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Prevalence and Antimicrobial Resistance of *Escherichia coli* Strains Isolated from Beef and Chicken Meat in Egypt

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ABSTRACT

The goal of this study was to investigate the prevalence and antimicrobial resistance of *Escherichia coli* isolated from 100 samples of raw beef meat (50) and chicken meat (50) Collected From supermarkets at Menofia, Cairo and El-Kalyobia governorates, Egypt. *E. coli* was recovered from 13 (26%) beef meat samples and 18 (36.00%) chicken meat samples and identified serology. the most common serovars (O26, O119, O111, O103, O91, O86, O146, O45, O121 and O 128). A total of 75 *E. coli* isolates were obtained and tested for susceptibility to 10 antimicrobials by the disk diffusion method. *E. coli* isolates of the current investigation revealed a high resistance against Tetracycline (33.3%), Ampicillin (26.6%), Kanamycin & Trimethoprim-Sulphamethoxazole (20%), Cefotaxime (12%), Chloramphenicol & Ciprofloxacin (8%) antimicrobial agents. Whereas; the lowest degree of resistance was noticed against Streptomycin (6.6%), Ceftazidime (5.3%) and Nalidixic acid (4%). These data suggest that antimicrobial-resistant *E. coli* isolates are widely distributed in meat samples and processing environment in Egypt, which can play a role in dissemination of antimicrobial resistance to other pathogenic and commensal bacteria.

Keywords: *E. coli, raw beef meat, chicken meat, Antimicrobial resistance, Egypt.*

INTRODUCTION

Foods of animal origin like chicken meat and beef are rich in proteins which are very essential to body growth and development. However, foods of animal origin also act as a vehicle and medium to transmit various microorganism causing health hazards, disease and death. Food borne diseases are a growing public unhealthiness everywhere the globe. which cause an estimated 48 million illnesses and 3,000 deaths every year within the us (Scallan *et al.,* 2011). In developed countries, up to 30% of the population suffer from food borne diseases every year, whereas in developing countries up to 2 million deaths are estimated each year (WHO, 2007).

Escherichia coli is a commensal bacterium and has a large variety of hosts. It is generally found in the atmosphere and is known to be an indicator of food and water fecal pollution. *E. coli* from other species in the ecosystem may develop, retain, and transfer resistance genes because of its human and animal ubiquity and its function as a pathogenic and commensal organism.

Despite improved technologies and hygiene practices in developed countries at all stages of the processing of poultry and beef meat, foodborne pathogens remain a continuous threat to human and animal health. *Escherichia coli* and *S. Enterica* serovars are the dominant members of *Enterobacteriaceae* causing foodborne infections. The expansion of antibiotic resistance in bacteria is also an emerging public health hazard due to the compromised efficacy in the treatment of infectious diseases (Helmy *et al.*, 2017)

The level of antimicrobial resistance in *E. coli* represents a useful indicator of resistance dissemination in bacterial populations and of selective pressure imposed by the antimicrobials used in treatment of food animals and humans (Elhaj *et al.*, 2007) and (Scott, *et al.* 2005). So, Burden of antimicrobial resistant bacteria affects the economy and health of people in both developed and developing countries. Globally, bacterial antimicrobial resistance is witnessing a rapid rise in both human and veterinary world (Aarestrup, 2004 and Ciˇzma, 2003)

Food is also an essential factor in the antimicrobial resistance transfer process. This transition will take place by means of antibiotic residues in foods such as poultry meat. (Jhonson *et al*., 2007), through the transfer of resistant food-borne pathogens or through the ingestion of resistant strains of the original food microflora and resistance transfer to pathogenic microorganisms (Pesavento *et al*., 2007). In the process of food production many kinds of antimicrobials are used for preventing and controlling diseases, enhancing growth and increasing feed efficiency in food producing animals (CDC, 2005). Increased occurrence of multiple drug resistance in *E. coli* (Khan *et al*., 2005 and Sharada *et al.*, 2010).

