



# Molecular Determinants of Multiple Antibiotic Resistant *E. coli* Isolated from Food of Animal Origin

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**Abstract** | Multiple antibiotic-resistant *E. coli* usually contaminates foods of animal origin representing a major threat to public health. The purpose of this study was to demonstrate multiple antibiotic-resistant *E. coli* as a serious food contaminating bacteria in milk, meat and their products. One hundred and twenty five (125) samples were collected from meat, milk and their products from different markets in Beni-Suef and Giza Governorates, Egypt. The isolated *E. coli* were subjected to antimicrobial sensitivity testing against ampicillin, tetracycline and streptomycin and resistance genes were detected using polymerase chain reaction (PCR). Out of 125 samples, 41 *E. coli* were isolated. Serogrouping of *E. coli* isolates revealed O157, O44, O119, O168, O6, O158, O164, O126 and O125 serogroups. Most of *E. coli* isolates were multidrug resistant (MDR). *E. coli* isolates were resistant to ampicillin, amoxicillin, kanamycin, tetracycline and streptomycin with percentages of 63.4, 61, 53.7, 41.5 and 14.6%, respectively. Out of tetracycline resistant isolates ( $n=17$ ), 14 isolates harbored *tetB* gene (82.4%), while six isolates harbored *tetA* gene (35.3%). Meanwhile, both *tetA* and *tetB* were harbored in 4 isolates with a percentage of 23.5%. Ampicillin resistance gene *blaTEM* found in 76.5% of the ampicillin resistant isolates. Streptomycin resistant genes *aadA1* and *aadA2* were represented in 50% and 0% of the isolates, respectively. Foods of animal origin is considered an important source of multiple antibiotic resistant *E. coli* that can be a major source of food-borne diseases.

**Keywords** | *E. coli*, MDR, Food of animal origin, Resistance genes, PCR

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## INTRODUCTION

Food-borne illness is one of the major public health problems all over the world. Foods of animal origin are considered serious sources of food-borne diseases worldwide. Moreover, the situation worsens in developing countries due to difficulties in achieving optimum hygienic food handling practices (Schlundt *et al.*, 2004; Newell *et al.*, 2010; Badi *et al.*, 2018). Foodborne pathogens cause high economic losses in developing countries due to medical care and social costs (Fratmico *et al.*, 2005). The food safety unit of WHO (World Health Organization) recorded that about 1.8 million children died from diarrhea caused by contaminated food and drinking water

in 2005 (WHO, 2011). Antibiotics make revolution in the treatment of diseases. They are either used for prophylactic and/or therapeutic purposes in humans and animals. Some antibiotics are used as animal growth promoters. Unfortunately, similar antibiotics are being used in both veterinary and humans practice. The correlation between extensive use of antibiotics and the development of multiple antibiotic resistant bacteria is well authenticated (Garipcin and Seker, 2015; Zdolec, 2016; Jans *et al.*, 2018). Due to the misuse of antimicrobials in animal production, multiple antibiotic resistant bacteria have been increasing over the last decades, in addition to the spread of the resistant foodborne zoonotic pathogens (Teuber, 2001; Zdolec, 2016). There is a great possibility for transmission

of resistant genes to the food-related commensal bacteria or opportunistic pathogens which represents a potential hazard to consumers (Aarestrup et al., 2008) bacteria may represent an important threat to public health, as the antibiotic resistance genes can be transferred from resistant bacteria to other bacteria infecting humans. The acquisition and spread of resistance genes may be due to the misuse of antibacterial agents or through horizontal gene transfer (Threlfall et al., 2000; Chiu et al., 2002). *Escherichia coli* is recognized as an important pathogen of livestock such as cattle and poultry. It may cause severe human diseases such as hemorrhagic colitis and hemolytic uremic syndrome; a serious complication may lead to death (Diercke et al., 2014; Bryan et al., 2015; Markland et al., 2015). Due to its ubiquity, the antibiotic resistant *E. coli* can transmit its resistance genes from other Enterobacteriaceae species in the environment (Pitout, 2013; Hooper and Jacoby, 2015). Therefore, *E. coli* could be used as a monitoring for the misuse of antibiotics either in food producing animals or in treatment of humans (Laarem et al., 2017). Many studies in recent years undertaken to assess the antibiotic resistance of bacteria in food of animal origin such as meat, raw milk, and their products (Bhoomika et al., 2016; Ranjbar et al., 2018). Molecular analysis of antibiotic-resistant mobile elements and antibiotic resistance genes indicates similar elements in bacterial isolates from both animals and humans, which explain the role of food of animal origin in the dissemination of resistant bacteria to humans through food chain (Teuber, 2001; Bhoomika et al., 2016).

