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Enterobacteriaceae in Beef Products from Retail Outlets in Alexandria

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Key words

Enterobacteriaceae, *E.coli*, Salmonellae, *Yersinia enterocolitica*, beef products

ABSTRACT:

This study aimed to determine the presence of Enterobacteriaceae in beef products as luncheon, pasterma, frankfurter and minced meat as these microbes are considered as major cause of foodborne illness.A total of 100 samples (25 of each beef product) were collected from different retail outlets. Each sample was kept in a separate sterile plastic bag and transferred in an ice box to the laboratory under complete aseptic conditions with a minimum of delay. All collected samples were bacteriologically examined for isolation and identification of Enterobacteriaceae.

We found that the most important bacteria that isolated from minced meat were E. coli (44 %), Enterobacter spp. Especially *Enterobacter aerogenes* (12 %), *Enterobacter intermedium* (4) and *Enterobacter gergoviae* (4 %), Citrobacter spp. that includes *Citrobacter amalonaticus* (4 %), *Citrobacter diversus* (4 %) and *Citrobacter freundii* (4 %), serratia spp especially *Serratia marcescens* 8 %), *Serratia ficaria* (8 %), *Serratia fonticola* (12 %), *Serratia liquefaciens* (4 %) and *Serratia rubidaea* (8 %), *Edwardsiella spp*. especially *Edwardsiella ictalori* (8 %) and *Edwardsiella hoshinae* (12 %), *Povidencia spp.* (8 %) especially *Providencia alcalifciens* (4 %), Klebsiella pneumoniae especially *Subsp. Ozanae* (4 %) and Proteus spp. especially *Proteus mirabilis* (16 %).

The most important bacteria that isolated from luncheon were E. coli (32 %), *Enterobacter spp.* Especially Enterobacter aerogenes (8 %), Enterobacter intermedium (4 %) and *Enterobacter gergoviae* (8 %), Citrobacter spp. that includes *Citrobacter amalonaticus* (12 %), *Citrobacter diversus* (4 %) and *Citrobacter freundii* (16 %), Serratia spp. especially *Serratia marcescens* (8 %), *Serratia ficaria* (12 %), *Serratia fonticola* (4 %), *Srratia liquefaciens* (4 %) and *Serratia rubidaea* (8 %), Edwardsiella spp. especially *Edwardsiella ictalori* (8 %) and *Edwardsiella hoshinae* (16 %), Providencia spp. especially *Providencia alcalifciens* (4 %), Klebsiella pneumoniae especially Subsp. Ozanae (12 %) and Proteus spp. especially *Proteus mirabilis* (8 %).

Also,the most important bacteria that isolated from pasterma were E. coli (40 %), Enterobacter spp. Especially *Enterobacter aerogenes* (8 %), *Enterobacter intermedium* (4 %) and *Enterobacter gergoviae* (12 %), *Citrobacter spp.* that includes *Citrobacter amalonaticus* (4 %), *Citrobacter diversus* (12 %) and *Citrobacter freundii* (4 %), *Serratia spp.* especially *Srratia marcescens* (4 %), *Serratia ficaria* (8 %), *Serratia fonticola* (4 %), *Serratia liquefaciens* (4 %) and *Serratia rubidaea* (8 %), *Edwardsiella spp.* especially *Edwardsiella ictalori* (12 %) and *Edwardsiella hoshinae* (8 %), providencia spp. especially providencia alcalifciens (8 %), Klebsiella pneumoniae especially subsp. Ozanae (8 %) and *Proteus spp.* especially *Poteus mirabilis* (12 %).