Antibiotics play a vital role comprising treatment and management of bacterial infections, leading to a reduction in morbidity and mortality of both human and animal patients. However, in agriculture, veterinary and medical companies, the abuse of antibiotics drives the selection of antibiotic-resistant bacteria that resist and overcome antibiotic action. Approximately 80% of all antibiotics used worldwide are in agriculture and aquaculture. protuberant role of antibiotics in livestock husbandry, antibiotics are used for prevention of infection or the simultaneous treatment of healthy and sick animals in a group during an outbreak of disease. It can also be used as antimicrobial feed additives (AFAs) to support growth and efficiency in animal production. (VuurenM Van, 2014). Subsequently, resistant pathogens enhance by many folds the probability of foodborne disease (FBD) outbreak and FBD associated with resistant *E. coli* has reached an alarming proportion (Jindal *et al.*2014) Further transfer of resistance to enteric and commensal bacteria is even a bigger problem. The emerging resistance to WHO classified critically important antimicrobial such as carbapenems, extended-spectrum cephalosporins (ESCs), aminoglycosides and fluoroquinolones (FQs) among Enterobacteriaceae remains worrisome (WHO, 2015)

Vast majority of antimicrobials are normally used to treat infections either in animals or humans caused by microorganisms. In the care of farm animals, the use of antimicrobials has been related to the development of multidrug-resistant microorganisms that are a threat to public health (Hoelzer *et al.*, 2017; Mouiche *et al.*, 2019). *Escherichia coli* isolated from beef meat has been identified of different resistances to a number of antimicrobials including erythromycin, tetracycline, ampicillin, gentamicin, suphamethoxazole/trimethoprim, chloramphenicol, cefuroxime and ceftriaxone (Adzitey, 2015a; Anning, Dugbatey, Kwakye-Nuako, & Asare, 2019; Aslam & Service, 2006 and Saud *et al.*, 2019).

In addition, in many countries, foods contaminated with antibiotic resistance bacteria are a serious public health problem due to the persistent circulation in the environment of resistant bacterial strains. The vast majority of Egyption population loved eating carcasses of chicken that were slaughtered and butched in small retail shops. Meat are available in open-air without adequate temperature control also the hygiene is always questionable.

The current research was achieved to determine the prevalence of *Escherichia coli* strains isolated from beef and chicken meat in Egypt and to evaluate the antimicrobial resistance and sensitivity pattern of the obtained isolate.

MATERIALS AND METHODS

Isolation of E. coli from beef meat and chicken meat samples:

This study included 100 random locally raw beef (50) and raw chicken (50) were collected from different super markets at Menofia, Cairo and El-Kalyobia governorates, Egypt in clean sterile containers and transported with a minimum of delay to the laboratory.

25 g of each beef and chicken meat was added into 225 ml of Tryptic Soy Broth and incubated overnight at 37 ^{o}C. Samples from the tryptic soy broth were then inoculated onto Eosin Methylene Blue agar (EMB) on different plates and incubated for 24–48 hours at 37˚C.The EMB is a selective enrichment media and differential for *E. coli*. One pure green metallic sheen colonies characteristic of *E. coli* on EMB were confirmed as presumptive isolates and stored in peptone broth awaiting identification. (APHA, 2001)

Serotyping E. coli isolates

The isolates were serologically identified according to *Kok et al.* (1996) by using rapid diagnostic *E. coli* antisera sets (DENKA SEIKEN Co., Japan) for detection of the *Escherichia coli* serovars.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing to 10 antimicrobials was carried out in 75 *E. coli* isolates by the disk diffusion method on Mueller–Hinton agar (Difco), according to the CLSI guidelines (CLSI, 2012). The concentration of the disks (Becton Dickinson, Sparks, USA) and abbreviation of antimicrobial agents which were used throughout this paper are: Ampicillin (AMP: 10 μg), Cefotaxime (CTX), Ceftazidime (CAZ), Kanamycin (K: 30 μg), Streptomycin (S: 10 μg), Tetracycline (TE: 30 μg), Ciprofloxacin (CIP: 5 μg), Nalidixic acid (NA: 30 μg), Trimethoprim-Sulphamethoxazole (SXT: 1.25/23.75 μg) and Chloramphenicol (C: 30 μg) were used as recommended by the CLSI.

Determination of antimicrobial minimum inhibitory concentration (MIC)

The MIC of antimicrobials to which the bacteria were either resistant or showed intermediate resistance by disk diffusion method, was determined by broth microdilution method, according to the CLSI guidelines (CLSI, 2012). The isolates were classified as susceptible and resistant according to the zone diameter and MIC interpretative standards recommendations by CLSI (2013).