Therefore, this study was designed to investigate the existence of multiple antibiotic-resistant *E. coli* and their molecular determinants in food of animal origin that collected from Beni-Suef and Giza Governorates markets.

## MATERIAL AND METHODS

### SAMPLES

One hundred and twenty five (125) samples were randomly collected from milk, meat and their products from Giza and Beni-Suef Governorates markets (Table 1). All samples were aseptically collected in sterile plastic bags separately and transferred immediately under hygienic measures in ice box to the laboratory to be examined for presence of *E. coli*.

### ISOLATION AND IDENTIFICATION OF *E. COLI*

The prepared samples were inoculated onto MacConkey agar plates (Oxoid). Pink colonies were picked up and streaked onto eosin methylene blue agar (Oxoid) to observe the metallic sheen appearance. Typical colonies were picked up onto nutrient agar and subjected to standard morphological and biochemical tests as well as serological identification of the identified isolates as described by Quinn et al. (2002).

### ANTIMICROBIAL SENSITIVITY TESTING FOR IDENTIFIED ISOLATES

The antimicrobial sensitivity test was done by disk diffusion technique using antibiotic discs including ampicillin (10 $\mu$ g), amoxicillin (10 $\mu$ g), amoxicillin-clavulanate (20/10 $\mu$ g), streptomycin (10 $\mu$ g), kanamycin (30 $\mu$ g), ciprofloxacin (5 $\mu$ g), Trimethoprim-sulfamethoxazole (1.25/ 23.75  $\mu$ g) and tetracycline (30 $\mu$ g) (Oxoid). The degree of sensitivity was interpreted according to the clinical laboratory standard institute (CLSI, 2017).

### GENOTYPIC DETECTION OF THE RESISTANCE ASSOCIATED GENES

Polymerase chain reaction was performed for detection of resistance genes in Biotechnology Center in the animal health institute according to Sambrook et al. (1989). The primers (Metabion, Germany) of target genes with their sequence and references are listed in Table 2.

## RESULTS AND DISCUSSION

### ISOLATION AND IDENTIFICATION OF *E. COLI* FROM DIFFERENT FOOD SAMPLES

The results showed in Table 3 revealed that *E. coli* was totally isolated with a percentage of 32.8 arranged as 30.8% and 35% from samples of (raw milk and milk products) and (meat and meat products), respectively.

Nearly the same recovery rate of *E. coli* from meat and meat products was obtained by many studies worldwide (Ali et al., 2010; Gousia et al., 2011; Bhoomika et al., 2016). A higher prevalence rate was obtained by Abdalslam et al. (2014) who identified *E. coli* from meat products with a percentage of 50%. While a lower prevalence rate was obtained by Al-Zogibi et al. (2015) who recovered 17 *E. coli* isolates (11.33%) from raw meat samples. The same lower recovery rate of *E. coli* was found in meat with a percentage of 20% (Peternel et al., 2014). In the present work the incidence of *E. coli* isolated from milk and milk products is approximately the same obtained by many studies (Zeinhom and Abdel-Latef, 2014; Al-Zogibi et al., 2015). A higher recovery rate was recorded by many studies (Abike et al., 2015; Bhoomika et al., 2016).