Eventually,the most important bacteria that isolated from frankfurter were *E. coli* (36 %), *Enterobacter spp.* Especially enterobacter aerogenes (4 %), enterobacter intermedium (4 %) and enterobacter gergoviae (8 %), *Citrobacter spp.* that includes *Citrobacter amalonaticus* (8 %), *Citrobacter diversus* (4 %) and *Citrobacter freundii* (4 %), *Serratia spp.* especially *Serratia marcescens* (4 %), *Serratia ficaria* (12 %), *Serratia fonticola* (4 %), *Serratia liquefaciens* (4 %) and *Serratia rubidaea* (4 %), *Edwardsiella spp.* especially edwardsiella ictalori (8 %) and Edwardsiella hoshinae (12 %), providencia spp. especially *Providencia alcalifciens* (4 %), *Klebsiella pneumoniae* especially subsp. Ozanae (8 %) and *Proteus spp.* especially *Proteus mirabilis* (8 %).

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1. INTRODUCTION

Meat is rich in nutrients required for microorganisms growth and may become contaminated from different sources; these sources may be originated from the environment, human handling, manipulation and/or the animal itself. Environmental contamination includes: Air and water which are the most important sources, it also includes dust, insects, rodents, vehicles, dirty

floors, tables, holding pens, equipments and knives. The incidence of carcasses contamination depends on various factors including stress during transportaion, time spent in lairages and hygienic level during slaughter (Marritto and Gravani, 2006).

The most important pathogenic microorganisms found in the intestinal tracts belong to the family Enterobactriaceae, as these microbes are responsible for causing many cases of foodborne illness all over the world for many years.The pathogenic contamination of meat and its products has prompted consumer fear and global concern, threatened trade and economic profit and stimulated ideas in developing new process control measures. Public awareness has increased, such that in recent surveys, food poisoning from meat was citied as the fifth biggest fear of U.S.A consumers (Smith et al., 2000).

The Public health hazard of isolated Enterobacteriaceae constituted in Escherichia coli causes symptoms caused by Shigella dysenteriae mainly in the young and elderly (APHA, 2001 and CDC, 2004). Also, Hiko et al. (2008) reported that Escherichia coli (verocytotoxigenic) including serotype O157: H7 are one such group causing severe chronic and potentially fatal illness such as hemorrhagic colitis, hemolytic uremic syndrome, thrombotic thrombocytopenic purpura and in severe cases death occur.

Salmonella is the second most common cause of foodborne illness, it responsible for millions of cases of foodborne illness every year (Bell and Kyriakides, 2002; Khaitsa et al., 2007; FSIS 2008; Yang 2010).

The symptoms of infection by Enterobacter spp., Citrobacter, Serratia spp., Edwardsiella, Providencia, Klebsiella and Proteus spp. differs according to age in which it causes diarrhea and gasteroenteritis in children and infants while it causes mesenteric lymphadenitis and abdominal pain on older children. Ehara et al. (2000) and Arnold (2004) stated that Enterobactericeae are most frequently isolated bacterial pathogens from human cases of gasteroenteritis, it causes gasterointestinal disorders ranging from mild diarrhea to mesenteric lymphadenitis. Ray et al. (2004); Huovinen et al., (2010) revealed that Enterobacteriacae is a zoonotic bacterial species that food transmitted infections with clinical manifestations like gastroenteritis and reactive arthritis.

Therefore, the study aimed to determine the presence of Enterobacteriaceae in beef products as luncheon, pasterma, frankfurter and minced meat as these microbes are considered as major cause of foodborne illness, it will also contain solutions and recommendations on how to obtain a wholesome meat products

2-MATERIALS AND METHODS

1-1-Materials

1.1.a- Collection of samples:

A total of 100 random samples of retailed meat products represented by luncheon, pasterma, frankfurter and minced meat (25 of each) were collected from different retail outlets in Alexandria province.

Each sample was kept in a separate sterile plastic bag and transferred in an ice box to the laboratory under complete aseptic conditions with a minimum of delay. All collected samples were bacteriologically examined for isolation and identification of Enterobacteriaceae.

2-Methods:

2.2.a. Preparation of samples :

To 25 grams of sample ,225 ml of sterile peptone water were added and homogenized thoroughly by using sterile blender for 2.5 minutes, from which ten fold serial dilutions were prepared up to 10^6.The prepared samples were subjected to the following examination.

