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Bacteriological and molecular studies on antibiotic resistant *Escherichia coli* isolated from meat and its products in Kaliobia, Egypt

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A B S T R A C T

The present study was performed on 175 random samples (about 250 g for each) of fresh meat and meat products viz: Sausage, beef burger, luncheon and Kofta (35 for each), were collected from different shops at Kaliobia Governorate, Egypt, for detection of *E. coli* and phenotypic characterization as well as virulence and antibiotic resistant genes in them. Bacteriological examination indicated the isolation of 11 (6.3%) isolates of *E. coli* from 175 samples as kofta, sausage samples (3= 8.6% for each) followed by fresh meat, beef burger samples (2=5.7% for each) and luncheon (1=2.9%). Accordingly, *E. coli* strains were serotyped as one O26:H11 from beef burger samples; four O_{55}:H_7 (one from each samples of fresh meat, luncheon, kofta and sausage); two O_{111}:H_4 (one from each samples fresh meat and kofta); one O_{119}:H_4 from sausage samples and three O_{125}:H_{18} (one from each samples beef burger, kofta and sausage). The antibiotic sensitivity profile revealed that ,the isolated *E. coli* strains were highly resistant for methicillin and oxacillin followed by amoxicillin; ampicillin; oxytetracycline; streptomycin; erythromycin; Nalidixic acid. Meanwhile, they were highly sensitive to enrofloxacin and gentamycin followed by norfloxacin; cefotaxime and ciprofloxacin. PCR declared that ***eae*A; *bla*TEM; *tet*A(A)** and ***aada*1** genes were amplified in all four studied *E. coli* strains giving product of 248 bp; 516 bp; 576 bp and 484 bp, respectively. The study concluded that; antibiotic resistances *E. coli* is meat-borne pathogen of public health important.

Key words: *Meat products, E. coli, antibiotic resistant genes*

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1. INTRODUCTION

Meat and meat products serve as important source of proteins for humans. However, recently the emerging antibiotic resistant foodborne pathogens combined with the injudicious use of antibiotics in animals bears considerate public health threats worldwide (Messele *et al*., 2017). *Escherichia coli* is commonly non-virulent but some strains have adopted pathogenic or toxigenic virulence factors that make them virulent to human and animals. It has become recognized as a serious food borne pathogen and has been associated with numerous out breaks of disease resulting from contaminated meat products (Gi *et al.*, 2009 and Datta *et al.*, 2012). Pathogenic *E. coli* strains have been divided into intestinal pathogenic *E. coli* and extra intestinal pathogenic *E. coli* (ExPEC) depending on the location of the infection they are causing. EPEC strains are responsible for a variety of infections, including bacteremia, urinary tract infections, neonatal meningitis, pneumonia, deep surgical wound infections, endovascular infections, vertebral osteomyelitis, and septicemia (Russo and Johnson, 2000 and Kaper *et al.*, 2004).

The wide spread and imprudent use of antibiotics in food animals is thought to be

accountable for the emergence and wider spreading of antimicrobial resistant (AMR) bacteria in humans (Aslam *et al*., 2009 and Messele *et al*., 2017). Antimicrobial resistant may be acquired or intrinsic resistance. Acquired antibiotic resistance, in which a previously sensitive bacterium becomes resistant and the majority of them are propagated through horizontal or lateral gene transfer between bacteria often due to the polymicrobial nature of infections and proximity of pathogens (Rodríguez *et al*., 2013 and Juhas, 2015). In addition; intrinsic resistance relates to the unique physiological properties of a microorganism, in which their metabolic activity is substantially unaffected by the presence of an antimicrobial compound. Such resistances are generally chromosomally encoded, and are typically responsible for observed differences in resistance observed between genera, species and strains of bacteria. It can be associated with differences in cell wall structures, the ability to pump antimicrobial compounds out of the bacterial cell using efflux pumps, or the production of enzymes capable of inactivating antimicrobial compounds within the bacterial cell (Russell, 2001 and Gilbert and McBain, 2003). Consumption of contaminated and/or uncooked meat poses the risks of acquiring foodborne *E. coli* strains (Frye and Jackson, 2013) causing a serious public health hazard. Such strains easily harbor antibiotic resistant genes from one another. This is because genes encoding AMR determinants that are carried on mobile genetic elements such as plasmids and transposes of some bacterial strains could be transferred to other bacteria strains during contact causing a threat to cure acute infections in man and animals (Van den Bogaard and Stobberingh, 2000).

