Incidence of *E. coli* in some beef and chicken meat products

Aboubakr M. Edris[1], Reham A. Amin[1], Hanan T. Elawandi[2], Aboubakr A. Abdelkader[2], Amina M. El-amin[2]

[1]Department of Food Control, Faculty of Veterinary Medicine, Benha University [2]Animal Health Research Institute, Zagazig Branch

A B S T R A C T

A grand total of 120 random samples of beef and chicken meat products were collected from different supermarkets in Sharkia governorate to be examined for detection of *E. coli*. Beef products were represented by 60 samples of pasterma, luncheon and beef burger (20 of each), while chicken meat products were represented by 60 samples of luncheon, shawerma and shish tawouq (20 of each). The incidences of *E. coli* were5%, 20% and 10% in the examined pasterma, beef luncheon and beef burger samples, while they were 10 %, 5% and 15 % of the examined chicken shawerma, chicken meat luncheon and shish tawouq samples, respectively. Moreover, the isolated serotypes of *E. coli* from the examined samples were O_{26}: H_{11}, O_{44}: H_{18}, O_{78}, O_{91}: H_{21}, O_{111}: H_2, O_{121}: H_7, O_{124}, O_{128}: H_2 and O_{153}: H_2 with various percentages. The obtained results revealed that *E. coli* O_{26}, O_{111} possess (4) virulence genes, *E. coli* O_{91} carry (3) genes stx1, stx2 and hlyA genes, another strain of *E. coli* O_{111} also carry (3) genes stx1, eaeA and hlyA genes. *E. coli* O_{78} possess (2) genes stx1 and stx2 genes, also *E. coli* O_{44}, O_{128} carry (1) gene stx1 gene, *E. coli* O_{121}, O_{153} carry (1) gene stx2 gene, while virulence genes were not detected in *E. coli* O_{124}. Concerning antimicrobial resistance profile of all isolated *E. coli* strains, Gentamicin (G) is the most susceptible one for *E. coli* strains then Kanamycin (K), while Erythromycin (E) and Cephalotin (CN) were the lowest susceptible antimicrobial for *E. coli* strains.

Keywords: *E. coli*, Beef Products, Chicken meat Products, PCR.

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1. INTRODUCTION

Beef and chicken meat products are considered as important products, which attract the consumers for its palatability and easily prepared than the fresh meat. However, it proved to be of high nutrient value, but it is also liable to harbor different types of microorganisms and constitute the largest potential source of food borne illness (Wendlandt et al., 2013). Beef and chicken meat products are subjected to contamination with several types of microorganism from different sources during slaughtering, dressing, preparation, processing, transportation and cooking (Madahi et al., 2014). Most of *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts. Insufficient cooking may result in survival of *E. coli* and subsequently causes food poisoning to consumers. However, *E. coli* is commonly non virulent, but some strains have adapted pathogenic or toxigenic virulence factors that make them serious for man and animals (Antown and Dapgh, 2009). Polymerase chain reaction (PCR) based methods have been identified as a powerful diagnostic tool for detection of pathogenic microorganisms (Wagner, 2008). The present study was conducted to detect the incidence of *E. coli* and detection of Virulence genes using bacteriological and molecular methods in beef and chicken meat products. Actually *E. coli* is commonly non virulent but some strains have adapted pathogenic or toxigenic virulence factors that make them virulent for man and animals These Pathogenic *E. coli* strains include enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enteroaggregative (EAEC), diffusely adherent (DAEC) and enterohemorrhagic (EHEC) types, of which *E. coli* O157:H7 is a member. These pathogenic strains caused human illnesses ranging from bloody diarrhea and hemorrhagic colitis to the life-threatening hemolytic uremic syndrome (HUS) (Madahi et al., 2014).