2.2.b. Total Enterobateriaceae Count (Gork, 1976):

One from each of the previously dilution was transferred into two separate Petri-dishes to which approximately 15 ml of sterile melted and tempered Violet Red Bile Glucose agar medium (VRBG) were added .the plates were incubated at 37 ^{o}c for 24 hours. All purple colonies surrounded by halo zones were then counted and the average number of colonies was determined. Hence, the Enterobacteriaceae count cfu/g was calculated.

2.2.C. Identification and isolation of Enterobacteriaceae (ICMSF, 1996):

2.3. Statistical analysis: The obtained results were statistically evaluated according to the guidelines recommended by SAS, (2004).

3- RESULTS AND DISCUSSION

The detection of total Enterobacteriaceae count in the examined meat products (luncheon, pasterma, frankfurter and minced meat) is important in examining the sanitary condition of the different meat products types at the retail level, Enterobacteriaceae contain many species, which have been reported to cause health hazard for the

consumer, some other species are important from the economic point of view as they may cause spoilage and deterioration of meat and meat products (ICMSF, 1980 and National Academy of science, 1985). The occurrence of high Enterobacteriaceae count indicated that, there were poor sanitary conditions during slaughtering, handling and preparation as that was reported by (Mulder and Krol (1976); Mira (1989).

The results of incidence of identified Enterobacteriaceae that isolated from minced meat that observed in Table (1) differ significantly ($P < 0.01$) among different isolated Enterobactericeae.

The most important bacteria that isolated were E. coli (44 %), Enterobacter spp. Especially *Enterobacter aerogenes* (12 %), *Enterobacter intermedium* (4) and *Enterobacter gergoviae* (4 %), Citrobacter spp. that includes *Citrobacter amalonaticus* (4 %), *Citrobacter diversus* (4 %) and *Citrobacter freundii* (4 %), serratia spp. especially *Serratia marcescens* 8 %), *Serratia ficaria* (8 %), *Serratia fonticola* (12 %), *Serratia liquefaciens* (4 %) and *Serratia rubidaea* (8 %), *Edwardsiella spp.* especially *Edwardsiella ictalori* (8 %) and *Edwardsiella hoshinae* (12 %), *Povidencia spp.* (8 %) especially *Providencia alcalifciens* (4 %), Klebsiella pneumoniae especially *Subsp. Ozanae* (4 %) and Proteus spp. especially *Proteus mirabilis* (16 %).

Also, the incidence of identified Enterobacteriaceae that isolated from luncheon samples that observed in Table (2) differ significantly ($P < 0.01$) among different isolated Enterobactericeae.

The most important bacteria that isolated were E. coli (32 %), *Enterobacter spp.* Especially Enterobacter aerogenes (8 %), Enterobacter intermedium (4 %) and *Enterobacter gergoviae* (8 %), Citrobacter spp that includes *Citrobacter amalonaticus* (12 %), *Citrobacter diversus* (4 %) and *Citrobacter freundii* (16 %), Serratia spp especially *Serratia marcescens* (8 %), *Serratia ficaria* (12 %), *Serratia fonticola* (4 %), *Srratia liquefaciens* (4 %) and *Serratia rubidaea* (8 %), Edwardsiella spp. especially *Edwardsiella ictalori* (8 %) and *Edwardsiella hoshinae* (16 %), Providencia spp. especially *Providencia alcalifciens* (4 %), Klebsiella pneumoniae especially Subsp. Ozanae (12 %) and Proteus spp. especially *Proteus mirabilis* (8 %).

The results of incidence of identified enterobacteriacae that isolated from pasterma that observed in Table (3) differ significantly ($P < 0.01$) among different isolated Enterobactericeae.

