

The Incidence of Enterobacteriaceae Causing Food Poisoning in Some Meat Products

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Abstract: A total of seventy five samples of meat products, 25 of each of kofta, sausage, and shawerma were collected from different supermarkets and shops in Giza governorate, Egypt to be examined bacteriologically to determine its sanitary quality. The current study was done at April to July 2009. The mean aerobic plate count values were 28.4×10^5 , 24.3×10^4 and 7×10^5 CFU/g, respectively. *Enterobacteriaceae* count was 10.14×10^5 , 5×10^4 and 37.8×10^4 CFU/g, respectively. While the Coliforms (MPN) count was 7×10^2 , 3×10^2 and 4.59×10^2 CFU/g, respectively. *Escherichia coli*, *Salmonella* spp, *Klebsiella* spp and *Proteus* spp were isolated from 7(28%), 0(0%), 4(16%) and 2(8%); 3(12%), 2(8%), 1(4%) and 2 (8%); 5 (20%), 1(4%), 3(12) and 2(8%) of kofta, sausage and shawerma samples respectively. Further, *Citrobacter* spp was isolated from 1(4%) of shawerma samples. 80% of *Escherichia coli* isolates were enteropathogenic (O_{166} , O_{78} , O_{126} , O_{55} , O_{26} , O_{20} , O_{25} : K_{11} , O_{119} , O_{125} : K_{70} , O_{146} , O_{126}) while 20% were untypable. *Salmonella* Buntantan was isolated from 8% of sausage samples while *Salmonella Kentucky* was isolated from 4% of shawerma samples.

Key words: *E. coli*, food poisoning, meat products, *Salmonella*

INTRODUCTION

Meat and meat products are considered as an excellent source of high quality animal protein, vitamins especially B complex, and certain minerals, especially iron (Gracey *et al.*, 1986). They are considered as an ideal culture medium for growth of many organisms because of the high moisture, high percentages of nitrogenous compounds of various degrees of complexity, plentiful supply of minerals, accessory growth factors and some fermentable carbohydrates (glycogen) of a favourable pH for most of the enteric microorganisms. Meat products may be contaminated with microorganisms from meat handlers, which carry of pathogenic microorganism during the processes of manufacturing, packing and marketing. Improper cooking, refrigeration or storage may lead to meat borne illness. Foodborne pathogens are the leading causes of illness And death in developing countries costing billions of dollars in medical care, medical and social costs (Fratmico *et al.*, 2005) In the United States, foodborne disease have been estimated to cause to 24 to 81 million of human illness (Archer and Kvenberg, 1985). Microbial pathogens in food cause an estimated 6.5 to 33 million cases of human illness and up to 9000 deaths annually (Council for Agricultural Science and Technology, 1994). In the developed world, frequent reports of food poisoning have increased public concern in relation to the potential presence of pathogenic organisms in food. Changes in eating habits, mass catering, unsafe food storage conditions and poor hygiene

practices are major contributing factors to food associated illnesses (Hedberg *et al.*, 1992) Contamination raw meat is one of the main sources of foodborne illnesses (Bhandare *et al.*, 2007; Podpecan *et al.*, 2007) *Salmonella* species remains a leading cause of food poisoning in the developed world, resulting in multiple cases of absenteeism, illness, hospitalization and death each year (CDC, 2006). *Salmonella* is one of the microorganisms most frequently associated with food-born outbreaks of illness. Meat products in general and poultry, in particular, are the most common sources of food poisoning by *Salmonella* (Antunes *et al.*, 2003). *Escherichia coli* are considered the most commensally living microorganism in the alimentary tract of nearly all domestic and wild animals as well as human. Enteropathogenic *E. coli* organisms usually lead to severe diarrhea in infants and it may also be the causal organisms in appendicular abscess, peritonitis and cholecystitis (Frazier, 1988; Mackie and Mecartney, 1989).

The Enterobacteriaceae group of bacteria is the most challenging bacterial contaminant to raw and processed meat products worldwide. *Salmonella*, *E. coli*, *Proteus*, and *Klebsiella* species are the most predominant species in all food poisoning cases associated with some meat products. Due to the rising incidence of food borne infections, there is an urgent need for control and/or prophylaxis for food poisoning outbreaks associated with meat products. It depends greatly on investigating the causative agents in food (meat products), eliminating them to ensure food safety and to protect public health from microbial contamination of food.