### SEROGROUPING OF THE IDENTIFIED ISOLATES

Serogrouping of *E. coli* isolates revealed nine types while seven of the tested isolates were untyped with available sera. For each of the following serogroups (O6, O119, O125, O158, and O168) two isolates were identified with a percentage of 4.9, four isolates for every one of O 44 and O164 with a ratio of 9.77%. At the same time, O157 and O126 represented the highest prevalence (six isolates for each one; 14.6%). *E. coli* O157 which represented the highest prevalence in the study is a zoonotic food-borne

**Table 1:** Total number of product samples collected from sale markets.

Total number of samples	Milk		Yoghurt		Kareesh		Minced meat		Burger		luncheon	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
125	28	22.4	18	14.4	19	15.2	20	16	20	16	20	16

%: Percentages were calculated according to the total number of samples.

**Table 2:** Oligonucleotide primer sequences of target genes specific for *E. coli*.

Target gene	Primer sequence (5' → 3')	Amplified product (bp)	References
TetA [A]	GGTCACTCGAACGACGTCA	576 bp	Randall et al., 2004
	CTGTCGACAAGTTGCATGA		
TetA [B]	CCTCAGCTTCTCAACCGTG	633 bp	
	GCACCTTGCTCATGACTCTT		
Bla <sub>TEM</sub>	ATCAGCAATAAACCAGC	516 bp	Colom et al., 2003
	CCCCGAAGAACGTTTC		
AadA1	TATCAGAGGTAGTTGGCGTCAT	484 bp	Randall et al., 2004
	GTTCCATAGCGTTAAGGTTTCATT		
AadA2	TGTTGGTTACTGTGGCCGTA	622 bp	Walker et al., 2001
	GATCTCGCCTTCACAAAGC		

**Table 3:** Prevalence of *E. coli* isolated from the different food products during the study.

Samples source	No. of examined samples	Recovered <i>E. coli</i> isolates	
		Number positive	Percent (%)
Milk	28	6	21.4
Yogurt	19	2	10.5
Kareesh cheese	18	12	66.7
Milk and milk products	65	20	30.8
Minced meat	20	11	55
Burger	20	4	20
Luncheon	20	6	30
Meat and meat products	60	21	35
Total	125	41	32.8

%: Percentages were calculated according to the corresponding number of examined samples.

**Table 4:** Antimicrobial susceptibility profile of *E. coli* isolates (n=41) in the examined samples during the study.

Antimicrobial agents	Sensitive		Intermediate		Resistant	
	No.	%	No.	%	No.	%
<b>Penicillin group</b>						
Ampicillin	13	31.7	2	4.9	26	63.4
Amoxicillin	13	31.7	3	7.3	25	61.0
<b>β-lactam/β-lactamase inhibitor combinations</b>						
Amoxicillin-clavulanate	37	90.2	2	4.9	2	4.9
<b>Aminoglycoside group</b>						
Streptomycin	25	61.0	10	24.4	6	14.6
Kanamycin	16	39.0	3	7.3	22	53.7
<b>Tetracycline group</b>						
Tetracycline	20	48.8	4	9.8	17	41.4
<b>Fluoroquinolones group</b>						
Enerofloxacin	39	95.2	1	2.4	1	2.4
Ciprofloxacin	41	100	0	-	0	-
<b>Folate pathway inhibitor</b>						
Trimethoprim-sulfamethoxazole	40	97.6	0	0	1	2.4

%: Percentages were calculated in relation to the total number of tested isolates.

pathogen of worldwide significance; elicits possibly life-threatening hemorrhagic colitis, hemolytic uremic syndrome and/or bloody diarrhea (Kanitpun et al., 2004). Previous studies in Brazil and Nigeria informed that most *E. coli* strains isolated from yoghurt raw milk and cheese belonged to enteropathogenic serogroups (Paneto et al., 2007; Abike et al., 2015). A former study in Egypt done by Ahmed and Shimamoto (2014) reported *E. coli* O157:H7 in cheese, chicken meat, beef and raw milk.

