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Study on Dispersal of *Escherichia coli* and *Salmonella enterica* in Retail Beef and Chicken Meat

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ABSTRACT

This study was performed to detect the dispersal of *Escherichia coli* and *Salmonella enterica* as well as reveal its antibiogram and spread of ESBL genes in the isolates from retail beef and chicken meat. Eighty-two beef and chicken meat samples have been picked up from butcher's stores and restaurants in Cairo and Giza, Egypt. *Escherichia coli* were isolated from 15% of examined beef and chicken meat samples, which was 52%, 41%, 12% and 1% from raw beef, raw chicken beef ready for consumption and chicken ready for consumption samples respectively. However, *Salmonella enterica* was detected from 1% from chicken meat samples ready for consumption only which constitute 5% from different meat samples. *Escherichia coli* isolates were insensitive to Clindamycin and Rifampin 100% each and showed high resistance to Ciprofloxacin, 78.9%. While *Salmonella enterica* isolate was unsusceptible to Ceftazidime, Cefepime, Cefotaxime, Clindamycin, Ciprofloxacin and Rifampin. All *Escherichia coli* isolates as well as, *salmonella enterica* isolate were phenotypically ESBL positive. All of *Escherichia coli* isolates contained bla_{TEM} gene while bla_{SHV} gene was detected in 85% and 60% in members of *Escherichia coli* recovered from raw and ready for consumption meat respectively. bla_{TEM} and bla_{SHV} both genes were detected in *Salmonella enterica* isolate. While Bla_{CTX} gene did not detect in any of the *Escherichia coli* and *salmonella enterica* isolates.

Key words: *Escherichia coli*, *Salmonella enterica*, antimicrobial resistance, retail meat

INTRODUCTION

Enterobacteriaceae, a huge family of gram-negative, nonspore-forming, rod-shaped, facultative anaerobes sugars fermenters (Ye, 2018). *Escherichia coli* and *Salmonella* species are the dominant members of Enterobacteriaceae causing foodborne infections which transmitted throughout beef and poultry meat (Helmy *et al.*, 2014). The extensive use of antibiotics establishes problems for antimicrobial resistance, which leads to an increase in treatment costs and even to therapy failure (Boucher *et al*., 2013).

Ready for consumption meat treated foodstuffs which have acquired reputation in recently because it could be consumed without further cooking (Rodriquez *et al*., 2010). The cross-contamination between raw meats and individuals or surfaces in the kitchen environment and the subsequent risk of contamination in ready for consumption foods has been previously documented (Humphrey *et al*., 2001).

Therefore, the purpose of the present research was to demonstrate the spreading, antibiogram and Extended Spectrum-β Lactamase (ESBL) *Escherichia coli* and *Salmonella enterica* in raw and ready for consumption meat with ESBL genes.

MATERIALS AND METHODS

Collection of samples

The study was conducted on different meat samples (n=82); raw beef (n=17), raw chicken (n=12), beef ready for consumption (n=31) and chicken ready for consumption samples (n=22) were collected from different butcher's stores and restaurants in Cairo and Giza. Samples were picked up into sterile plastic cups and immediately delivered to the laboratory in Microbiology department, Faculty of Veterinary medicine, Cairo University for bacteriological examination.

E. coli isolation

According (ISO Standards,2011) that 0.1% Buffered Pepton Water (BPW, Oxoid) was used as enrichment and after incubation at 37°C for 24 h, a loop full from the broth was spreader on MacConkey agar plates (MCA, Oxoid)

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and kept at 37°C for 24 h. Pink color colonies on the MacConkey agar surface subjected to confirmation by biochemical tests (catalase, IMVC and TSI).

***Salmonella* isolation**

Buffered peptone water (BPW, Oxoid) used as non-selective pre-enrichment and Rappaport-Vassiliadis soya peptone broth (RVS, Oxoid) Selective enrichment incubation for 24h/37C in both enrichments respectively. loopful from RVS broth was streaked on Selective agar plating Brilliant Green Agar (BGA, LabM) incubated 37C/24h, colonies which converted the color of the medium from yellow to red/pink were serotyped. Serotyping was performed using omnivalent O Antisera (MAST® ASSURE Salmonella Agglutinating Antisera) to confirm *Salmonella enterica* isolates. (ISO Standards, 2011).

