Original Paper

Traceability of *Escherichia coli* bacteria in retail meat

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ABSTRACT

Escherichia coli is one of the main pathogenic bacteria found in meat and its products. In this study, we investigated the prevalence and incidence of *E. coli* in raw fresh retail meat in some Egyptian markets. Total random samples of 90 fresh retail beef meat were collected from various butcher shops located in Cairo, Giza and Benha governorates (30 of each). The collected samples were subjected to bacteriological and biochemical analysis to assess their edibility for human consumption according to standard specifications. Serological examination was performed to identify strain types in positive samples. The results showed that the incidence of *E. coli* pathogen in the Cairo, Giza and Benha samples was 13.3, 16.7, and 26.7 %, respectively, with an average incidence 18.9 %. The identified serovars of pathogenic *E. coli* were O26:H11 (3.3%), O111:H2 (6.7%) and O125:H21 (3.3%) in Cairo collected samples, O44:H18 (3.3%), O111:H2 (3.3%), O117:H4 (3.3%) and O127:H6 (6.7%) in Giza collected samples and O15:H2 (3.3%), O26:H11 (10%), O86 (3.3%), O91:H21 (6.7%) and O159 (3.3%) in Benha collected samples. Variable serotypes detected in the collected examined samples indicates a wide range of *E. coli* contamination in raw beef meat samples. Periodical evaluation the hygienic status of Egyptian meat markets is very important as it incorporated with many of food poisoning bacteria with public health hazards

1. INTRODUCTION

Meat is an important compartment of the Egyptians diet. It is one of the main sources of protein, fats, minerals, and vitamins. Most meats have a high moisture content corresponding to a water activity of about 0.99 which is suitable for microbial growth (Rao et al., 2009).

Animal protein sources (meat, meat products, fish, and aquatic products) are generally considered a high-risk commodity in terms of pathogens, natural toxins and other potential contaminants and adulterants. Meat is a good media for bacterial multiplication. Its quality depends on the initial bacterial contamination. This contamination can cause meat to spoil, loss quality and sometimes disease can be caused by bacterial pathogens or their toxins passing through meat and meat products (Yousuf et al., 2008).

E. coli is one of the major bacteria in the human gut and, consider as part of the normal gut flora. Some of gut flora have numerous health benefits for the host, for example, they prevent harmful pathogens from colonizing the gut (FDA, 2012).

There are currently six recognized pathogen groups: enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), and diffusely adherent *E. coli* (DAEC). Of these, the first four are known to be transmitted through contaminated food or water. EHECs in particular are frequently associated with large foodborne outbreaks worldwide (FDA, 2012).

The study was applied to investigate the prevalence of *E. coli* in retailed raw fresh meat in Egyptian markets.

2. MATERIAL AND METHODS

2.1. Collection of samples

A total of 90 fresh retail meat (beef) samples were taken from different butcher shops located in Cairo, Giza and Benha governorates (30 of each). The collected samples were individually placed in sterile plastic bags, labeled, and placed in ice boxes and taken to the laboratory to assess suitability for human consumption according to Egyptian standards. All collected samples were bacteriologically, biochemically, and serologically for detection of *E. coli* pathogens.

2.2 Bacteriological examination:

2.2.1Preparation of samples (ISO 4833-1, 2013):

Under complete sterile conditions, 25 g collected sample was homogenized with 225 ml 2% sterilized peptone water for 2 m then left to stand for 5 minutes at room temperature. One ml from the homogenization was transferred into a separate tube containing 9 ml of sterile peptone water (0.1%) from which ten-fold serial dilution was prepared for enumeration of different groups of

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microorganisms. The prepared samples were subjected to the following examinations:

2.2.2. Screening for Enteropathogenic E. coli:
2.2.2.1. Pre-enrichment (ISO 16649-2, 2001):
From the initial dilutions, one ml was inoculated into MacConkey broth tubes with inverted Durham's tubes. Inoculated tubes were incubated at 37°C for 24 hrs.

2.2.2.2. Enrichment broth:
One ml positive MacConkey tube was inoculated into another MacConkey broth tubes and incubated at 44 °C for 24 hrs.

2.2.2.3. Plating media:
Samples from positive MacConkey broth tubes were individually streaked onto Eosin Methylene Blue agar media (E.M.B.) and incubated at 37 °C for 24 hrs. Suspected colonies (metallic green) were purified and inoculated into inclined nutrient agar tubes for further identification.

2.2.2.4. Morphological identification:
2.2.2.4.1. Microscopically examination (ISO, 1995):
Films from suspected pure cultures were gram stained and examined microscopically. Gram negative, medium size, uniformly colored cocci were suspected of *E. coli*.

2.2.2.4.2. Motility test:
Motility medium was inoculated to a depth of 5 mm by spotting or stabbing technique and then incubated at 37 °C for 24 hrs. A circular growth from residual line represented a positive one.

