet*C, and *tet*D). The results showed that three *E. coli* serovars out of five (60%) were positive for (*tet*B); 2 serovars of EHEC: O111:H4 and 1 serovar O26:H11. While (40%) from total *E. coli* isolates were positive for both (*tet*C) and (*tet*D); 1 serovar of EHEC O26:H11 (20%) and 1 serovar of EPEC: O125:H21 (20%) respectively (Table 4). *Salmonella* isolates were screened for antibiotic-resistant genes ($bla_{CMY}$ and $bla_{CTX}$). The results showed that two serovars of *S. enteritidis*, one *S. typhimurium* and *S. virchow* hold $bla_{CTX}$ genes (represented as 50% from total *Salmonella* isolates), while two serovars (*S. typhimurium* and one *S. infantis* hold $bla_{CMY}$ genes (37.5 %) (Table 5).

*IV. Antimicrobial susceptibility test*:

The antibiotic resistance of *E. coli and Salmonella* isolates against the tested 14 antibiotics with different disc potency was determined by disc diffusion method and the results showed that all *E. coli* isolates were resistant to Streptomycin (10mg/disc) and Cephalothin (30mg/disc). A high percentage (80%) exhibited resistance to Penicillin (10mg/disc), Oxacillin (10mg/disc) and Erythromycin (15mg/disc). Nalidixic acid and Sulfamethoxazole resistance were observed in almost 60%. Furthermore, 60% of isolates were sensitive Kanamycin (30mg/disc) while 80% sensitive for Gentamicin (10mg/disc) (Table 4). All *Salmonella* isolates were resistant to Nalidixic acid (30mg/disc), while 88% were resistant to Oxacillin (10mg/disc) and Neomycin (30mg/disc). Streptomycin, Penicillin (10mg/disc) and Erythromycin (15mg/disc) resistances were observed in almost 75%. Cephalothin (30mg/disc) resistance was 62.5%. Kanamycin (30mg/disc), Sulfamethoxazole (25mg/disc) and Ampicillin (10mg/disc) resistance were 50%. Furthermore, 50% of *Salmonella* isolates were sensitive to Oxytetracycline (30mg/disc), Gentamicin (10mg/disc) and Ciprofloxacin (15mg/disc) (Table 5).

**Table 4:** Resistance genes detected in antibiotic resistant *E. coli* isolates.

| **Phenotype of Antibiotic (%) [a]** | | | | **Genes detected by PCR** | |
|---|---|---|---|---|---|
| **Antimicrobial agent (mg/disc)** | **R** | **M** | **S** | **Resistance genes** | **No. of positive serotype[b] / %** |
| **S-10** | **100** | **0** | **0** | ***tet*B** | **3:** (2) O111:H4; O26:H11 / (60%) |
| **CN-30** | **100** | **0** | **0** | ***tet*C** | **1:** O26:H11 / (20 %) |
| | | | | ***tet*D** | **1**: O125:H21 / (20%) |
| **P-10** | **80** | **20** | **0** | | |
| **OX-10** | **80** | **0** | **20** | | |
| **E-15** | **80** | **0** | **20** | | |
| **NA-30** | **60** | **40** | **0** | | |
| **SXT-25** | **60** | **20** | **20** | | |
| **AM-10** | **40** | **60** | **0** | | |
| **T-30** | **40** | **40** | **20** | | |
| **CP-15** | **40** | **40** | **20** | | |
| **N-30** | **40** | **20** | **40** | | |
| **C-30** | **40** | **0** | **60** | | |
| **K-30** | **20** | **20** | **60** | | |
| **G-10** | **20** | **0** | **80** | | |

Antimicrobial susceptibility of *E. coli* isolates against *β*-lactams group. [a]: R: Resistant; M: Moderate; S: Sensitive; NA*:* Nalidixic acid; OX: Oxacillin; N: Neomycin; S: Streptomycin; P: Penicillin; E: Erythromycin;

CN: Cephalothin; K: Kanamycin; SXT: sulfamethoxazole; AM: Ampicillin; C: Chloramphenicol; T: Oxytetracycline; G: gentamycin; CP: Ciprofloxacin. 10, 15, 25 and 30: antibiotic concentration (mg/disc). [b]: Number of positive serovars for each detected gene and its percentage compared to the total *E. coli* isolates.