Phenotypic antimicrobial resistance characterization of isolates

The sensitivity of 19 *Escherichia coli* and one *Salmonella* isolates to 13 antimicrobial drugs (7 antimicrobial drug classes) was achieved as stated by the Clinical and Laboratory Standards Institute (CLSI) guidelines (2013): Ampicillin/Sulbactam (20 µg), Amoxicillin/Clavulinic (20 µg), Azotreonam (30 µg), Cefepime (30 µg), Cefotaxime (30 µg), Ceftriaxone(30 µg), Ceftazidime (30 µg), Gentamicin (10 µg) Azithromycin (15 µg), Clindamycin (2 µg), Ciprofloxacin (10 µg), Sulphamethoxazole/ trimethoprim (25 µg) and Rifampin (50 µg). The antimicrobial resistance profiles were performed as mentioned previously (Gomez-Zorrilla *et al*., 2014).

ESβL confirmatory test

The test was carried out as shown by CLSI, 2013 by the double-disc synergy technique with disks containing Cefotaxime (30 µg) and Ceftazidime (30 µg) alone and then in combination with Clavulinic acid and (Ceftazidime, Ceftazidime/Clavulinic acid 30/10 µg) when the zone diameter of either antimicrobial drug investigated in combination with Clavulanic acid was increased versus the diameter of the drug examined alone above or equal to 5 mm the bacteria were considered to be ESBL.

Surveying of ESBL genes

Bacterial DNA was retrieved using the QIAamp DNA mini Extraction Kit, as described by the manufacturing company. Examination of ESBL genes was done by PCR techniques. Primers were designated for amplification of the bla_{TEM}, bla_{SHV} and Bla_{CTX} (Fig. 3).

RESULTS

Spreading of *Escherichia coli* and *Salmonella enterica* from deferent meat samples

A total 19 isolates (15%) from variety of meat samples were recovered in this study, were positive to *E. coli* including raw beef 52% (n=9), raw chicken 41% (n=5) and beef ready for consumption 12% (n=4) chicken ready for consumption samples. While there was one isolate of *Salmonella enterica* (1%) from variety of meat samples this isolate was from ready for consumption chicken meat samples in a percentage of 5% in ready for consumption chicken meat samples Fig. 1.

Phenotypic Antimicrobial Resistance Characterization

The findings of antimicrobial sensitivity testing of the 19 *Escherichia coli* isolates and one *Salmonella enterica* are shown in figure 2. It was found that *Escherichia coli* isolates were 100% resistance to Clindamycin and Rifampin each and showed high resistance to Ciprofloxacin in a percentage of 78.9%. *Salmonella enterica* isolate was resistance to Ceftazidime, Cefepime, Cefotaxime, Clindamycin, Ciprofloxacin and Rifampin.

It was noticed that 89% of *Escherichia coli* isolates and *Salmonella enterica* isolate were MDR (multidrug resistant) Table 2.

It is observed that all isolates of *E. coli* and *Salmonella enterica* were phenotypically Extended Spectrum-β Lactamase (ESBL) positive.

Result of surveying of ESBL genes

The result of surveying of ESBL genes. All examined *Escherichia coli* contained bla_{TEM} gene. *Escherichia coli* isolates from raw meat and ready for consumption meat harboured bla_{SHV} gene in a percentage of 85% and 60% respectively figure 2. *Salmonella enterica* isolate was contain both bla_{TEM} and bla_{SHV} genes. However, Bla_{CTX} gene couldn't be detected either in *Escherichia coli* or *salmonella enterica* isolates.

DISCUSSION

The present study revealed the spreading of *Escherichia coli* was 52% while *Salmonella enterica* couldn't be isolated in any retail raw beef samples. The previous studies Angkititrakul *et al*., 2013 reported that *S. enterica* and *E. coli* isolated from beef in a percentage of 52% and 56% respectively and Adzitey, 2015 noticed that the in-beef samples the average prevalence of *Escherichia coli* and *Salmonella* species were 56% and 31% respectively.