RESULTS

Prevalence of Escherichia coli strains isolated from beef and chicken meat.

A total of 100 random locally raw beef and chicken meat samples, including (n = 50) beef and (n = 50), chicken meat specimens were collected from different super markets at Menofia, Cairo and El-Kalyobia governorates, Egypt in clean sterile containers and transported with a minimum of delay to the laboratory. Of these, *Escherichia coli* samples were recovered from 13 (26%) beef 's meat samples,18 (36.00%) chicken's meat samples, after further identification of these samples the Prevalence of *Escherichia coli isolates was* 31isolates (62%) beef 's meat and 44 isolates (88%) chicken's meat (Table 1). From these results, it is clear that the chicken meat is the most affected by *Escherichia coli* followed by beef. Out of 100 meat samples 75 isolates *E. coli* by (75%).

Serovars of E. coli isolates isolated from raw beef meat

In this investigation, 13 (26%) beef meat samples were proved to be positive for *E. coli*. the suspected isolates were serologically identified for detection of *Escherichia coli* serovars.

31 *E. coli* isolates were found 10 serovars. the most common (O26, O119) by 12. 9% followed by (O111, O103, O91, O86, O146, O45, O121) by 9. 6% and the last (O 128) by 6. 4% as in table 2.

Serovars of E. coli isolates isolated from raw chicken meat

In this investigation, 18 *E. coli* samples were isolated from chicken meat were serologically identified according to *Kok et al.* (1996) by using rapid diagnostic *E. coli* antisera sets (DENKA SEIKEN Co., Japan) for detection of the *Escherichia coli* serovars. From 44 *E. coli* isolates were found 9 serovars. the most common (O119) by 18.2 % followed by (O45) by 15.9 % and (O 111 and O91) by 13.6 %; (O146) by 11.4%; (O26, O 121) by 9% and (O128, O86) by 4.5% as in table 3.

Table (1): Prevalence of *E. coli* in raw beef and Chicken meat

Samples	No. of examined samples	No. of +ve *E. coli* samples	No. of +ve *E. coli* isolates
Beef meat	**50**	13 (26%)	31 (62%)
Chicken meat	**50**	18 (36%)	44 (88%)
Total	100	31 (31%)	75 (75%)

Table (2): Serovars of *E. coli* isolates isolated from raw beef meat

Serovar	No.	%
O111	3	9.6
O26	4	12.9
O103	3	9.6
O91	3	9.6
O119	4	12.9
O128	2	6.4
O86	3	9.6
O146	3	9.6
O45	3	9.6
O121	3	9.6
Total	31	**100**

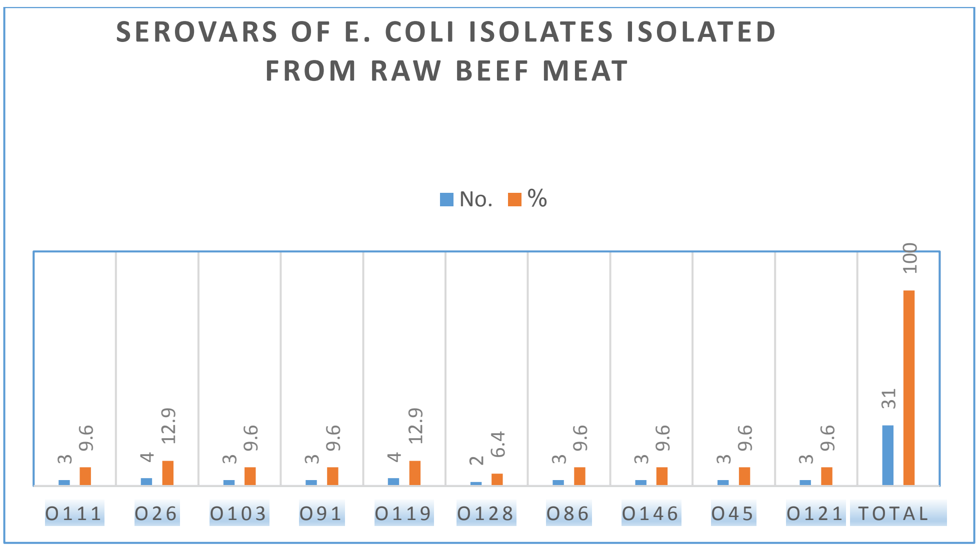
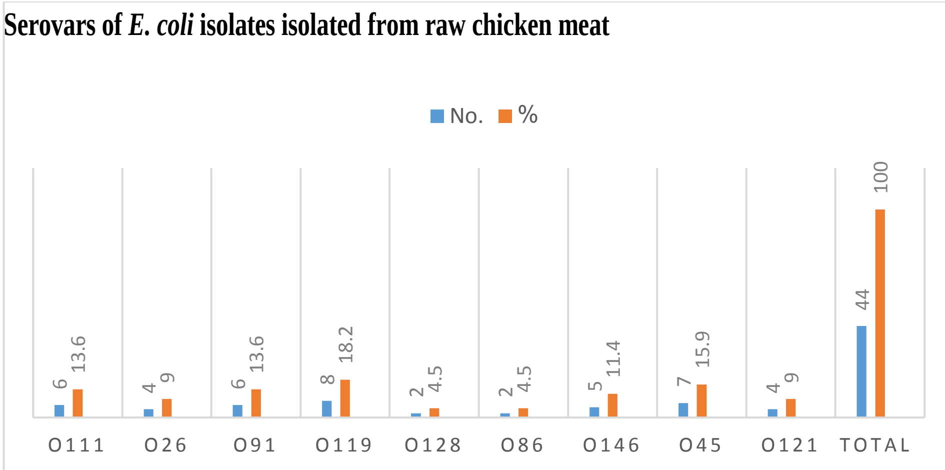


