Challenge of multi drug resistant stx1 harboring *E. Coli* in meat and fast foods

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A B S T R A C T

A total of 120 random samples of fresh (30) and processed (90) beef and poultry meat products were collected from different supermarkets in Menoufia governorates .these samples were examined for the presence of multi –drug resistant Stx1 harboring *E. Coli*, the obtained results revealed that the highest incidence of *E.COLI* in processed samples were recorded in chicken fahita (46.66 %), while beef burger showed the lowest incidence(20%),but the highest incidence in fresh samples were recorded in chicken thigh(40%),on the other hand, chicken breast and fresh beef were the same incidence (20%).The illustrated results showed that the serovars O2:H6 producing Stx1,while the serovars O91:H21,O86,O153:H2 and O44:H18 which isolated from different products producing Stx2, these serovars showed multi-drug resistance to major group of antimicrobials.

KEYWORDS: Meat products, E. *coli*, Stx1, Antibiotic resistance.

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1. INTRODUCTION

Meat and meat products are one of major sources of high quality proteins containing most essential amino acids which built and repair body tissues for maintenance of life, meat also contains vitamins and minerals(Abd-Allah,2005). They are good media for many organisms to grow because their high moisture contents, rich in nitrogenous compounds (amino acids, peptides, proteins) furthermore, they have some fermentable carbohydrates usually glycogen and keep favorable *PH* for growth of microorganisms (Galvez *et al.*,2010). The presence of *E. coli* in raw food of animal origin can be expected because of the close contact of the food with the animal environment and contamination of the carcass from fecal material, hide during slaughtering and dressing procedures. These organisms destroyed by heat processing of food, thus, the presence of *E.coli* in a heat treated food means either process failure or more commonly post processing contamination from equipment, employees or from contact with contaminated raw food(National Academy of Science ,1985). Pathogenic *E. coli* have been broadly classified into two major categories; the diarrheagenic *E. coli* and the extra intestinal pathogenic *E. coli*. Among the diarrheagenic *E. coli*, there are currently six categories including enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), entero-invasive *E. coli* (EIEC), entero-aggregative *E. coli* (EAEC), diffusively adherent *E. coli* (DAEC) and enterohemorrhagic *E. coli* (EHEC)/Shiga toxin-producing *E. coli* (STEC) (Xiaodong, 2010). Shiga toxin–producing *E. coli* (STEC), also known as verotoxin-producing *E. coli* (VTEC) or enterohaemorrhagic *E. coli* (EHEC), have been known as a group of highly pathogenic *E.coli* strains producing one or more Shiga toxins (Monaghan *et al., 2011*),the resistance of various serotypes of *E.COLI* to different antimicrobials revealed that *E.COLI* were completely resistant to trimethoprim , sulfamethoxazole and gentamycin(100%)*(*Harakeh *et al.,2005).* Contamination of such meat products with some foodborne microorganisms as *E. coli* during further processing make us in need to use rapid and accurate methods for their detection and to establish appropriate control measures to get red of such organisms.

2. MATERIAL AND METHODES

2.1. Collection of samples:

A total of 120 random samples divided to fresh and processed samples included Beef samples (Sausage, Burger and Shawrma) (15 of each) and fresh beef (10)samples and Chicken samples(shawrma, pane and fahita) (15 of each) and fresh thigh& breast(10 for each) were

collected from different markets in Ashmoun city, Menofia governorate. The collected samples were transferred in an ice box to the laboratory without undue delay.

2.2. Preparation of the samples (APHA, 2004)

25 grams of the examined meat product samples were transferred to 225 ml of sterile buffered peptone water (0.1%) then homogenized by stomacher (Seward stomacher 80 Biomaster, serial No. 46464, England) for 2 minutes to provide a homogenate of 1/10 dilution.