This study reported that *Escherichia coli* had been recovered from 48% of examined raw chicken meat although *Salmonella enterica* didn't be isolated from raw chicken meat. Adeyanju and Ishola, 2014 mentioned that they isolated *Escherichia coli* and *Salmonella* species from chicken in a percentage of 47.2% and 32.1% respectively. Yulistiani *et al*., 2017 showed that *Escherichia coli* and *Salmonella* species were isolated by 77.5% and 85% from chicken meat, however Sonavane and Sankarankutty, 2018 mentioned that all examined chicken meat samples were harbor E. *coli* and *Salmonella* spp.

Escherichia coli and *Salmonella enterica* were isolate from 12% and zero% respectively of examined beef ready for consumption and were 1% each in chicken ready for consumption samples. In prior studies Hassanin *et al*., 2014 detected *E. coli* and *Salmonella* spp. From ready to eat beef in percentage of 40% and 31% respectively as well as 31% and 22% respectively in ready to eat chicken. Al-Humam, 2019 detected *E. coli* at a rate of 5.56% and *Salmonella* species didn't isolate from his examined samples.

In this survey 89% of *Escherichia coli* isolates and *Salmonella enterica* isolate were MDR.

This study recorded that all isolated *Escherichia coli* bacteria were resistance to Clindamycin and Rifampin each and showed high resistance to Ciprofloxacin in a percentage of 78.9%. *Salmonella enterica* isolate was

Table 1: Oligonucleotide primers sequences sources

Gene	Primer sequence (5'-3')	Length of amplified product	Reference
bla$_{TEM}$	ATCAGCAATAAACCAGC CCCCGAAGAACGTTTTC	516 bp	Colom *et al*., 2003
bla$_{SHV}$	AGGATTGACTGCCTTTTTG ATTTGCTGATTTCGCTCG	392 bp	
Bla$_{CTX}$	ATG TGC AGY ACC AGT AAR GTK ATG GC TGG GTR AAR TAR GTS ACC AGA AYC AGC GG	593 bp	Archambault *et al*., 2006

Table 2: Antimicrobial resistance profiles *E. coli* and *Salmonella enterica* isolated from meat to various antibiotics

Antibiotics	n= of antibiotics	n= of antibiotic classes	n= of isolates	n= of XDR	n= of MDR	n= of PDR
E. coli						
Raw beef						
DA, RA	2	2	2	2	0	0
DA, CIP, RA	3	3	1	0	1	0
CAZ, DA, CIP, RA	4	4	1	0	1	0
SAM, AMC, DA, CIP, RA	5	4	1	0	1	0
SAM, CTX, DA, CIP, RA	5	4	1	0	1	0
AMC, CN, AZM, DA, CIP, RA	6	6	1	0	1	0
SAM, AMC, CTX, DA, CIP, RA	6	4	1	0	1	0
SAM, AMC. ATM, CTX, CRO, CAZ, CN, AZM, DA, CIP, SXT, RA	12	7	1	0	1	0
Raw chicken						
AMC, DA, CIP, SXT, RA	5	5	2	0	2	0
CAZ, CN, AZM, DA, RA	5	5	1	0	1	0
SAM, AMC, CAZ, DA. CIP, RA	6	4	1	0	1	0
AMC, CN, AZM, DA, CIP, SXT, RA	7	7	1	0	1	0
Ready for consumption beef						
DA, RA	2	2	1	0	1	0
DA, CIP, RA	3	3	1	0	1	0
AMC, DA, CIP, RA	4	4	1	0	1	0
SAM, AMC, CAZ, DA, CIP, RA	6	4	1	0	1	0
Ready for consumption chicken						
AMC, CAZ, DA, CIP, RA	5	4	1	0	1	0
Salmonella enterica						
Ready for consumption chicken						
FEP, CTX, CAZ, DA, CIP, RA	6	4	1	0	1	0

SAM; Ampicillin/Sulbactam, AMC; Amoxicillin/ Clavulinic, AZM; Azotreonam, FEP; Cefepime, CTX; Cefotaxime, CRO; Ceftriaxone, CAZ; Ceftazidime, CN; Gentamicin, AZM; Azithromycin, DA; Clindamycin, CIP; Ciprofloxacin, SXT; sulphamethoxazole /trimethoprim, RA; Rifampin. XDR: Extensively Drug Resistant, MDR: Multidrug Resistance, PDR: Pandrug Resistance.

