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 (Youssf 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), enteraggregative *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 retail fresh meat in 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.
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, 2001):

2.2.2.5.1. Indole test:
One ml of ether added to the culture 1% peptone water then incubated for 48 hrs. at 37°C. The tubes were strongly shaken then left and 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 a 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% KNO3, the isolates was inoculated and incubated at 37°C for 96 hrs., one ml of solution containing 8 g sulphamic 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 Ornithin deacylase (ODC):
Suspected colonies were inoculated into ornithin deacylase 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 deacylase (LDC)
Suspected colonies were inoculated into L-lysine deacylase 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 deacylase (ADH)
Suspected colonies were inoculated into arginine deacylase 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
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%.

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

<table>
<thead>
<tr>
<th>Strain</th>
<th>No.</th>
<th>%</th>
<th>No.</th>
<th>%</th>
<th>No.</th>
<th>%</th>
<th>Strain characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>O15:H2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>3.3</td>
<td>ETEC</td>
</tr>
<tr>
<td>O26:H11</td>
<td>1</td>
<td>3.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>EHEC</td>
</tr>
<tr>
<td>O44:H18</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>3.3</td>
<td>-</td>
<td>-</td>
<td>EAECC</td>
</tr>
<tr>
<td>O86</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>3.3</td>
<td>EPEC</td>
</tr>
<tr>
<td>O91:H21</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>6.7</td>
<td>-</td>
<td>-</td>
<td>EHEC</td>
</tr>
<tr>
<td>O111:H2</td>
<td>1</td>
<td>3.3</td>
<td>1</td>
<td>3.3</td>
<td>-</td>
<td>-</td>
<td>EAEC</td>
</tr>
<tr>
<td>O117:H4</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>3.3</td>
<td>-</td>
<td>-</td>
<td>EHEC</td>
</tr>
<tr>
<td>O125:H21</td>
<td>1</td>
<td>3.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ETEC</td>
</tr>
<tr>
<td>O127:H6</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>6.7</td>
<td>-</td>
<td>-</td>
<td>ET EEC</td>
</tr>
<tr>
<td>O159</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>3.3</td>
<td>EIEC</td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>13.3</td>
<td>5</td>
<td>16.7</td>
<td>8</td>
<td>26.7</td>
<td></td>
</tr>
</tbody>
</table>

In unsanitary conditions of water, food, equipment and tools, water, hands of workers, clothing, meat 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.

5. CONCLUSION

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 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).

REFERENCES