2. MATERIAL AND METHODS

2.1. Collection of samples:

A total of 120 random samples of beef and chicken meat products (25 grams of each) were collected from different supermarkets in Sharkia governorate to be examined for detection of *E. coli*. Beef products were represented by 60 samples of pasterma, luncheon and beef burger (20 of each), while chicken meat products represented by 60 samples of luncheon, shawerma and shish tawuq (20 of each). The collected samples were kept in separate plastic bags and aseptically transferred in an insulated ice box to the laboratory as rapidly as possible for isolation and identification of *E. coli*.

2.2. 2.2 Preparation of samples: according to APHA (American Public Health Association) (2004)

To 25 grams of the sample, 225 ml of sterile peptone water (0.1%) were added and thoroughly mixed using sterile blender for 1 – 1.5 minutes, from which ten folds' serial dilutions were prepared. The prepared samples were subjected to the following examinations: -

2.3. Isolation and Identification of E. coli: according to International Organization of Standardization "ISO" (2003)

2.3.1. Morphological examination: according to ISO (2003)

2.3.2. Biochemical identification according to (Collins et al., 1991)

2.3.3. Serotyping identification:

The applied technique recommended by Varnam and Evans (1991) was used . The isolated strains of *E. coli* were identified serologically by using rapid diagnostic *E. coli* antisera sets (DENKA SEIKEN Co., Japan) for diagnosis of the Enteropathogenic types.

2.4. Detection of Virulence genes of isolated E. coli strains by mutiplex PCR:

Application of PCR for identification of shiga toxins (stx1 & stx2), intimin (eaeA) and haemolysin (hylA) genes of *E. coli* was performed essentially by using primers (Pharmacia Biotech) as shown in the following table (A):

Table (A): Primers sequences, target genes and amplicon size of the used genes:

Primer	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
stx1 (F)	5′ ACACTGGATGATCTCAGTGG ′3	614	Dhanashree and Mallya (2008)
Stx1 (R)	5′ CTGAATCCCCCTCCATTATG ′3		
Stx2 (F)	5′ CCATGACAACGGACAGCAGTT ′3	779	Dhanashree and Mallya (2008)
Stx2 (R)	5′ CCTGTCAACTGAGCAGCACTTTG ′3		
eaeA (F)	5′ GTGGCGAATACTGGCGAGACT ′3	890	Mazaheri et al. (2014)
eaeA(R)	5′ CCCCATTCTTTTTCACCGTCG ′3		
hylA (F)	5′ ACGATGTGGTTTATTCTGGA ′3	165	Fratamico et al. (1995)
hylA (R)	5′ CTTCACGTGACCATACATAT ′3		

2.5. Antibiogramme for antibiotic:

Sensitivity of isolated strains of antimicrobial susceptibility was tested by the single diffusion method according to Mary and Usha (2013) for *E. coli.* Sensitivity discs with variable concentrations were used to determine the susceptibility of the isolated *E. coli* strains (Oxoid Limited, Basingstoke, Hampshire, UK).

3. RESULTS

It is evident from the results recorded in table (1) that the incidences of *E. coli* were 5%, 20% and 10% in the examined pasterma, beef luncheon and beef burger samples, while they were 10 %, 5% and 15 % in chicken shawerma, chicken meat luncheon and shish tawouq samples, respectively. Beef luncheon in beef product and shish tawouq in chicken meat products showed the highest incidence of E. *coli*. Results achieved in table (2) showed the serological identification of *E. coli* serotypes isolated from the examined beef product samples were *E. coli* O_{26} in pasterma and lunchen samples, O_{111}, O_{121} and O_{153} in luncheon samples, while O_{128} and O_{44} were in beef burger samples. Results achieved in table (3) showed the serological identification of *E. coli* serotypes

isolated from the examined chicken meat product samples were *E. coli* O_{78} and O_{91} in shawerma, O_{124} in luncheon, while O_{128}, O_{26} and O_{111} in shish tawouq. The results in table (4) revealed that the isolated *E. coli* strains were highly sensitive to Gentamicin (G) 92.3%, Neomycin (N) and Kanamycin (K) 69.2%, Ciprofloxacin (CP) 53.8% and Sulphamethoxazol (SXT) 46.2%., *E. coli* strains were resistant to Erythromycin (E) and Cephalotin (CN) 100%, Ampicillin (AM) 84.6%, Oxacillin (OX) 76.9%, Chloramphenicol (C) 69.2%, while Enrofloxacin (EN) and Oxytetracycline (T) were 53.8%.