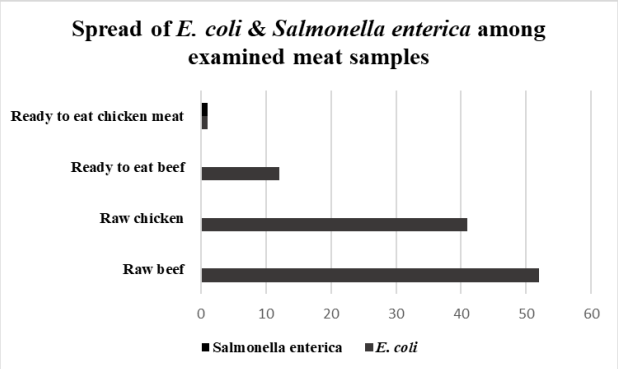

Fig. 1: Showed spreading of *E. coli* & *Salmonella enterica* among examined meat samples.

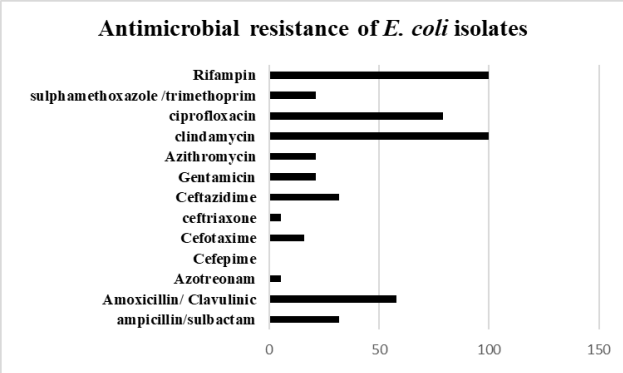

Fig. 2: Showed antimicrobial resistance pattern of *E. coli* isolates.

resistance to Ceftazidime, Cefepime, Cefotaxime, Clindamycin, Ciprofloxacin and Rifampin. 100% of *Escherichia coli* and 80% of *Salmonella enterica* isolate harbored bla$_{TEM}$ and *bla*$_{SHV}$ genes.

Rahman *et al*., 2014 mentioned that totally 50% of studied *Escherichia coli* of food origin were multi drug resistant. Sonavane and Sankarankutty, 2018 found that examined *Escherichia coli* and *Salmonella* species were resistant Ampicillin, Gentamicin, Ciprofloxacin and Ceftriaxone.

Moawad *et al*., 2017 mentioned that obtained *Escherichia coli* were unsusceptible to Ampicillin, Trimethoprim/Sulphamethoxazole and Cefotaxime in a percentage of 71.4%, 61.9% and 33.3% respectively, while *bla*TEM and *bla*CTX-M detected in 52.4% and 42.9%. *S. enterica* isolates were resistant to ampicillin 86.7%, Cefotaxime 80%, Cefpodoxime 60%, Trimethoprim/ Sulphamethoxazole 53% and Tetracycline 40% respectively, however, *blaCTX*-M and *bla*TEM were 73.3% and 73.3%.

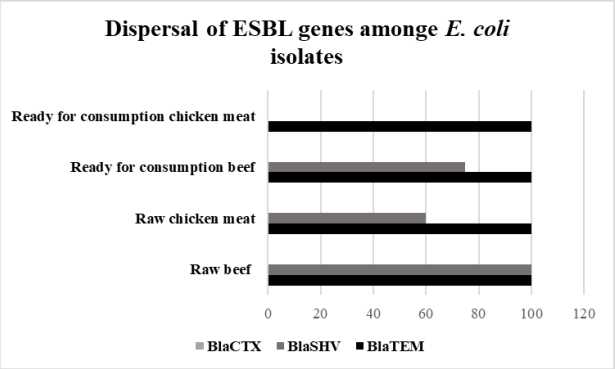

Fig. 3: Showed dispersal of ESBL genes among *E. coli* isolates.

Conclusions

This study showed high contamination of in raw meat (chicken meat and beef) and food ready for consumption by *E. coli* as foodborne pathogens. The antibiogram of *E. coli* and *Salmonella* enterica isolates revealed high antimicrobial resistance and considered of public health concern in Egypt. Molecular survey on the presence of ESBL genes among *E. coli* and *Salmonella enterica* reveal presence of ESBL genes in food of animal origin and that Represent a hazard for human.

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