The most important bacteria that isolated were E. coli (40 %), Enterobacter spp. Especially *Enterobacter aerogenes* (8 %), *Enterobacter intermedium* (4 %) and *Enterobacter gergoviae* (12 %), *Citrobacter spp.* that includes *Citrobacter amalonaticus* (4 %), *Citrobacter diversus* (12 %) and *Citrobacter freundii* (4 %), *Serratia spp.* especially *Srratia marcescens* (4 %), *Serratia ficaria* (8 %), *Serratia fonticola* (4 %), *Serratia liquefaciens* (4 %) and *Serratia rubidaea* (8 %), *Edwardsiella spp.* especially *Edwardsiella ictalori* (12 %) and *Edwardsiella hoshinae* (8 %), providencia spp. especially providencia alcalifciens (8 %), Klebsiella pneumoniae especially subsp. Ozanae (8 %) and *Proteus spp.* especially *Poteus mirabilis* (12 %).

The results of incidence of identified enterobacteriacae that isolated from frankfurter that observed in Table (4) differ significantly ($P < 0.01$) among different isolated enterobacteriaceae.

The most important bacteria that isolated were *E. coli* (36 %), *Enterobacter spp.* Especially Enterobacter aerogenes (4 %), Enterobacter intermedium (4 %) and Enterobacter gergoviae (8 %), *Citrobacter spp* that includes *Citrobacter amalonaticus* (8 %), *Citrobacter diversus* (4 %) and *Citrobacter freundii* (4 %), *Serratia spp* especially *Serratia marcescens* (4 %), *Serratia ficaria* (12 %), *Serratia fonticola* (4 %), *Serratia liquefaciens* (4 %) and *Serratia rubidaea* (4 %), *Edwardsiella spp.* especially edwardsiella ictalori (8 %) and Edwardsiella hoshinae (12 %), providencia spp. especially *Providencia alcalifciens* (4 %), *Klebsiella pneumoniae* especially subsp. Ozanae (8 %) and *Proteus spp.* especially *Proteus mirabilis* (8 %).

It is clear from the previous results that the *Enterobacteriaceae* counts seem to be high and this draws our attention to the contamination from enteritis sources so it can be used as proof for enteric contamination (Mercuri and Cox, 1979).

It is also clear that carelessness during animal evisceration lead to intestinal rupture and releasing of intestinal contents which will lead to heavy contamination of different carcass parts by *Enterobacteriaceae.*

The presence of high *Enterobacteriaceae* counts in minced meat indicates poor sanitary conditions inside the butcher's shops especially for mincing machines which were used for meat mincing without periodical washing or cleaning and also workers hands which carry heavy contamination and contaminate meat by bad handling.

The presence of coliforms on meat surface is common and has been isolated from different sites

in variable numbers as reported by Hess (1970) and Mira (1989). Also, our results cleared that, the occurrence of high members of *Enterobacteriaceae* and *Coliforms* on the meat surfaces is important in reflecting the hygienic quality of meat and the test for *Coliform bacilli* is considered of much greater value in assessing its quality Voetsch et al.,(2004) reported that *Salmonella* is a zoonotic enteric pathogen with significant public health implications,resulting in approximately 1.4 million illness, 16,000 hospitalizations, and between 400 and 600 deaths annually in the U.S.A alone.

From the obtained results in this present work, we can conclude that retailed meat of different types (luncheon, pasterma, frankfurter and minced meat) has been exposed to bacterial contamination from different sources during selling and marketing in butchers shops. The equipments, knives, water, cloths, manure, intestinal contents, bad handling, dirty floors and surfaces used for cutting meat inside butchers shops act as good sources for retailed meat contamination. The neglection of sanitation, lack of experience and education especially for workers in retail outlets are major reasons for contamination of retailed meat. The results achieved revealed that level of contamination was very high by some members of the family *Enterobacteriaceae* which are considered to be dangerous to the public health. Therefore, we must pay great attention to the hygienic measures to ensure maximum safety and lowering meat contamination. Also, this study recommended that, all knives and equipments should be sterilized, workers and meat handlers meat must wear protective clothes and informed about hand washing before meat cutting and handling, daily cleaning and periodical disinfection of out retails and daily washing of mincing machines, and never left overnight with remnants of minced meat.