Beside the antimicrobial resistance among *E. coli* strains, the pathogenicity of them could be attributed to their virulence factors including those encoding for adhesions (F1, P, and *stg* fimbriae, curli, and *eae*A); anti-host defense factors (*omp*A, *iss*, lipopolysaccharide, and K1); iron acquisition systems (aerobactin, Iro proteins, yersiniabactin, and the Sit iron acquisition locus); auto transporters (*tsh*, *vat* and *aat*A); the phosphate transport system, sugar metabolism, and the *ibe*A protein (Germon *et al*., 2005; Zhao *et al*., 2009; Bisi-Johnson *et al*., 2011; Le Bouguenec and Schouler, 2011 & Van der and Bragg, 2012).

As food-borne bacteria specially antimicrobial resistant ones constitutes serious problems for consumers, therefore, the present study was conducted to detect *E. coli* in meat and meat products (luncheon; kofta; beef burger and sausage) at Kaliobia Governorate, beside the phenotypic characterization of the isolates and determination of virulence and their antibacterial resistant genes.

2. MATERIALS AND METHODS

2.1. Samples

A total of 175 random samples (about 250 g for each) of fresh meat and meat products *viz*: Sausage, beef burger, luncheon and Kofta (35 for each), were collected from different shops at Kaliobia Governorate

2.2. Bacteriological examination

Twenty five grams of each sample were prepared for bacteriological examination according to APHA (2001).

2.2. Isolation and identification of E. coli (Quinn *et al*., 2002)

One ml of prepared sample was inoculated into nutrient broth and incubated aerobically at 37ºC for 12 hours. A loopful from incubated nutrient broth was streaked on MacConkey's agar plates and incubated for 24 hours at 37ºC. Suspected lactose fermented colonies were picked up and streaked on the following media: Eosin methylene blue (EMB); Brilliant Green agar (BG) and Xylose Lysine Deoxycholate (XLD) agar and incubated for another 24-48 hours at 37ºC. Suspected colonies (colonies with metallic green sheen on EMB; yellow colonies on BG and bright yellow colonies and agar on XLD) were picked up and kept in Semi-solid nutrient agar. The purified isolates of *E. coli* were morphologically identified by Gram stain; biochemical tests and serologically by slide agglutination test using *E. coli* antisera of DENKA SEIKEN CO., LTD.TOKYO, Japan according to Edward and Ewing (1972) and Quinn *et al*. (2002).

2.3. *In-Vitro* anti-microbial sensitivity test

The isolated *E. coli* strains were subjected to the sensitivity test against different antibiotics) using the disc and agar diffusion method

(Koneman *et al.*, 1997) and interpretation of results were carried out according to NCCLS (2007).

2.4. Detection of Virulence and antibiotic resistant genes of E. coli by PCR

PCR was applied by using four sets of primers for detection of four virulence and antibiotic resistant genes that may play a role in virulence of *E. coli*. These genes were intimin or *E. coli* attaching and effacing gene (*eae*A); β-lactamase ampicillin resistance gene (*bla*TEM); tetracycline resistant A gene (*tet*A) and streptomycin resistant gene (*aada*1).
It was applied on four random isolated *E. coli* that showed antibiotic resistant by disk diffusion method to the same studied strains following QIAamp® DNA Mini Kit instructions (Catalogue no. 51304), Emerald Amp GT PCR mastermix (Takara) with Code No. RR310A and 1.5% agarose gel electrophoreses (Sambrook *et al*., 1989) using the Primers sequences, target genes, amplicons sizes and cycling conditions showed in Table (2).