Table (1): Incidence of *E. coli* in some beef and chicken meat products(n=20):

	+ve samples	
	No	Percentage
1-Beef products		
• Pasterma	1	5%
• Luncheon	4	20%
• Beef burger	2	10%
2-Chicken meat products		
• Shawerma	2	10%
• Luncheon	1	5%
• Shish tawouq	3	15%

Table (2): *E. coli* serotypes isolated from the examined samples of beef products. (n=20)

E. coli	Pasterma		Beef Luncheon		Beef burger		
strains	No.	%	No.	%	No.	%	*Strains*
O26 : H11	1	5	1	5	0	0	(EHEC)
O111 : H2	0	0	1	5	0	0	(EHEC)
O121 : H7	0	0	1	5	0	0	(EPEC)
O153 : H2	0	0	1	5	0	0	(EPEC)
O44 : H18	0	0	0	0	1	5	(EPEC)
O128 : H2	0	0	0	0	1	5	(ETEC)

N.B: % was calculated according to number of samples of each product

Table (3): *E. coli* serotypes isolated from the examined samples of chicken meat products. (n=20)

E.coli	Shawerma		Luncheon		Shish tawouq		
strains	No.	%	No.	%	No.	%	Strains
O78	1	5	0	0	0	0	(EPEC)
O91 : H21	1	5	0	0	0	0	(EHEC)
O124	0	0	1	5	0	0	(EIEC)
O26 : H11	0	0	0	0	1	5	(EHEC)
O111 : H2	0	0	0	0	1	5	(EHEC)
O128 : H2	0	0	0	0	1	5	(ETEC)

N.B: % was calculated according to number of samples of each product

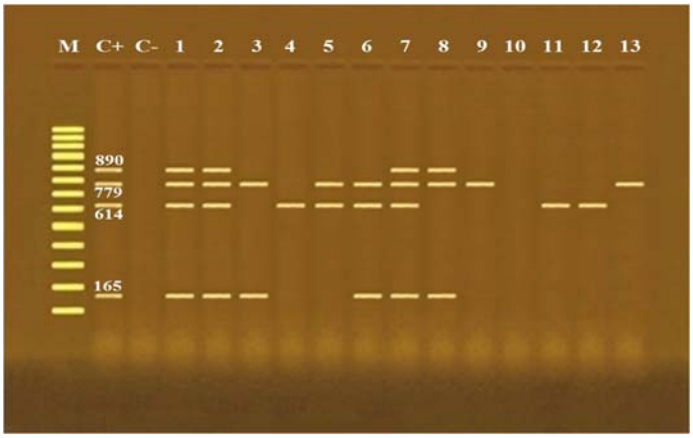
Table (4): Antimicrobial susceptibility of *E. coli* serotypes isolated from the examined samples of beef and chicken meat products (n=13).

Antimicrobial agent	S		I		R	
	NO	%	NO	%	NO	%
Erythromycin (E)	-	-	-	-	13	100
Cephalotin (CN)	-	-	-	-	13	100
Ampicillin (AM)	1	7.7	1	7.7	11	84.6
Oxacillin (OX)	-	-	3	23.0	10	76.9
Chloramphenicol (C)	1	7.7	3	23.0	9	69.2
Enrofloxacin (EN)	2	15.4	4	30.8	7	53.8
Oxytetracycline (T)	4	30.8	2	15.4	7	53.8
Cloxacillin (CL)	4	30.8	3	23.0	6	46.2
Norfloxacin (NOR)	3	23.0	5	38.5	5	38.5
Sulphamethoxazol (SXT)	6	46.2	3	23.0	4	30.8
Ciprofloxacin (CP)	7	53.8	2	15.4	4	30.8
Neomycin (N)	9	69.2	1	7.7	3	23.0
Kanamycin (K)	9	69.2	3	23.0	1	7.7
Gentamicin (G)	12	92.3	-	-	1	7.7