Table 1: Incidence of some species of the family *Enterobacteriaceae* in the sample meat products

Types of samples	No. of samples	<i>Escherichia coli</i>		<i>Salmonella</i>		<i>Klebsiella</i>		<i>Proteus</i>		<i>Citrobacter</i>		Total	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Kofta	25	7	28	0	0	4	16	2	8	0	0	13	52
Sausage	25	3	12	2	8	1	4	2	8	0	0	8	32
Shawerma	25	5	20	1	4	3	12	2	8	1	4	12	48
Total	75	15	20	3	4	8	10.66	6	7.7	1	1.33	33	44

Therefore, the present study was planned to investigate the incidence of *Escherichia coli*, Enteropathogenic *Escherichia coli*, *Proteus* spp, *Klebsiella* spp and *Salmonella* spp in some meat products.

MATERIALS AND METHODS

Sampling: A total of seventy five samples of meat products including 25 samples from each of kofta, sausage and shawerma were collected from different supermarkets and shops in Giza governorate, Egypt. Each sample was packed in sterile plastic bag and transferred immediately to the laboratory in an ice box with a minimum period of delay to be examined bacteriologically.

Sample processing: Samples were prepared according to the technique recommended by ICMSF (1978). Briefly, 10 g from each sample were transferred under aseptic condition to a sterile polyethylene bag containing 90 mL of sterile Ringer's solution. The content of the bag was then homogenized using stomacher (Lab. Blender 400, Seward Lab, London) to have a dilution of 10^{-1} . Such homogenate was used for all bacterial investigation. Further, 10 fold serial dilutions were prepared up to 10^{-4} .

Bacteriological examination: The Enterobacteriaceae colony count surface spread plate technique was adopted from the method described by ICMSF, (1978). Total coliform and fecal coliform counts (Most Probable Number) (MPN) for the selected meat product samples were done according to FAO (1992). The criteria used for confirmation of the retrieved isolates were based on microscopical examination of Gram stained smears, biochemical profiling (Indole production, methyl red, Vogus Proskauer, Simmon's citrate utilization) and serological profiling (FAO, 1992; Garrity, 2001). Selective isolation of *Salmonella* species were done according to FAO (1992). Full identification of the *Salmonella* suspect isolates were done after matching the achieved morphological, biochemical and serological results against standard methods reported by Kerig and Holt (1986) and Garrity (2001). Serological identification of the strains was carried out in Clinical Microbiology Department, Central Health Laboratories of Ministry of Health on Egypt.

Table 2: Incidence of the isolated Enteropathogenic *Escherichia coli* strains from examined samples (n = 25)

Type of samples	Isolated <i>Escherichia coli</i> strains	Enteropathogenic <i>Escherichia coli</i>	
		No.	Percentage (%)
Kofta	7	6	85.7
Sausage	3	3	100
Shawerma	5	3	60
Total	15	12	80

Table 3: Serotyping of the Enteropathogenic strains of *Escherichia coli* (EPEC) isolated from some meat products

Type of samples	No. of positive sample	Serovars	Enteropathogenic strains of <i>Escherichia coli</i>	
			No.	%
Kofta	7	O166	1	14.14
		O78	1	14.14
		O126	1	14.14
		O55	1	14.14
		O26	1	14.14
		O20	1	14.14
		untypable	1	14.14
Sausage	3	O25 : K11	2	66.66
		O119	1	33.33
Shawerma	5	O125 : K70	1	20
		O146	1	20
		O126	1	20
		untypable	2	40
Total	15	Typed	12	80
		Untypable	3	20

RESULTS

Incidence of some species of family Enterobacteriaceae in the sampled meat products: *Escherichia coli*, *Salmonella* spp, *Klebsiella* spp and *Proteus* spp were isolated from 7(28%), 0(0%), 4(16%) and 2(8%); 3(12%), 2(8%), 1(4%) and 2 (8%); 5 (20%), 1(4%), 3(12) and 2(8%) of kofta, sausage and shawerma samples respectively (Table 6). Further, *Citrobacter* spp was isolated from 1(4%) of shawerma samples (Table1).