**Table 5:** Resistance genes detected in antibiotic resistant *Salmonella* isolates.

| Phenotype of Antibiotic (%) [a] | | | | Genes detected by PCR | |
|---|---|---|---|---|---|
| **Antimicrobial agent (mg/disc)** | **R** | **M** | **S** | **Resistance genes** | **No. of positive serotype[b] / %** |
| **NA-30** | **100** | **0** | **0** | ***bla*CTX** | **4:** (2) *S. enteritidis*, (1) *S. typhimurium*, (1) *S. Virchow* / (50%) |
| **OX-10** | **88** | **12** | **0** | ***bla*CMY** | **3**: (2) *S. typhimurium*, (1) *S. infantis* / (37.5%) |
| **N-30** | **88** | **12** | **0** | | |
| **S-10** | **75** | **25** | **0** | | |
| **P-10** | **75** | **12.5** | **12.5** | | |
| **E-15** | **75** | **0** | **25** | | |
| **CN-30** | **62.5** | **12.5** | **25** | | |
| **K-30** | **50** | **37.5** | **12.5** | | |
| **SXT-25** | **50** | **25** | **25** | | |
| **AM-10** | **50** | **0** | **0** | | |
| **C-30** | **37.5** | **25** | **37.5** | | |
| **T-30** | **37.5** | **12.5** | **50** | | |
| **G-10** | **25** | **25** | **50** | | |
| **CP-15** | **12.5** | **12.5** | **50** | | |

Antimicrobial susceptibility of *Salmonella* isolates against *β*-lactams group. [a]: R: Resistant; M: Moderate; S: Sensitive; NA*:* Nalidixic acid; OX: Oxacillin; N: Neomycin; S: Streptomycin; P: Penicillin; E: Erythromycin; CN: Cephalothin; K: Kanamycin; SXT: sulfamethoxazole; AM: Ampicillin; C: Chloramphenicol; T: Oxytetracycline; G: gentamycin; CP: Ciprofloxacin. 10, 15, 25 and 30: antibiotic concentration (mg/disc). [b]: Number of positive serovars for each detected gene and its percentage compared to the total *Salmonella* isolates.

## Discussion

The presence of *Escherichia coli* in ready-to-eat meat indicates non- hygienic conditions for food preparations (Nyabundi et al., 2017). *E. coli* as Enteropathogenic in meat products provide an evidence of contamination of fecal or water origin. In this study (Table 2)., the percentage of *E. coli* in Kofta samples were (6.6%) which lower than that obtained by Ahmed and Shimamoto (2014) Regarding to percentage of *E. coli* in the Burger (6.6 %), this result was lower than incidences recorded by Kalantari et al. (2012) (16.4%). On the other hand, the present study for Shawirma samples were (3.3%) that was different from those reported by Vazgecer et al. (2004) (31%). While these results in agreement with the results obtained by Hemeg (2018) who reported the prevalence of *E. coli* O111, O55 and O26 in food samples. Interestingly, the absence of *E. coli* in this study was from liver samples (Kibda) which disagree with Zaghloul et al. (2014), who isolate *E. coli* from 32% of examined liver sandwiches. The absence of *E. coli* in heat-treated food as RTE liver sandwiches indicates an adequate cooking hygiene without post-processing contamination. On the other hand, the presence of these microbes in liver sandwiches can be linked to improper handling and processing, use of contaminated raw materials or the use of dirty processing utensils like knife and trays (Salma et al., 2015). On the other hand, *Salmonella* is responsible for disease in humans. It has been the leading cause of many outbreaks and infections around the world and is considered as one of the major causes of human gastroenteritis worldwide (Rasschaert et al., 2005)**.** The current results of Kofta showed the highest percentage (13.3%) of *Salmonella* spp. that

were nearly like that recorded by Zaghloul et al. (2014). Moreover, the incidence of *Salmonella* isolated from Kibda (6.6%) were like the results from Shaltout et al. (2013). On the other hand, only one *Salmonella* isolate was detected in Shawirma and Burger samples that represented (3.3%) and these results was higher than those obtained by Al-Mutairi (2011). While, In Lebanon, Harakeh et al. (2005) reported prevalence's of *Salmonella* 7.4 % in meat pies and Shawirma.