S: sensitive I: intermediate R: resistant

N.B: % was calculated according to positive number of samples

Photo (1): Agarose gel electrophoresis of multiplex PCR of stx1 (614 bp), stx2 (779 bp), eaeA (890 bp) and hlyA (165 bp) genes for characterization of *E. coli.*



Lane M: 100 bp ladder as molecular size DNA marker. Lane C+: Control positive *E. coli* for stx1, stx2, eaeA and hlyA genes. Lane C-: Control negative. Lanes 1, 2 (O26) & 7 (O111): Positive *E. coli* for stx1, stx2, eaeA and hlyA genes. Lane 3 (O26): Positive *E. coli* for stx2 and hlyA genes. Lanes 4 (O44), 11 & 12 (O128): Positive *E. coli* for stx1 gene. Lane 5 (O78): Positive *E. coli* for stx1 and stx2 genes. Lane 6 (O91): Positive *E. coli* for stx1, stx2 and hlyA genes. Lane 8 (O111): Positive *E. coli* for stx1, eaeA and hlyA genes. Lanes 9 (O121) & 13 (O153): Positive *E. coli* for stx2 gene. Lane 10 (O124): Negative *E. coli* for stx1, stx2, eaeA and hlyA genes.

4. DISCUSSION

The presence of *E. coli* in raw products of animal origin may be due to contamination of the carcass from the fecal material, hide during slaughtering and dressing procedures. Thus, the presence of *E. coli* may be due to processing failure or more commonly, post processing contamination from equipment, employees or from contact with contaminated raw products (Wendlandt et al., 2013). The current results of the examined beef samples agreed to some extent, with those reported by Mohammed et al. (2014) (10% in beef burger) and Awadallah et al. (2014) (20% in beef luncheon). Higher results were detected by Torky

(2004) (25% in beef burger) and Mohamed (2014) (33% in beef burger), Armany (2016) (20% in pasterma). While Lower results were obtained by Ismail (2008) (zero in pasterma), Antown and Dapgh (2009) (4% in beef burger) & (zero in luncheon) and Ahmed-Neveen (2016) (6% in beef burger). The results of *E. coli* incidence in chicken meat products agreed to some extent to those recorded by Edris-Shimaa (2012) (14% in shish tawouq), while higher results were obtained by Sharaf and Sabra (2012) (20% of the chicken meat shawarma) and Awadallah et al. (2014) (10% in chicken meat luncheon). Lower results were obtained by Khalifa and Hassan (2005) (zero in luncheon) and Osaili et al. (2014) (zero in shish tawouq). The variations in the results may be due to the differences in manufacture practices, handling from producers to consumers and the effectiveness of hygienic measures applied during production and storage condition and shelf life of each product. Therefore, *E. coli* is considered as an indicator of fecal contamination, besides, it may induce severe diarrhea in infants and young (Osaili et al., 2014). The obtained results concluded that beef luncheon samples were the highest contaminated products which be similar to results those obtained by Mostafa (2015) . Beef products can be easily contaminated with different microorganisms, if not properly handled and preserved, it will support the growth of pathogenic bacteria, causing potential public health problems. The serotypes of *E. coli* isolated in this study as shown in (table2 & 3) were 9 *E. coli* strains belonged to following serotypes: O_{26}, $O_{91,\ O111}$ (EHEC), O_{44}, O_{78}, O_{121}, O_{153} (EPEC), O_{128} (ETEC) and O_{124} (EIEC) which were similar to those isolated by Mostafa (2015) who could isolate O_{128}, O_{26}, O_{111}, O_{91} and O_{121} and Ahmed-Neveen (2016) who could isolate O_{128}, O_{26}, O_{111} and O_{124}. These pathogenic strains as EHEC caused human illnesses ranging from sudden onset of crampy abdominal pain followed by watery diarrhea which later on become grossly bloody (Madahi et al., 2014).