Table (3): Serovars of E. coli isolates isolated from raw chicken meat

Serovar	No.	%
O111	6	13.6
O26	4	9
O91	6	13.6
O119	8	18.2
O128	2	4.5
O86	2	4.5
O146	5	11.4
O45	**7**	15.9
O121	4	9
Total	44	100



Antimicrobial resistance of Escherichia coli isolated from beef and chicken meat.

Antimicrobial	No. (%) of resistant *E. coli* isolates		
	Beef meat (n = 31)	Chicken meat (n = 44)	Total (n = 75)
AMP	11 (35.5)	9(20.4)	20 (26.6)
C	2 (6.4)	4 (9)	6 (8)
CAZ	1 (3.2)	3 (6.8)	4 (5.3)
CIP	2 (6.4)	4 (9)	6 (8)
CTX	9 (29)	-	9 (12)
K	8 (25.8)	7 (15.9)	15 (20)
NA	3 (9.7)	-	3 (4)
S	2(6.4)	3 (6.8)	5 (6.6)
SXT	14 (45.1)	1 (2.3)	15 (20)
TE	18 (58)	7 (15.9)	25 (33.3)

AMP = Ampicillin (10µg), C =Chloramphenicol (30µg), CAZ = Ceftazidime (30 µg), CIP = Ciprofloxacin (5µg), CTX = Cefotaxime (30µg), K = Kanamycin (30 µg), NA = Nalidixic acid (30µg), S = Streptomycin (10 µg), SXT = Trimethoprim-Sulphamethoxazole (1.25/23.75µg), T = Tetracycline (30 µg)

Table (4) displayed the degree of antimicrobial resistance of 75 *E. coli* strains recovered from various types of uncooked beef and chicken meat samples. Based on our findings, *E. coli* isolates of the current investigation revealed a strong resistance against Tetracycline (33.3%), Ampicillin (26.6%), Kanamycin & Trimethoprim-Sulphamethoxazole (20%), Cefotaxime (12%), Chloramphenicol & Ciprofloxacin (8%) antimicrobial agents. Whereas; the lowest degree of resistance was noticed against Streptomycin (6.6%), Ceftazidime (5.3%) and Nalidixic acid (4%).

MAR index of this antibiotic calculated as follow If indexing is applied to a sample from which several isolates were taken, the index of the sample would be **a/ (b * c),** where **a** is the aggregate antibiotic resistance score of all isolates from the sample, **b** is the number of antibiotics, and **c** is the number of isolates from the sample. So, MAR index of *E. coli* in this investigation was (14.4%)

The percentage of *E. coli* strains with a MAR index > 0.2 was 75/100 (75%). Consequently, *E. coli* recuperated from beef meat and chicken meat is amazingly safe against most of antimicrobial specialists with uncommon estimations of MAR record.