3. RESULTS

The recorded results in Table (3) revealed a total of 11 (6.3%) isolates of *E. coli* were recovered from 175 samples and were isolated from, kofta, sausage samples (3= 8.6% for each) followed by fresh meat, beef burger samples (2=5.7% for each) and luncheon (1=2.9%).
The recovered isolates are Gram-negative, medium sized rods, arranged singly, pairs and groups and motile. They grow well on different media and showed rounded, non-pigmented colonies on Nutrient agar medium, while on MacConkey's agar medium showed rounded, non-mucoid bright pink colonies (lactose fermenter) on the surface of the medium. On Eosin methylene blue agar (EMB) showed a distinctive greenish metallic sheen colonies; yellow colonies on Brilliant Green agar (BG) and bright yellow colonies and agar on Xylose Lysine Deoxycholate (XLD) agar.
The results of biochemical identification showed that, all isolates had characteristic biochemical reaction to be *E. coli*, where, all the 11 isolates were positive for indole test; Methyl red test; catalase test; sugar fermentation test; nitrate reduction test; Eijkman test. . Meanwhile, they were negative for oxidase; Voges-Proskauer; Urease; citrate utilization and gelatin hydrolysis tests. The results of serological examination revealed that, *E. coli* strains were typed as one O26:H11 (9.1%) from beef burger samples; four O55:H7 (36.4%) from fresh meat, luncheon, kofta and sausage samples; two O111:H4 (18.2%) from fresh meat and kofta samples; one O119:H4 (9.1%) from sausage samples and three O125:H18 (27.3%) from beef burger, kofta and sausage samples.
In-vitro sensitivity test (Table, 4) revealed that the *E. coli* isolates were highly resistant for methicillin and oxacillin (90.9%) followed by amoxicillin; ampicillin and oxytetracycline (81.8% for each); streptomycin (72.7%) and erythromycin, Nalidixic acid (63.6% for each). Meanwhile, they were highly sensitive to enrofloxacin and gentamycin (90.9%) followed by norfloxacin (81.8%) and cefotaxime, ciprofloxacin (72.7% for each). Moreover, they were intermediate sensitive to trimethoprim/ sulphamethoxazol (63.6%).
PCR results showed that, *eae*A; *bla*TEM; *tet*A (A) and *aada*1 genes were amplified in all four studied *E. coli* strains giving product of 248 bp.; 516 bp.; 576 bp. and 484 bp., respectively as shown in Fig. (1-4).

Table (1): Antisera used in serological identification of *E. coli*

Polyvalent Sera	Monovalent sera						
Polyvalent 1	O1	O26	O86a	O111	O119	O127a	O128
Polyvalent 2	O44	O55	O125	O126	O146	O166	
Polyvalent 3	O18	O114	O142	O151	O157	O158	
Polyvalent 4	O6	O27	O78	O148	O159	O168	
Polyvalent 5	O20	O25	O63	O153	O167		
Polyvalent 6	O8	O15	O115	O169			
Polyvalent 7	O28ac	O112ac	O124	O136	O144		
Polyvalent 8	O29	O143	O152	O164			

Table (2): Oligonucleotide primers sequences source Metabion (Germany)

Target M.O.	Gene	Primer sequence (5'-3')	Length of amplified product	Reference
E. coli	***TetA(A)***	GGTTCACTCGAACGACGTCA CTGTCCGACAAGTTGCATGA	576 bp.	**Randall *et al.* ,2004**
	Aada1	TATCAGAGGTAGTTGGCGTCAT GTTCCATAGCGTTAAGGTTTCATT	484 bp.	
	blaTEM	ATCAGCAATAAACCAGC CCCCGAAGAACGTTTTC	516 bp.	**Colom *et al.*, 2003**
	eaeA	ATG CTT AGT GCT GGT TTA GG GCC TTC ATC ATT TCG CTT TC	248 bp.	**Bisi-Johnson *et al.*, 2011**

Table (3): Prevalence of *E. coli* strains in the examined samples (n=35 for each sample)

Samples	*E. coli* strains	
	NO.	%*
Fresh meat	2	5.7
luncheon	1	2.9
Kofta	3	8.6
Beef Burger	2	5.7
Sausage	3	8.6
Total (175)	11	6.3

* Percentage in relation to total No. of each examined samples (35 for each).