PCR based methods, as multiplex PCR is very useful as it allows the simultaneous detection of several pathogens by introducing different primers to amplify DNA regions coding for specific genes of each bacterial strain targeted (Ghodousi et al., 2015). The use of Multiplex PCR with specific primers for Stx1, Stx2, eaeA and hylA genes revealed the presence or absence of such genes in the tested isolates. These 9 *E.* coli strains were investigated by using multiplex PCR to detect presence of virulence genes stx1, stx2, eaeA and hlyA genes.

The results in photo (1) showed that *E. coli* O_{26}, O_{111} possess (4) virulence genes, *E. coli* O_{91} carry (3) genes stx1, stx2 and hlyA genes, *E. coli* O_{111} also carry (3) genes stx1, eaeA and hlyA genes. *E. coli* O_{78} possess (2) genes stx1 and stx2 genes, also *E. coli* O_{44}, O_{128} carry (1) gene stx1 gene, *E. coli* O_{121}, O_{153} carry (1) gene stx2 gene, while virulence genes were not detected in E. coli O_{124}. These results were nearly similar to those recorded by Mostafa (2015) and Ahmed-Neveen (2016). The previous results showed that PCR technique is considered as rapid and less labor technique for detection of *E. coli* as it can be detected within few hours with very accurate results. PCR can be applied to fixed tissues (frozen), reducing the potential dangers involved in handling of specimen with live virulent pathogen. Also, rapid and sensitive detection techniques for foodborne pathogens are important to food industry instead of using traditional detection methods rely on bacterial cultural in combination with biochemical tests which takes 4-7 days to complete. PCR as rapid detection methods will be very beneficial in microbial food poisoning outbreak to detect all foodborne pathogenic bacteria and detect its virulence. The disadvantages of PCR are it can't allow the microorganisms to be retained for further cultivation beside that the high cost of PCR. Also it depends upon the efficient of DNA extraction, PCR method haven't the ability to distinguish between the DNA of dead and viable cells. PCR method consider as expensive technique.

Concerning antimicrobial resistance profile of all isolated *E. coli* strains, Gentamicin (G) is the most susceptible antimicrobial for *E. coli* strains, then Kanamycin (K), while Erythromycin (E) and Cephalotin (CN) were the lowest susceptible antimicrobial for *E. coli* strains followed by Ampicillin (AM). The development of bacterial antimicrobial resistance is neither unexpected nor a new phenomenon and it will affect human health. Although traditionally , *E.coli* has been one of the most widely antibiotic susceptible member of *Enterobacteriaceae*, recently, horizontal gene transfer has allowed for the rise of highly resistant strains Osaili et al. (2014). Antimicrobial resistance (AMR) threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, without effective antibiotics, the success of major surgery and cancer chemotherapy would be compromised. The cost of health care for patients with resistant infections is higher than care for patients with non-resistant infections due to longer duration of illness, additional tests and use of more expensive drugs. Resistance of *E. coli* to one of the most widely used antibiotics for the treatment of urinary

tract infections (fluoroquinolone) is very widespread. There are countries in many parts of the world where this treatment is now ineffective in more than half of patients. Regarding to these results we could conclude that variation between results of beef products and chicken meat products, this might be mainly attributed to the manner of handling each product, the number of processing operations that the product subjected to them, amount of post processing contamination and storage condition and shelf life of each product. therefore, the need to use modern and rapid technique for detection of such microorganisms by PCR which is accurate and time saving technique. The good manufacturing practice must be followed in order to assure safety and high quality products.

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