#### ANTIMICROBIAL SUSCEPTIBILITY OF THE ISOLATED *E. COLI*

The results of *E. coli* sensitivity test (Table 4) revealed that most of the isolates were multidrug resistant (MDR). They were resistant to ampicillin, amoxicillin, kanamycin, tetracycline and streptomycin with percentages of 63.4%, 61%, 53.7%, 41.5% and 14.6%, respectively. On the other hand, high sensitivity pattern was observed to ciprofloxacin (100%), trimethoprim-sulfamethoxazole (97.6%), enerofoxacin (95.2%) and amoxicillin-clavulanate (90.2%). MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories (Magiorakos et al., 2012). Similar MDR *E. coli* isolates were recovered by many recent investigations (Hinthong et al., 2017; Lahuerta-Marin et al., 2017; Martínez-Vázquez et al., 2018; Ranjbar et al., 2018). Nearly the same findings of resistant *E. coli* against ampicillin, amoxicillin, kanamycin, tetracycline and streptomycin were obtained by many studies (Lei et al., 2010; Rashid et al., 2013; Rasheed et al., 2014; Laarem et al., 2017).

#### GENOTYPIC DETECTION OF RESISTANCE ASSOCIATED GENE

The accurate and rapid detection of antibiotic resistance genes is extremely important in preventing the spread of infections. PCR-based molecular technique is preferred for detection of the antibiotic resistance genes. It is a rapid, specific, and accurate method for detection of bacteria with resistant genes of interest (Hinthong et al., 2017; Lahuerta-Marin et al., 2017; Martínez-Vázquez et al., 2018; Ranjbar et al., 2018).

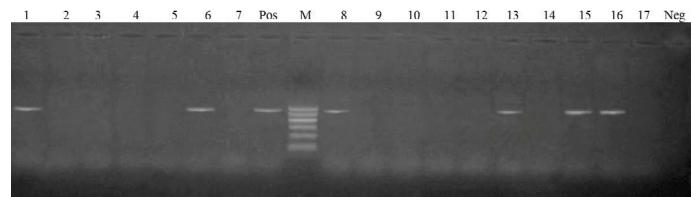
#### AMPLIFICATION *TETA* AND *TETB* (GENES IN TETRACYCLINE RESISTANT ISOLATES)

PCR was carried for amplification of *tetA* (576 bp) and *tetB* (633 bp) genes in tetracycline resistant isolates. Out of 17 tetracycline resistant isolates, 14 isolates harbored *tetB* gene (82.4%), while 6 isolates harbored *tetA* gene (35.3%). Meanwhile, both *tetA* and *tetB* were harbored in 4 isolates with a percentage of 23.5% (Figures 1 and 2).

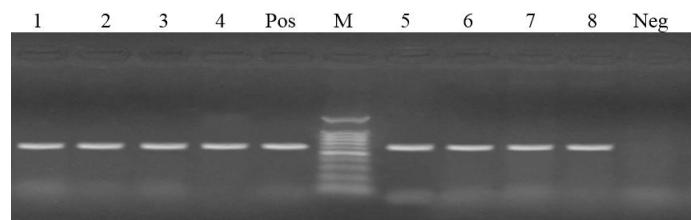
#### AMPLIFICATION OF *BLA<sub>TEM</sub>* GENE IN AMPICILLIN RESISTANT ISOLATES

Antimicrobial susceptibility revealed that 26 isolates were resistant to ampicillin, a total of 17 isolates were selected

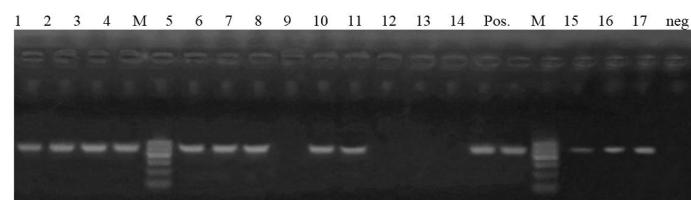
for genotypic detection of *bla<sub>TEM</sub>* gene (516 bp), about 76.5% (13/17 isolates) harbored *bla<sub>TEM</sub>* gene (Figure 3).



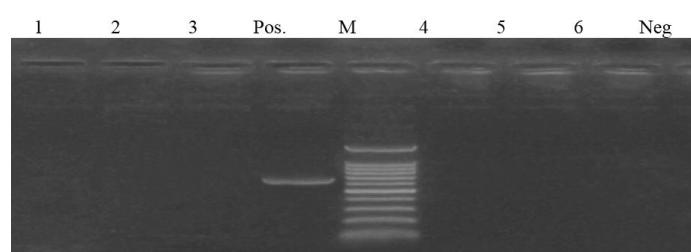
**Figure 1:** Lanes 1, 6, 8, 13, 15, 16: positive amplification of *tetA* gene at 576 bp  
Pos.: positive control; Neg.: negative control; M: DNA ladder.