2.3. Isolation and identification of E.coli (APHA, 2004)

2.4. Identification of Enteropathogenic E.coli: Suspected isolation of *E.coli* were identified according to MacFadden (2000)

2.4.1. Morphological examination

2.4.2. Staining (Cruickshank et al., 1975)

2.4.3. Motility test (ICMSF, 1996)

2.4.4. Biochemical identification (Kreig and Holt, 1984)

2.4.5. Serological identification of isolated E. coli (Kok et al., (1996)

2.5. Identification of shiga toxin (Stx) by (PCR).

2.5.1. Extraction of DNA

2.5.2. Preparation of PCR Master Mix according to 2X DreamTaq Green mastermix kit

2.5. 3. Preparation of duplex PCR Master mix for stx1 and stx2 genes

2.5.4. Cycling conditions of the primers during cPCR

2.5.5. Agarose gel electrophoreses (Sambrook et al., 1989) with modification

2.6. Anti-microbial sensitivity test for the isolated stx harboring E.coli strains: according to Finegold and Martin (1982)

3. RESULTS

Table (1) reported that the incidence of *E.coli* in examined beef products of beef burger, sausage, shawrma and fresh Samples were 20% ,33.33%, 33.33%, 20%, respectively. In addition, the serotyping of isolated *E. coli* were O26:H11(33.33%), O111:H2(33.33%), O127: H6(33.33%) in beef burger, O26:H11 (40%), O55:H7 (20%), O127:H6 (20%), O124 (20%) in beef sausage, (2) O111:H2(40%) O91:H21(20%) , O113:H7(20%) O86 (20%) in beef shawrma andO26:H11(50%), O55:H7 (50%) in fresh beef. Also, the illustrated results revealed that the serovar O91:H21 producing Stx2 (20%) and sensitive to streptomycin (S) and neomycin (N), while resistant to amoxycilin clavuilinc acid (AMC), streptomycin(S) and norfloxacin(NOR), in addition to serovar O86 also producing Stx2 (20%) and resistant to amoxycilin clavuilinc acid (AMC), norfloxacin (NOR) and doxycyclin (DO), but sensitive to streptomycin (S), neomycin (N), erythromycin (E) and gentamycin (CN). Table (2) showed the incidence of *E. COLI* in the examined poultry products of; chicken, pane, Shawrma, fahita, chicken breast, chicken thigh was 26.66%,40% ,46.66%,20%, and 40%, respectively. Also, The serotyping of the isolated *E. COLI*, O153:H2(25%)O2:H6 (50%)O142:H6 (25%) in chicken pane O2:H6 (16.66%), O119:H6(33.33%), O44:H18(16.66%) ,O26:H11 (16.66%) , O78 (16.66%) in chicken shawrma and O153:H2 (14.28%), O1:H7 (14.28%) O44:H18 (28.57%) , O126:H21 (14.28%) and O78(28.57%) in chicken fahita, while, in chicken breast O153:H2 (50%) , O78 (50%) in chicken breast ,but O2:H6 (25%) ,O1:H7 (25%)O78 (50%) in chicken thigh. Moreover , the incidence of Stx1 and Stx2 from the isolated serovars of poultry products by using PCR technique, of O2:H6(50%) from chicken panne,O2:H6 of chicken shawrma (16.66%) and,O2:H6 from chicken thigh (25%)were Stx1 producing and resisting to (amoxicillin clavulinic acid, doxycyclin) and sensitive to (streptomycin erythromycin, gentamycin) but O44:H18(28.57%),O153:H2(14.28%)from chicken fahita , ,O153:H2 (50%) from chicken breast were producing Stx2, the sensitivity of the Stx2 harboring E.COLI of poultry productes showed multi drug resistance to (AMC,DO,S and E),but sensitive to (neomycin) but other strains resistant to (AMC and DO), and sensitive to (streptomycin, E and gentamycin).

4. DISCUSSION

The results of the incidence of *E.COLI* in processed meat products were agreed to some extent with what reported by Fathi and Thabet (2001), Ouf–Jehan (2001), Saleh (2001), Zaki – Eman (2003),but Abou–Hussien and Reham (2004) recorded incidence 12%, 64% in sausage and burger respectively , this results differ from this study in which the incidence is 33.33%, 20% in sausage and burger respectively, EL-eiwa-Nasreen (2003) their results differ to large extent to this study due to they don't detect *E.COLI* in beef burger at all and in sausage the incidence was 12% ,Lee *et al.*(2009) recorded incidence 4% ,6% in fresh beef and fresh poultry respectively these results lower greatly than that recorded in this study .

Table (1): Multi-drug resistant Stx1 *E.coli* contamination in beef and beef products.