Table 1. Incidence of identified Enterobacteriaceae isolated from examined Minced meat samples (n=25).

Type of organism	Number	%
E.coli	11	44
Enterobacter spp.		
Enterobacter aerogenes	3	12
Enterobacter intermedium	1	4
Enterobacter gergoviae	1	4
Citrobacter spp.		
Citrobacter amalonaticus	1	4
Citrobacter diversus	1	4
Citrobacter freundii	1	4
Serratia spp.		
Serratia marcescens	2	8
Serratia ficaria	2	8
Serratia fonticola	3	12
Serratia liquefaciens	1	4
Serratia rubidaea	2	8
Edwardsiella spp.		
Edwardsiella ictalori	2	8
Edwardsiella hoshinae	3	12
Providencia spp.		
Providence alcalifaciens	2	8
Klebsiella pneumonia.	1	4
Subsp .ozanae		
.Proteus mirabilis.	4	16

$Chi^2 = 55.35^{**}$ ** = significant at ($P < 0.01$)

Table 2. Incidence of identified Enterobacteriaceae isolated from examined Lunchen samples (n=25).

Type of organism	Number	%
.E.coli	8	32
Enterobacter spp.		
Enterobacter aerogenes	2	8
Enterobacter intermedium	1	4
Enterobacter gergoviae	2	8
Citrobacter spp.		
Citrobacter amalonaticus	3	12
Citrobacter diversus	1	4
Citrobacter freundii	4	16
Serratia spp.		
Serratia marcescens	2	8
Serratia ficaria	3	12
Serratia fonticola	1	4
Serratia liquefaciens	1	4
Serratia rubidaea	2	8
Edwardsiella spp.		
Edwardsiella ictalori	2	8
Edwardsiella hoshinae	4	16
Providencia spp.		
Providence alcalifaciens	1	4
Klebsiella pneumonia.	3	12
Subsp .ozanae		
proteus mirabilis.	2	8

$Chi^2 = 60.47^{**}$ ** = significant at ($P < 0.01$)

Table 3. Incidence of identified Enterobacteriaceae isolated from examined Pasterma samples (n=25).

Type of organism	Number	%
.E.coli	10	40
Enterobacter spp.		
Enterobacter aerogenes	2	8
Enterobacter intermedium	1	4
Enterobacter gergoviae	3	12
Citrobacter spp.		
Citrobacter amalonaticus	1	4
Citrobacter diversus	3	12
Citrobacter freundii	2	8
Serratia spp.		
Serratia marcescens	1	4
Serratia ficaria	2	8
Serratia fonticola	1	4
Serratia liquefaciens	1	4
Serratia rubidaea	2	8
Edwardsiella spp.		
Edwardsiella ictalori	3	12
Edwardsiella hoshinae	2	8
Providencia spp.		
Providence alcalifaciens	2	8
Klebsiella pneumonia.	2	8
Subsp .ozanae		
proteus mirabilis.	3	12

$Chi^2 = 59.60^{**}$ ** = significant at (P < 0.01)

Table 4. Incidence of identified Enterobacteriaceae isolated from examined Frankfurter samples (n=25)]

Type of organism	Number	%
E.coli	9	36
Enterobacter spp.		
Enterobacter aerogenes	1	4
Enterobacter intermedium	1	4
Enterobacter gergoviae	2	8
Citrobacter spp.		
Citrobacter amalonaticus	2	8
Citrobacter diversus	1	4
Citrobacter freundii	1	4
Serratia spp.		
Serratia marcescens	1	4
Serratia ficaria	3	12
Serratia fonticola	1	4
Serratia liquefaciens	1	4
Serratia rubidaea	1	4
Edwardsiella spp.		
Edwardsiella ictalori	2	8
Edwardsiella hoshinae	3	12
Providencia spp.		
Providence alcalifaciens	1	4
Klebsiella pneumonia.	2	8
Subsp .ozanae		
proteus mirabilis.	2	8

$Chi^2 = 53.55^{**}$ ** = significant at (P < 0.01)

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