DISCUSSION

Foodborne illnesses caused by *E. coli* represented a major public health problem worldwide and transmitted mainly through consumption of contaminated food and the presence of *E. coli* in beef and chicken meat has relevant public health implications (Sousa, 2008)

Prevalence of *Escherichia coli* in the beef and chicken meat are presented in Table (1). Nearly similar results were obtained by Maarouf and Nassif (2008), Abdaslam *et al.,* (2014), Ramadan (2015), Saif (2015) and Shawish (2015). The colonial appearance and the biochemical profile of *E. coli* isolated was similar to those previously reported such as the fermentation of certain sugars or enzymatic reaction (Quinn *et al.,* 2002, and Ezzat *et al.,* 2014) The contamination by *E. coli* of meat samples suggests that lapses occurred during the animal slaughter and transport and sale of meat. [Ritchie and Roser, 2019**]**. This is because a non-diseased living animal's muscle is indispensably sterile. The muscles are exposed until the animals are slaughtered and may be contaminated by microorganisms. In the gastrointestinal tract of farm animals, E. coli is known to naturally harbor [Feng P et al,2017]. As the gastrointestinal tract ruptures during evisceration, they cross-contaminate meat. During sampling, it was found that knives used to cut meat were not intermittently sterilized. Arslan and Ayi (2011) reported 42.9 % prevalence from retail meat sample including chicken meat. Our results indicate that there is a high prevalence of *E. coli* in chicken meat and beef which suggest that the production and processing of these foods are not hygienic. Relatively higher numbers of *E. coli* isolates were obtained in conventional method (37.87%) than molecular technique (35.94%). Similar findings by Rahman et al (2017)

Regarding to, the serological identification of 31 *E. coli* isolates were found 10 serovars. the most common (O26, O119) by 12, 9% followed by (O111, O103, O91, O86, O146, O45, O121) by 9, 6% and the last (O 128) by 6, 4% (Table, 2 and 3). These results similar with those of Kalchayan and *et al.,* (2012), Windham et al, (2013) and Abd El-Salam (2014) and Shawish (2015). These results coincided with the fact of Woody *et al.,* (1998) who recorded that the same serogroups were Enteropathogenic *E. coli* and causing infantile enteritis, hemorrhagic colitis, hemorrhagic gastroenteritis and diarrheal illness in different settings.

Multi- drug resistant E. coli isolates were tested for 10 used available antibiotics. Although we did not check the pathogenicity of the isolates, the gene responsible for multi-drug resistance may transfer to consumer via food and results in serious public health hazard as because Boarlin et al., (2005) reported antimicrobial resistance is more frequent in pathogenic than in other E. coli strains, and also shows that the resistance genes found in ETEC isolates are different from those of other E. coli isolates and that clear associations exist between specific resistance and virulence genes. Jhonson et al. (2007) also reported that the drug resistant human isolates were similar to poultry isolates and thus, concluded that many drug-resistant human fecal E. coli isolates may be originated from poultry. This resistance occurs due to possessing of resistant gene found in single and multiple size plasmids in E. coli isolates.

The highest prevalence of multi-drug resistant E. coli isolates was obtained from chicken meat 76%. Adesiyun et al. (2007) reported E. coli which was resistant to at least five or more antimicrobial agents. Álvarez-Fernández et al. (2013) reported that 91.7 % E. coli isolates of poultry were multi-drug resistant. Indiscriminate use, improper selection, improper dose, incorrect duration of antibiotics at flock level may be responsible for such a higher occurrence of MDR. Hassan et al. (2013) reported 22.7% MDR E. coli isolates from bird samples.

In our study, E. coli isolates of the current investigation revealed a strong resistance against Tetracycline (33.3%), Ampicillin (26.6%), Kanamycin & Trimethoprim-Sulphamethoxazole (20%), Cefotaxime (12%), Chloramphenicol & Ciprofloxacin (8%) antimicrobial agents. Whereas; the lowest degree of resistance was noticed against Streptomycin (6.6%), Ceftazidime (5.3%) and Nalidixic acid (4%). Hence in therapeutic decision these drugs should be used with caution and only after antibiotic sensitivity testing which is similar to the findings of Jhonson et al. (2007).

CONCLUSIONS

The higher prevalence of *E. coli* in beef meat and chicken meat indicates unhygienic production and processing of these foods. Presence of multi-drug resistant *E. coli* in these foods may pose serious public health threats.

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