Incidence of enteropathogenic *Escherichia coli*: Enteropathogenic *Escherichia coli* retrieved from the examined samples were recorded in Table 2. 7 *Escherichia coli* isolates were retrieved from kofta samples, 6 out of 7 *Escherichia coli* strains (85.7%) were Enteropathogenic. 3 Enteropathogenic *Escherichia coli* strains (100%) were isolated from sausage samples. Only 5 *Escherichia coli* isolates were retrieved from shawerma samples. 3 out of 5 *Escherichia coli* isolates were Enteropathogenic with an incidence of 60%.

Serotyping of the Enteropathogenic strains of *Escherichia coli*: Serotyping of the 15 retrieved *Escherichia coli* isolates indicated that 12 of them were Enteropathogenic (EPEC) with an incidence of 80%

Table 4: Incidence of *Salmonella* serovars in the examined samples

Type of samples	No. of samples	<i>Salmonella</i> serovars	Positive samples	
			No	%
kofta	25	----	0	0
sausage	25	<i>S. buntantan</i>	2	8
shawerma	25	<i>S. kentucky</i>	1	4
Total	75		3	

Table 5: Incidence of *Proteus* spp in the examined samples

Type of samples	No. of examined samples	Positive samples	
		No.	%
Kofta	25	2	8
sausage	25	2	8
Shawerma	25	2	8
Total	75	6	

Table 6: Incidence of *Klebsiella* spp in the examined samples

Type of samples	No. of examined samples	Positive samples	
		No	%
kofta	25	4	16
sausage	25	1	4
shawerma	25	3	12
Total	75	8	

(O₁₆₆, O₇₈, O₁₂₆, O₅₅, O₂₆, O₂₀, O₂₅; K₁₁, O₁₁₉, O₁₂₅; K₇₀, O₁₄₆, O₁₂₆) while the remaining three isolates were untypable (Table 3).

Incidence of *Salmonellae*: Two *Salmonella* isolates were retrieved from a total of 25 sausage samples with an incidence of 8%. The two retrieved isolates were serotyped as *Salmonella* butantant. Only one *Salmonella* isolate was retrieved from a total of 25 shawerma samples with an incidence of 4%. This isolate was serotyped as *Salmonella* kentucky. No *Salmonella* isolates were detected in kofta samples (Table 4).

Incidence of *Proteus* species: A total of 6 *Proteus* isolates were retrieved from a total of 75 meat product samples. Retrieved isolate incidence was distributed as 2(8%) in kofta, 2(8%) in sausage and 2(8%) in shawerma (Table 5).

Incidence of *Klebsiella*: A total of 8 *Klebsiella* isolates were retrieved from a total of 75 meat product samples. The retrieved isolate incidence was distributed as 4(16%) in kofta, 1(4%) in sausage and 3(12%) in shawerma (Table 6).

DISCUSSION

Meat products are recognized as a major source of food born pathogens that cause food poisoning in humans. The source of infection is not determined in the majority of food born disease outbreaks. Currently the most important pathogens associated with meat products are *Salmonella* spp., *Campylobacter* spp., *Clostridium* perfringens and *Escherichia coli* (Borch *et al.*, 1996)

Enterobacteriaceae group has an epidemiological interest and importance as some of them are pathogenic

and may cause serious infections and/or food poisoning. The obtained data posed high contamination level of Enterobacteriaceae in kofta, sausage and shawerma samples with no significant differences between them. Enterobacteriaceae are useful indicators of hygiene and post processing contamination of processed foods (i.e., from dirty machinery). Furthermore, Enterobacteriaceae count can be taken as an indicator of possible enteric contamination in the absence of Coliforms (Mercuri and Cox, 1979). Although the bacterial count was used to reflect the hygienic quality of meat, however it is evident that the test for Coliforms is of much greater value in assessing its quality (Cruick Shank *et al.*, 1975).

The highest Coliforms count was found in kofta rather than in sausage and shawerma samples with no significant differences between them. Meanwhile, all examined meat products samples showed high contamination level of Coliforms.