Table (4): In-Vitro anti-microbial Sensitivity test for isolated *E. coli*

Antimicrobial agents	Disk concentrations	Sensitive		Intermediate		Resistant		
		No.	%	No.	%	No.	%	AA
Amoxicillin	25µg	1	9.1	1	9.1	9	81.8	R
Ampicillin	20 µg	1	9.1	1	9.1	9	81.8	R
Cefotaxime	30 µg	8	72.7	2	18.2	1	9.1	S
Ciprofloxacin	5 µg	8	72.7	3	27.3	0	0.0	S
Enrofloxacin	5 µg	10	90.9	0	0.0	1	9.1	S
Erythromycin	15 µg	2	18.2	2	18.2	7	63.6	R
Gentamicin	10 µg	10	90.9	0	0.0	1	9.1	S
Methicillin	5 µg	0	0.0	1	9.1	10	90.9	R
Nalidixic acid	30 µg	3	27.3	1	9.1	7	63.6	R
Norfloxacin	10 µg	9	81.8	2	18.2	0	0.0	S
Oxacillin	1 µg	0	0.0	1	9.1	10	90.9	R
Oxytetracycline	30 µg	1	9.1	1	9.1	9	81.8	R
Streptomycin	S/10	1	9.1	2	18.2	8	72.7	R
Trimethoprim/ Sulphamethoxazol	(1.25/ 23.75) mcg	2	18.2	7	63.6	2	18.2	IS

No.: Number of isolates AA: Antibiogram activity
% Percentage in relation to total number of isolated *E. coli* (11)

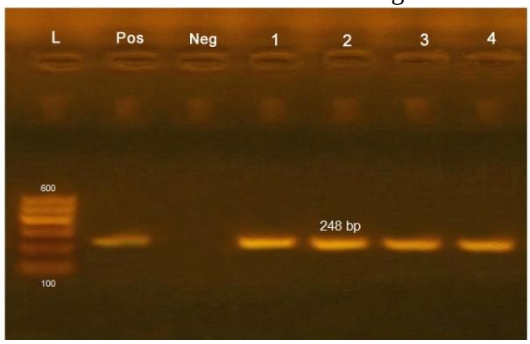

Fig. (1): Agarose Gel electrophoresis of Intiman or *E. coli* attaching and effacing **(*eae*A)** gene**.**
Lane L: 100-600 bpDNA Ladder.
Neg.: Negative control.
Pos.: Positive control (at 248 bp).
Lane 1 -4: *E. coli* (Positive).

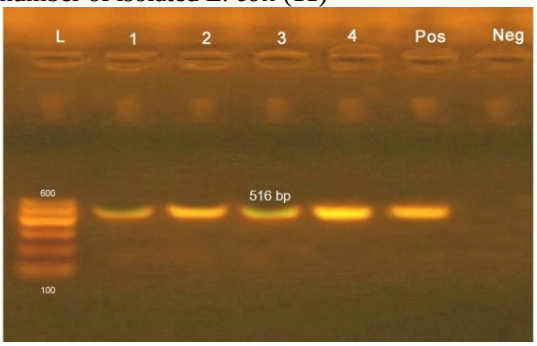

Fig. (2): Agarose Gel electrophoresis of β-lactamase ampicillin resistance gene **(*bla*TEM).**
Lane L: 100-600 bp DNA Ladder.
Neg.: Negative control.
Pos.: Positive control (at 516 bp).
Lane 1 - 4: *E. coli* (Positive).