Shiga toxin-producing *E. coli* (STEC) may cause food-borne infections leading to dangerous diseases in humans. The outcomes of STEC infections may range from asymptomatic carriage to uncomplicated diarrhea to the severe symptoms of hemorrhagic colitis (HC) and hemolytic-uremic syndrome (HUS). Owing to their human pathogenicity, some STEC strains are also designated as enterohemorrhagic *E. coli* (EHEC) (Levine, 1987). EHEC strains comprise a subgroup of STEC and are characterized by certain serotypes, which are frequently associated with outbreaks and severe clinical illness (Bugarel et al., 2010). In recent years, cumulative evidence from numerous countries has indicated that up to 30 to 60% of human EHEC infections are caused by non-O157 EHEC (EFSA, 2013). Remarkably, the current study regarding the serotypes of *E. coli* from the examined Kofta and burger samples were Enterohemorrhagic *E. coli* (EHEC, O26:H11, 20%) and (O111:H4, 40%) respectively. Some *E. coli* strains were isolated from different ready-to-eat meat products by Ahmed and Shimamoto (2014) who isolated *E. coli* O157:H7 (1.6 %) from fresh beef samples, and this results in contrast with current study which detected O125: H21(20%) and EHEC O26: H11 (20%), while in Shawirma O55: H7 (20%). Moreover, in Burger O111: H4 (40%) were identified in this study (Table 3). (Al-Mutairi, 2011) reported that out of seven isolates of *E. coli* recovered from Kofta sample, six isolates (85.7%) were serologically typed as Enteropathogenic *E. coli*, also from five isolates of *E. coli* from Shawirma sample, three isolates were Enteropathogenic with an incidence of 60%. Out of the 15 strains, 12 *E. coli* serovars isolated from meat product samples belonged to the serovars: O166; O78; O126; O55; O26; O20; O25: K4; O114; O125: K70 and O116. Similar *E. coli* serotypes were isolated from meat products were previously recorded by Hessain et al. (2015); Zaghloul et al. (2014); Ahmed and Shimamoto (2014). Regarding the *Salmonella* isolates in this study, *S. enteritidis* were (37.5%), S. *typhimurium* (25%), *S. virchow*, *S. infantis* and *S. malade* were (12.5%) (Table 3). This result was higher than the results obtained by Shaltout et al. (2013) who isolated *S. enteritidis* (4%) and *S. typhimurium* (4%) from cooked meat. The variation in the results may be due to the differences in the production process, handling from producers to consumers and the effectiveness of hygienic measures applied during production. Most of Enterobacteriaceae human diseases due to strains that produce either Shiga toxin 1 (*stx*1) and/or Shiga toxin 2 (*stx*2) (Brudzinski and Harrison, 1998). The pathogenicity of *E. coli* strains was due to genes expression for Shiga toxins (*stx* genes) and intimin; a virulence factor that is an outer membrane protein (*eae*A) (Beutin et al., 2004). The predominance of *stx*2 either alone or in combination with *stx*1 considered to be the most important virulence factor (Kahali et al., 2004**).** In current study, detection of virulence genes clarified the presence of *stx*1, *stx*2 and *eae*A genes in O111:H4 isolates, while the presence of *stx*2, *eae*A genes in O55: H7