High contamination level of Coliforms in examined meat products may indicates unsanitary conditions of raw meat production from which produced. They are indicators of fecal pollution at slaughterhouse which begin from skinning and direct contact with knives and workers hands. Also, during evisceration and washing, contamination may come from intestinal contents as well as from water during rinsing and washing of carcasses. Undercooked meat products have caused many food poisoning incidents associated with *Escherichia coli* which is present in the faeces, intestines and hide of healthy cattle from where it can potentially contaminate meat during the slaughtering process (Duffy *et al.*, 2003). Coliforms count is a reliable indicator of inadequate processing and post processing contamination of such products (ICMSF, 1996). In addition, Coliforms in processed meat may be responsible for inferior quality resulting in economic losses beside their presence in high count may give rise to public health hazard (Moreno *et al.*, 1997).

In the present study out of 75 samples of meat products (Kofta, Sausage and Shawerma (25 each) 15 (20%) *Escherichia coli* positive samples were recorded. The *Escherichia coli* incidence (Table1) among the examined samples was 7 (28%); 3 (12%); and 5 (20%), respectively. The *Escherichia coli* isolated in kofta samples were 7 (28%), which is markedly lower than that reported by El-Mossalami (2003) (40%). However, lower values were recorded by El-Taher-Omya (1998) (25%) and Saleh (2001) (16%). The incidence of *Escherichia coli* in the examined sausage samples was lower than the findings recorded by (44%); Zaki-Eman (1990) (48%); El-Khateib *et al.* (1994) (47.37%). The variation in the results obtained by different investigators may be due to difference in manufacturing practices, handling and difference in time of exposure. The present study also indicates that the incidence of *Escherichia coli* (Table1)

Isolated was higher than those obtained by Darwish *et al.* (1991) (12%) and Yassien *et al.* (1998) (0%). Out of 25 examined shawerma samples 5 (20%) were *Escherichia coli* positive samples. This result was remarkably different from those previously reported by El-Gohary (1993) (78%) and Vazgecer *et al.* (2004) (31%).

Enteropathogenic *Escherichia coli* can be defined as any strain of *Escherichia coli* that has the potential to cause diarrheal disease. The presence of *Escherichia coli* as Enteropathogens in meat and its products provides an evidence of contamination of faecal or water origin. It also reflects the unsatisfactory hygienic condition during manufacturing and handling of these products by human carriers (Mehlman *et al.* 1976 and Niazi and Refai, 1988). From the results in Table 2 it was found that out of 7 isolates of *Escherichia coli* recovered from kofta sample, 6 strains (85.7%) were serologically typed as Enteropathogenic *Escherichia coli*, also all isolated strains from sausage samples were Enteropathogenic *Escherichia coli*; Among 5 isolated *Escherichia coli* strains from shawerma sample, 3 strains were Enteropathogenic with an incidence of 60%.

Out of the 15 strains 12 *Escherichia coli* serovars isolated from meat product samples belonged to the serovars: O166; O78; O126; O55; O26; O20; O25; K4; O114; O125; K70 and O116 (Table 3). Similar *Escherichia coli* serovars were isolated from meat products were previously recorded by Gobran (1985) O126; O26 and O26; EL-Mossalami *et al.* (1989) O26 and O111; O128; Niazi and Refai (1988) O119; O26; O128 and O111; Taha (1989) O26; O111; O119; O127 and O26; Fahem (1993) O111; O126; O125 and O26 and Saad *et al.* (1994) O128. The rest of the isolate strains were untypable. The public health importance of isolated Enteropathogenic serovars had been attributed to its enterotoxin, which is implicated in causing gastroenteritis, epidemic children diarrhea, and sporadic diarrhoea in children as well food poisoning (Bryan, 1980). Moreover, Cliver (1990) stated that three principle syndromes caused by Enterohemorrhagic *Escherichia coli* have been linked to *Escherichia coli* O157: H7 which are hemorrhagic colitis with bloody stool, hemolytic uremic syndrome leading to renal failure in children; thrombotic thrombocytopenic purpura syndrome causing brain damage and high mortality rate.

In many countries, *Salmonellae* are very important causative agent of zoonotic and food born disease. The recorded results in Table 4 have revealed that the incidence of *Salmonella* species in examined kofta, sausage and shawerma samples were (0%), (8%) and (4%), respectively. From the obtained result, it was revealed that the identified *Salmonella* serotypes isolated from the examined sausage samples were two strains of *Salmonella buntantan* while the only *Salmonella* strain isolated from shawerma samples was *Salmonella*

Kentucky. *Salmonella* could not be recovered from the examined kofta samples. *Salmonella* were recovered from meat products in nearly similar incidence to the obtained results by many investigators, such as Abd El-Aziz (1987) (10%); Ahmed (1988) (8%); El-Mossalami *et al.* (1989) (6%) and El-Mossalami (2003) (5%); Torky (2004) (5%); Siriken *et al.* (2006) (7%).