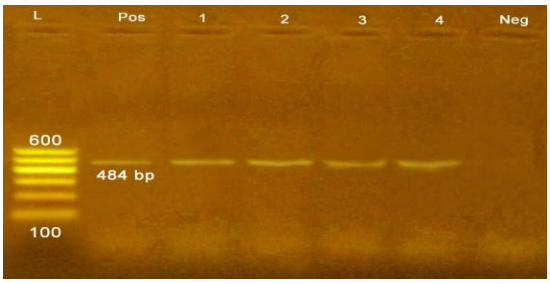

Fig. (3): Agarose Gel electrophoresis of tetracycline resistant **(*tet*A(A)** gene**.**
Lane L: 100-600 bp DNA Ladder.
Neg.: Negative control.
Pos.: Positive control (at 576 bp).
Lane 1 - 4: *E. coli* (Positive).

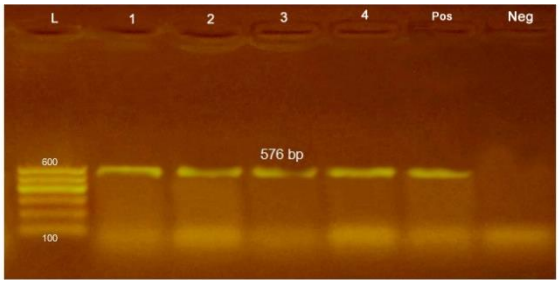

Fig. (4): Agarose Gel electrophoresis of streptomycin resistant (aada1) gene.
Lane L: 100-600 bp DNA Ladder.
Neg.: Negative control.
Pos.: Positive control (at 484 bp).
Lane 1 -4: E. coli (Positive).

4. DISCUSSION

Escherichia coli is one of the most important bacterial pathogens in meat and its products that are responsible for food-borne infections, illness and death all over the world especially antimicrobial resistant ones (Binsy *et al*., 2016). The results of bacteriological examination of examined samples (Table, 3) revealed that *E. coli* were isolated from, kofta, sausage samples (8.6% for each), fresh meat, beef burger samples (5.7% for each) and luncheon (2.9%). Nearly similar results were obtained by Abd El-Salam-Azza (2014); Armany *et al.* (2016) ; Tarabees *et al.* (2015) and El-Rais, Eman(2018).Meanwhile, these results were disagreed with those of Phillips *et al.* (2006); Nychas *et al.* (2008); Abdaslam *et al.* (2014); Gwida *et al.* (2014); Abd El-Tawab *et al.* (2015) ; Adwan *et al.* (2015) and Ramadan (2015) who isolated *E. coli* from raw meat and meat products with high incidence. In addition, the results were disagreed with Wehab and Hegazy (2007) who could not isolate *E. coli* from beef burger samples with Siriken *et al.* (2006) who failed to isolate *E. coli* from beef sausage samples.

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 (Burbutashvili *et al.*, 2007; Raji *et al.*, 2007 and Surendraraj *et al.*, 2010). In addition, the results of serological examination revealed that, *E. coli* strains were typed as one O26:H11 (9.1%) from beef burger samples; four O55:H7 (36.4%) from fresh meat, luncheon, kofta and sausage samples; two O111:H4 (18.2%) from fresh meat and kofta samples; one O119:H4 (9.1%) from sausage samples and three O125:H18 (27.3%) from beef burger, kofta and sausage samples. These results came in harmony with

those of Kalchayanand *et al.* (2012); Mansour (2013); Mohammed *et al.* (2014); Abd El-Tawab *et al*.(2015) and Tarabees *et al.* (2015) who detected the same serotypes of *E. coli* from meat and meat product samples.