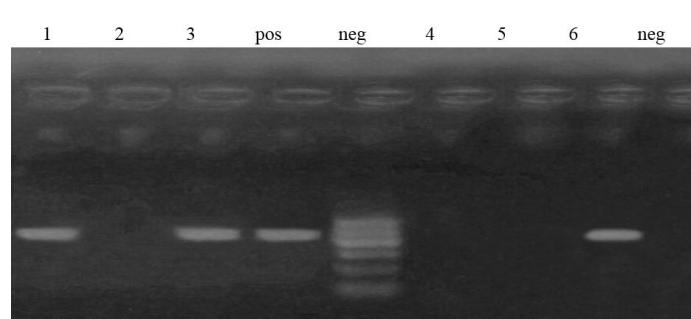
**Figure 2:** Lanes 1-8: positive amplification of *tetB* gene at 633 bp.  
Neg: negative control; pos: positive control; M: DNA ladder.



**Figure 3:** Lane 1-7, 9, 10, 14, 15- 17: positive amplification of *bla<sub>TEM</sub>* gene at 516pb fragment.  
Neg: negative control; pos: positive control; M: DNA ladder.



**Figure 4:** Lane 1- 6 negative amplification of *aadA2* gene at 622 bp.  
Neg: negative control; pos: positive control; M: DNA ladder.



**Figure 5:** Lane 1, 3, 6 positive amplification of *aadA1* gene at 484 bp.  
Neg: negative control; pos: positive control; M: DNA ladder.

## AMPLIFICATION OF *AADA1* AND *AADA2* GENES IN STREPTOMYCIN RESISTANCE ISOLATES.

Out of 6 streptomycin resistant isolates, *aadA1* and *aadA2* genes were detected in a percentage of 50% (3 isolates) and 0%, respectively (Figures 4 and 5).

Many researchers identified resistant genes among *E. coli* isolates (Sunde and Norstrom, 2006) and reported that *aadA1*, *bla<sub>TEM</sub>*, *tetA* and *tetB* genes are responsible for streptomycin, ampicillin, and tetracycline resistance.

Multi-drug resistance was recognized in 32% of the *E. coli* isolates by Guerra et al. (2003). The most prevalent resistances were detected against tetracycline, sulfamethoxazole, ampicillin, streptomycin, and spectinomycin, the predominant resistance genes were: ampicillin, *bla<sub>TEM</sub>* 1-like (92%), streptomycin, *aadA1* (61%), tetracycline, *tetA* (66%) and *tetB* (42%). One-third of the *E. coli* isolates recovered by Jouini et al. (2009) had MDR phenotypes. The highest resistance was detected against ampicillin, tetracycline and streptomycin. Different variants of *bla<sub>TEM</sub>*, (*tet* - *aadA*), and *aac* (3) genes were recovered from most *E. coli* isolates resistant to ampicillin, tetracycline and streptomycin, respectively. High-resistance profile was observed in most *E. coli* isolated by Martínez- Vázquez et al. (2018), about 92.4% of the isolates showed a multi-drug resistant phenotype with resistance to ampicillin (92%) and tetracycline (75%), *tetA* and *tetB* were detected in 56% of isolates while *aadA* in 17%. Recent study concluded that the highest prevalence of resistant *E. coli* isolated from milk and dairy products was against ampicillin (100%), gentamicin (100%) and tetracycline (96.87%), the most commonly detected antibiotic resistance genes was *tetA* gene (76.56%) (Ranjbar et al., 2018).

## CONCLUSIONS

Based on the previous results, it was concluded that milk, meat and their products may play a role in the spread of MDR *E. coli*, which is considered a reservoir of many antibiotic resistance genes that can be conveyed to other pathogenic bacteria representing a potential public health threat.

## AUTHORS CONTRIBUTION

All authors contributed equally.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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