On the contrary, very high incidence of *Salmonella* in sausage was recorded by Banks and Board (1983) (55%); and Moreno *et al.* (1997) (18.2%) Abraham *et al.* (1998) (20%). Yet, some investigators failed to detect *Salmonella* in meat products as El-Khateib (1982), Vazgecer *et al.* (2004) and Ismail (2008). Infection of food with *Salmonella* is not solely sufficient to cause food poisoning. It is necessary that the infected food to be moist and stored long enough under conditions allowing heavy growth of the bacteria e.g., for 24 h in a warm area of a kitchen or several days in a cool larder. Also, the food should not subject to thorough cooking before eating.

In the present study, *Proteus* spp. were present in kofta, sausage and shawerma samples with an incidence of (8%), (8%) and (8%), respectively (Table 5). The same observation was recorded by Ahmed (1988) who detect *Proteus* spp. in (12.5%) of sausage samples and (10%) of hamburger samples. Many researchers have isolated different *Proteus* spp. Roushdy (1971) had isolated *Proteus* spp with an incidence of (24%); Gobran (1985) had isolated *Proteus rettgeri* with an incidence of 5(20%), *Proteus morganii* 1(4%) and *Proteus vulgaris* 4(16%). From a total of 25 samples of sausage. Lofti *et al.* (1990) had isolated *Proteus rettgeri* with an incidence of 2%. Moreover, Ammar (2005) found *Proteus* with an incidence of 42.7% from the total examined samples which is higher than the present samples. The incidence of the isolated *Proteus* from the examined meat products (8%) were much lower than those reported by other investigator like Ammar (2005), Yet, it is still considered as an indicator of contamination of meat product during any of the processing, handling, storage stages. If the optimal condition for the isolated *Proteus* were existed, typical cases of food poisoning, urinary infection and other *Proteus* related human illnesses could happen due to rapid proliferation of the pathogen.

Klebsiella were isolated in different rates of incidence. These incidences were (16%) in kofta, (4%) in sausage and (12%) in shawerma (Table 6). Roushdy (1971) had isolated *Klebsiella* spp with an incidence of (12%); El-Khateib (1982) had isolated *Klebsiella* spp from sausage in incidence of (47.23%). Further were Gobran (1985) has isolated *Klebsiella* spp from sausage and basterma samples in incidence of (20%), Lofti *et al.* (1990) had isolated *Klebsiella* with an incidence of (12%), Ammar (2005) isolated 24% of *Klebsiella oxytoca*, and these are higher than the data reached from the present work. Despite the fact that the mentioned investigators (El-Khateib, 1982; Gobran, 1985;

as well as Ammar, 2005) have shown higher incidence of *Klebsiella* in meat product than those present in the current study. Yet, our result still indicates that the examined meat product sample is not edible or even unsafe for human consumption. This is due to the rapid proliferation of such pathogen in meat product regardless their count is high or low. However, the presence of high count of such pathogen in any of the meat products will quicken its spoilage and consequent food poisoning if eaten by human.

CONCLUSION AND RECOMMENDATION

From the obtained results, we can conclude that contamination by *Escherichia coli*, *Salmonella*, *Klebsiella* spp. and *Proteus* spp. was found in kofta, sausage and shawerma samples collected from different supermarkets and shops in Giza governorate.

The following suggestive measures should be considered to keep the examined products free from pathogens as possible:

- Routine microbiological examination should be adopted in meat product factories, butchers shops, groceries and other food rendering outlet with a consequent certificate of nil presence food born bacteria.
- Hygienic awareness should be applied for personnel whom involved in handling and preparing of food at factories, home or restaurants.
- Careful handling and thorough cooking of raw and frozen meat, regardless of market source by the consumers is required to prevent food born illness.
- Finally, Hazard Analysis Critical Control Point (HACCP) procedures should be adopted during all steps of manufacture, handling as well as storage of meat products to produce safe and high quality products as well as ensuring compliance with legislation.

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