In- vitro sensitivity tests for the isolated *E. coli* (Table, 4) showed that, they were highly resistant for methicillin and oxacillin followed by amoxicillin; ampicillin; oxytetracycline; streptomycin; erythromycin and Nalidixic acid. Meanwhile, they were highly sensitive to enrofloxacin and gentamycin followed by norfloxacin; cefotaxime and ciprofloxacin. Moreover, they were intermediate sensitive to trimethoprim/ sulphamethoxazol. Nearly similar were obtained by Altalhi *et al*. (2010); Li *et al.* (2011); Amosun *et al*. (2012); Zhao *et al*. (2012) and Abd El-Tawab *et al*. (2015). Moreover, the results proved that multiple antibiotic resistances are widely spread among isolated strains of *E. coli*. These observations agreed with the reports of Raji *et al.* (2007). It is of serious concern because these drugs are still considered the most recommended for the treatment of colibacillosis in both animal and human. In addition, antibiotic resistance in *E. coli* is of particular concern because it is the most common Gram-negative pathogen in humans, the most common cause of urinary tract infections, a common cause of both community and hospital-acquired bacteremia, as well as a cause of diarrhea(Kaper *et al*., 2004) .In addition, resistant *E. coli* strains have the ability to transfer antibiotic resistance determinants not only to other strains of *E. coli*, but also to other bacteria within the gastrointestinal tract and to acquire resistance from other organisms(Österblad *et al*., 2000).

The PCR technique is capable of identifying the pathogenic *E. coli* isolates based on the fact that virulence and antibiotic resistant genes varies not only among different species but also among strains of the same species. Thus, numerous studies have been conducted to identify virulence factors and antibiotic resistant genes of isolated *E. coli* strains (Kaper *et al*. ,2004; Ryu *et al*. ,2012 and Ahmed and Shimamoto ,2015). These genes were intimin or *E. coli* attaching and effacing gene (*eae*A); β-lactamase ampicillin resistance gene (*bla*TEM); tetracycline resistant A gene (*tetA*(A) and streptomycin resistant gene (*aada*1). The results of PCR cleared that, *eae*A; *bla*TEM; *tet*A(A) and *aada*1 virulence genes were detected in all 4 studied strains.

Regarding to the *E. coli* attaching and effacing *eae*A gene, the results of PCR amplification of *eae*A gene in *E. coli* isolates (Fig. ,1) showed that, the *eae*A gene was amplified in all four studied *E. coli* strains giving product of 248 bp. Similar findings were recorded by Kaper *et al*. (2004); Ayse *et al.* (2007); Ojo *et al*.(2010) and Bisi-Johnson *et al*.(2011). Meanwhile, for β-lactamase ampicillin resistance (*bla*TEM) gene, the results of PCR amplification of *bla*TEM gene in *E. coli* isolates (Fig., 2) showed that, the *bla*TEM gene was amplified in all of the four studied *E. coli* strains giving product of 516 bp. Similar detection of *bla*TEM gene in *E. coli* strains were recorded by Colom *et al*. (2003); Sunde and Norstrom (2006); Van *et al.* (2008); Aslam *et al.* (2009); Ryu *et al*. (2012); Hemati *et al*. (2014) and Ahmed and Shimamoto (2015). For tetracycline resistant *tet*A (A)gene , the results of PCR amplification of *tet*A(A) gene in *E. coli* isolates (Fig., 3) showed that, the *tet*A(A)gene was amplified in all four studied *E. coli* strains giving product of 576 bp .Similar detection of *tet*A(A)gene in *E. coli* strains were recorded by Randall *et al*. (2004); Sunde and Norstrom (2006); Van *et al.* (2008); Aslam *et al.* (2009); Gao *et al*. (2012); Momtaz *et al*. (2012) and Ryu *et al*. (2012). Moreover, for streptomycin resistant *aada*1 gene, the results of PCR amplification of *aada*1 gene in *E. coli* isolates (Fig. , 4) showed that, the *aada*1 gene was amplified in all four studied *E. coli* strains giving product of 484 bp. Similar detection of *aada*1 gene in *E. coli* strains were recorded by Randall *et al*. (2004); Sunde and Norstrom (2006); Van *et al.* (2008) ;Aslam *et al.* (2009) and Ryu *et al*. (2012).

Finally, the results proved that multiple antibiotic resistances are widely spread among isolated strains of *E. coli* and decided the fact of Shalini and Rameshwar (2005) that the food chain can be considered as the main route of transmission of antibiotic resistant bacteria between the animal and human populations. Therefore, it was concluded that*; E. coli* especially antibiotic resistances ones are meat-borne pathogens of public health important.

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