2.2.2.5. Biochemical identification (McFadden, 2000):
2.2.2.5.1. Indole test:
One ml of ether added to the culture1% peptone water then incubated for 48 hrs. at 37 °C. The tubes were strongly shaken then left and until ether floats on surface. In each tube 0.5 ml of the Kovac's reagent was dropped from the side of the tube. A red ring (surface layer) after 10 minutes was a positive reaction.

2.2.2.5.2. Methyl red test:
Pure culture inoculated into 5 ml buffered glucose broth tubes and incubated at 37 °C for 24 hrs. Five drops of methyl reed reagent were added to each tube. A red appearance was considered a positive test.

2.2.2.5.3. Voges – Proskauer test:
After 48 hrs. of incubation at 37 °C in 5 ml buffered glucose phosphate broth, one ml was placed in a test tube and 0.6 ml of alpha–naphthol (alcohol dissolved) and 0.2 ml of 4% KOH solution were added. The tubes were left for 24 hrs. The pink color of the mixture was positive one.

2.2.2.5.4. Utilization of citrate test:
Prick slanted and blunt Simon citrate agar tubes from pure cultures and incubated at 37 °C for 48 hrs. appearance of blue color indicated using of citrate.

2.2.2.5.5. Urease test:
Suspect isolates were inoculated into Christensen's medium and incubated at 37 °C for 24 hrs. the appearance of pink color indicates urea hydrolysis. Negative tubes recheck after an additional 24 hrs. of incubation.

2.2.2.5.6. Hydrogen sulfite production test:
On Triple Sugar Iron media agar, the detached organisms are pierced into the bottom of the flask with a needle and then pulled through the bevel to create sufficient surface growth. The inoculated tubes were incubated for 24 hrs. at 37 °C. Hydrogen sulfite production was observed by blacking the medium.

2.2.2.5.7. Gelatin hydrolysis test:
Nutrient gelatin stab cultures were grown at room temperature and observed daily after cooling to about 18°C.

2.2.2.5.8. Nitrate reduction test:
To 5 ml of peptone broth containing 0.1% KNO_3, the isolates was inoculated and incubated at 37 °C for 96 hrs., one ml of solution containing 8 g sulphanilic acid in 100 ml of 5 N acetic acid was put and mixed, then a solution containing 5 g of alpha –naphthylamine in 100 ml of 5 N acetic acid was added drop by drop. Appearance of red color indicated positive samples.

2.2.2.5.9. Detection of Ornithine decarboxylase (ODC):
Suspected colonies were inoculated into ornithine decarboxylase medium just below the surface. One ml of sterile mineral oil was added to the top of the medium and incubated at 37 °C for 24 hrs. Turbidity and violet color after incubation indicate a positive ODC.

2.2.2.5.10. Detection of L- lysine decarboxylase (LDC)
Suspected colonies were inoculated into L-lysine decarboxylase medium just below the surface. One ml of sterile mineral oil was added to the top of medium and incubated at 37 °C for 24 hrs. Turbidity and violet color after incubation indicate a positive LDC.

2.2.2.5.11. Detection of Arginine decarboxylase (ADH)
Suspected colonies were inoculated into arginine decarboxylase medium just below the surface. One ml of sterile mineral oil was added to the top of the medium and incubated at 37 °C for 24 hrs. Turbidity and violet color after incubation indicate a positive ADH.

2.2.2.5.12. Detection of β- galactosidase (ONPG):
The suspect colonies were inoculated into a sterile tube containing 2.5% NaCl solution and mixed. A drop of toluene was added, tube was shaken, placed in water bath at 37°C and allowed to stand for about 5 min. 0.25 ml of the reagent was added for detection of β- galactosidase (2-ortho-Nitrophenyl-β-D-galacto-pyranoside) and mixed. The tube was put back in the water bath set at 37 °C and left to stand for 24 hrs. then examined from frequently. A yellow color indicates positive reaction.

2.2.2.5.13. Fermentation of sugars:
To 5 ml peptone water (1%) with 0.2% bromocresol purple indicator, 1% of the following sugars were added (lactose, glucose, sucrose, dulcitol, salicin, arabinose, inositol, and xylose). Durham's tubes were inverted into the test tubes for a collection of a gas. After incubation at 37 °C, the reaction of the inoculated tubes was observed every day for seven successive days. Appearance of pink color indicates positive result.

2.2.2.6. Serological identification of E. coli:
The isolates were serologically identified according to Kok et al. (1996) by using rapid diagnostic *E. coli* antisera sets

(DENKKA SEIKEN Co., Japan) for diagnosis of the Enteropathogenic types.

The diagnostic *E. coli* antisera sets used for identification include Set 1: O- antisera, Polyvalent antisera 1, 2, 3, 4, 5, 6, 7, 8. Set 2: H- sera. H2, H4, H6, H7, H11, H18 and H21.

3. RESULTS

The obtained results in table (1) indicated that the incidence of *E. coli* was 13.3, 16.7, and 26.7 % in Cairo, Giza and Benha collected samples, respectively with mean incidence of 18.9 %.