Products	No of examined samples	Positive samples NO	%	Serotypes	No	%	Stx1	Stx2	%	Antibiotic Resistance
Beef	15	3	20	O26:H11O111:H2O127:H6	1	33.33	-	-	0	
burger					1	33.33	-	-	0	
					1	33.33	-	-	0	
Beef	15	5	33.33	O26:H11O55:H7	2	40	-	-	0	
sausage				127:H6O	1	20	-	-	0	
				O124	1	20	-	-	0	
					1	20	-	-	0	
Beef shawrma	15	5	33.33	O111:H2	2	40	-	-	0	R
				O91:H21	1	20	-	+	20	(AMC,S,NOR
				O113:H7	1	20	-	-	0	), S(E, N)
				O86	1	20	-	+	20	R (AMC,
										DO,NOR)
										S(S,E,CN,N)
Fresh beef	10	2	20	O26:H11	1	50	-	-	0	
				O55:H7	1	50	-	-	0	
Total	55	15	27.27							

AMC; amoxycillin clavulinic acid, N(neomycin),CN (gentamycin), DO (doxycyclin), S(streptomycin), E (erythromycin), NOR(norfloxacin).

Table (2) Multi –drug resistant Stx1 *E.COLI* contamination in chicken and chicken products

Products	No of examined samples	Positive samples NO	%	Serotypes	No	%	Stx1	Stx2	%	Antibiotic Resistance
Chicken pane	15	4	26.66	O153:H2 O2:H6 O142:H6	1 2 1	25 50 25	- + -	+ - -	25 50 0	R(AMC,S,E,DO) S(CN,N) R(AMC,DO) S(S,E,CN)
Chicken shawrma	15	6	40	O2:H6 O119:H6 O44:H18 O26:H11 O78	1 2 1 1 1 1	16.66 33.33 16.66 16.66 16.66	+ - - - -	- - + - -	16.66 0 16.66 0 0	R(AMC,DO) S(S,E,CN) R(AMC,S,DO,CN) S(N)
Chicken fahita	15	7	46.66	O153:H2 O1:H7 O44:H18 O126:H21 O78	1 1 2 1 2	14.28 14.28 28.57 14.28 28.57	- - - - -	+ - + - -	14.28 0 28.57 0 0	R(AMC,S,E,DO) S(CN,N) R(AMC,S,DO,CN) S(N)
Chicken breast	10	2	20	O153:H2 O78	1 1	50 50	- -	+ -	50 0	R(AMC,S,E,DO) S(CN,N)
Chicken thigh	10	4	40	O2:H6 O1:H7 O78	1 1 2	25 25 50	+ - -	- - -	25 0 0	R(AMC,DO) S(S,E,CN)
Total	65	23	35.38							

AMC; amoxycillin clavulinic acid, N (neomycin),CN (gentamycin), DO (doxycyclin), S(streptomycin), E (erythromycin), NOR(norfloxacin).

The serological identification of *E.COLI* which reported by Abou-Hussien and Reham (2004), Hassan (2007), Lee *et al.*(2009), Mohmmed (2012), Ursula *et al*. (2012), Mohmmed *et al.*(2014),Abou–Hussien and Reham (2007) were agreed to large extent to this study, but Hassan (2007), Azoz –Afaf (2009), Fantelli and Stephan (2001) couldn't detect serotype O157:H7 in their samples these also agreed to the present study , on the other hand Sarimehmetoglu *et al.*(2009), Mewafy and Abeer (2012) isolate O157 from fresh beef, sausage ,burger and ground beef and also producing Stx1,Stx2 but this results differ than the present study due to we cannot detect O157 in fresh beef. The results of poultry products are agreed to large extent to that reported by Lee *et al.* (2009), the current study at which the sensitivity of *E.COLI* to gentamycin are 56.75%, in contrast to that recorded by Harakeh *et al.*(2005) ,they found that the isolated *E.COLI* were 100% susceptible to gentamycin ,also ,in the present study the sensitivity of *E.COLI* to norfloxacin is 56.75%, however, Pavithra and Ghosh (2013) detect that the isolated serotypes were completely resistant to norfloxacin.

5. CONCLUSION

This study revealed that the highest incidence of *E.COLI* were from chicken fahita (46.66%) that producing Stx1 and showed multi drug resistant to most types of the used antimicrobials.

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