isolates. Also, O26: H11 isolates were positive for both *stx*1 and *eae*A and isolates of O125:H21 contain only *stx*1 gene (Table 3). Meanwhile, these results nearly in agreement with Mohammed et al. (2014) who detected *stx* and *eae*A genes from the isolates of O55:H7. Also, (Díaz-Sánchez et al. (2012) who found that *E. coli* serovars O166:O28 proved to have the two genes (*stx*1, *stx*2), and O119 serovars were positive for *stx*1, *stx*2 genes. Also, O86 isolates had the only *stx*2 gene. Most of the genes required for *Salmonella* virulence are clustered within five *Salmonella* pathogenicity islands (SPI-1–SPI-5), which contributes to its success as an intracellular pathogen (Marcus et al., 2000). Some virulence genes such as the chromosomally encoded *stn* (*Salmonella* enterotoxin gene) are not located on SPIs. These virulence genes act via maintenance of *Salmonella* membrane composition and integrity to play an important role in the virulence of *Salmonella* (Nakano et al., 2012; 2015)**.** The PCR results of *salmonella* isolates for the presence of *inv*A, *hil*A and *stn* in *S. enteritidis, S. typhimurium* and *S. virchow* while the presence of *inv*A and *hil*A in *S. malade*. Also, *inv*A and *stn* were positive in other isolates of *S. enteritidis* and *S. infantis* and this result was illustrated in Table (3). In current study, where the *stn* gene was prevalent among *Salmonella* isolates as evidenced by PCR (90%) and this result nearly in agreement with Ammar et al. (2016) who reported a wide distribution of this gene (100%) had also been recorded earlier among *Salmonella* isolates. The *inv*A gene has been widely used in studies for the detection of *Salmonella* in food samples (Chacón et al., 2010). In a study on the prevalence of virulence genes in non-typhoidal *Salmonella* isolated from humans, animals and food products in developing countries, Bangera et al. (2018) observed the high prevalence of strains carrying *inv*A gene and this percentage in agreement with current study (100%). The *hil*A gene encodes an OmpR/ToxR transcriptional regulator that activates the expression of invasion genes and has an important role in *Salmonella* pathogenicity (Crâciunaș et al., 2012). In a study conducted by Cardona-Castro et al. (2002), the *hil*A gene was detected in all the tested *Salmonella* isolates. In our results, hilA gene has been detected in all isolated *Salmonella* serovars except *S. infantis* (isolated from shawarma) and one isolate of *S. enteritidis* (isolated from Burger) (Table 3). Interestingly, routine PCR test confirmed the presence of all detected virulence genes (*stn, inv*A, and *hil*A) in most of the isolated *Salmonella*. On the other hand, Bradford (2001) who indicated that up to 90% of ampicillin resistance in *E. coli* is due to the production of inactivating *β*- lactams enzymes such as TEM-1. Also, Naber et al. (2008) who demonstrated the resistance to ciprofloxacin in *E. coli* was 21.6%. These results are almost in agreement with our results (Table 4). But also, our result different with the results reported by (Mohammed et al., 2014) who observed that the resistance to one or more antimicrobial agents was found in 22 isolates of *E. coli* (14.7%) detected from the total of 150 samples and a pattern of multiple drug resistance was observed. The distribution of resistance determinants for tetracycline was assessed by PCR in the resistant isolates. The most common resistance determinants were *tet*B (60%), *tet*C and D (20%) (Table 4). In this study, the presence of multidrug-resistant *E. coli* to *B*-lactams groups is attributable to the acquired ability of the strains to produce *B*-lactamase, which hydrolyzes *B*-lactams ring, rendering the entire compound inactive. Osaili et al. (2014) reported that most *Salmonella* isolates were resistant to the majority of antibiotics tested. Harakeh

et al. (2005) also found that 86 and 57% of the *Salmonella* isolates from meat-based fast food in Lebanon were resistant to trimethoprim-sulfamethoxazole and gentamicin, respectively. Moreover, (Diarra and Malouin, 2014) also reported a similar pattern of resistance against *B*-lactams antibiotics such as ampicillin. This resistance was associated with various antibiotic resistant gene determinants ($bla_{CMY}$-1, $bla_{CMY}$-2, and $bla_{CTX}$) (Liebana et al., 2013, Clemente et al., 2013). The previous results are in agreeing with our results where $bla_{CTX}$ and $bla_{CMY}$ genes were observed in 50% and 37.5% serovars, respectively (Table 5).

In conclusion, the prevalence's of both *Salmonella* and *E. coli*, at ready to eat meat products in Luxor city- Egypt, were high compared with those in other countries in the region, as earlier was published; consequently, obligatory implementation of strict food safety regulations in restaurants that sell high-risk meat products should be incessantly imposed and checked by regulatory agencies in Egypt for food safety.

**Conflict of interest statement**

The authors declare that they have no conflict of interest.

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