The identified serovars of pathogenic *E. coli* were O26:H11 (3.3%), O111:H2 (6.7%) and O125:H21 (3.3%) in Cairo collected samples, O44:H18 (3.3%), O111:H2 (3.3%), O117:H4 (3.3%) and O127:H6 (6.7%) in Giza collected samples and O15:H2 (3.3%), O26:H11 (10%), O86 (3.3%), O91:H21 (6.7%) and O159 (3.3%) in Benha collected samples.

Table (2) declared that 86.7%, 83.3% and 73.3% of the examined samples of retail meat in Cairo, Giza and Benha were acceptable. However, the unaccepted samples were 13.3%, 16.7% and 26.7%, respectively

Table 1 Incidence and serotyping of *E. coli* isolated from the examined retail meat samples at Egyptian markets (n=30)

Retail meat	Cairo		Giza		Benha		Strain characteristics
Strains	No.	%	No.	%	No.	%	
O15:H2	-	-	-	-	1	3.3	ETEC
O26:H11	1	3.3	-	-	3	10	EHEC
O44:H18	-	-	1	3.3	-	-	EAEC
O86	-	-	-	-	1	3.3	EPEC
O91:H21	-	-	-	-	2	6.7	EHEC
O111:H2	2	6.7	1	3.3	-	-	EHEC
O117:H4	-	-	1	3.3	-	-	EHEC
O125:H21	1	3.3		-	-	-	ETEC
O127:H6	-	-	2	6.7	-	-	ETEC
O159	-	-	-	-	1	3.3	EIEC
Total	4	13.3	5	16.7	8	26.7	

EPEC = Enteropathogenic *E. coli* ETEC = Enterotoxigenic *E. coli*. EIEC = Enteroinvasive *E. coli* EHEC = Enterohaemorrhagic *E. coli*. EAEC = Enteroadherent *E. coli*

Table 2 Edibility of the examined retail meat samples based on their contamination with *E. coli* (n=30).

Retail meat	*E. coli* /g*	Accepted samples		Unaccepted samples	
		No.	%	No.	%
Cairo	Free	26	86.7	4	13.3
Giza	Free	25	83.3	5	16.7
Benha	Free	22	73.3	8	26.7
Total (90)		73	81.1	17	18.9

* Egyptian Organization for Standardization (2013)

4. DISCUSSION

Bacteriological evaluation is one of the most important ways indicate the suitability and safety of food. Literatures extended over many years were pointed out that bovine meat during slaughtering can be contaminated with various kinds of microorganisms from different sources. The presence of microorganisms on meat surface and their initial number determines the safety, shelf-life, and hygienic quality of meat (Mackey et al., 1993).

Among all microorganisms *E. coli* is a regular contaminating organism and is an indicator of fecal contamination in unsanitary conditions of water, food, milk, and other dairy products (Soomro et al., 2002).

There were a differences between Cairo, Giza and Benha unaccepted samples thus the unaccepted samples in Benha were the highest followed by Giza and finally Benha. However, it is usually difficult to make comparisons between surveys because of differences in objectives, but we suggested the difference because the differences in hygienic status and may Benha city close the agricultural places. Moreover, The identified serovars of pathogenic *E.coli* were O26:H11 (3.3%), O111:H2 (6.7%) and O125:H21 (3.3%) in Cairo collected samples, O44:H18 (3.3%), O111:H2 (3.3%), O117:H4 (3.3%) and O127:H6 (6.7%) in Giza collected samples and O15:H2 (3.3%), O26:H11 (10%), O86 (3.3%), O91:H21 (6.7%) and O159 (3.3%) in Benha collected samples. These results were nearly close to results demonstrated by Phillips et al. (2001) and Barhoma (2016) (spleen samples) and Mohammed 2018 (butcher shop collected samples) while lower than results demonstrated by Nicoline et al. (2015), Nyamakwere et al. (2016), Barhoma (2016) in liver and

kidney samples, Kimassoum et al. (2017) and Mohammed (2018) (in abattoir collected samples). Moreover, the results were higher than results revealed by Corney et al. (2006), Milness et al. (2008), Kumar et al. (2014), Barhoma (2016) (in meat collected samples) and Mohammed (2018) (in hypermarkets collected samples).

According to ES (2013), 86.7%, 83.3% and 73.3% of the examined samples of retail meat in Cairo, Giza and Benha were accepted, however, the unaccepted samples were 13.3%, 16.7% and 26.7% in such governorates were unaccepted, respectively as shown in table (2). Meat is frequently contaminated with aerobic bacteria from various sources during processing as hide, floor washings, viscera (intestinal contents), abattoir environmental and processing equipment and tools, water, hands of workers, clothing, gum boots, aprons and tables (Zweifel et al., 2008).

5. CONCLUSION

Escherichia coli is one of the most important pathogenic bacteria incorporated in meat and its products. In this study the incidence of *E. coli* pathogens was 13.3, 16.7, and 26.7 % in Cairo, Giza and Benha collected samples, respectively with mean incidence of 18.9 %. Periodical evaluation the hygienic status of Egyptian meat markets is very important as it incorporated with many food poisoning bacteria with public health hazards.

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