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Enteropathogens of Public Health Importance in Imported Frozen Meat and Chicken

Fathi A. Elnawawi, Osama A. Attala and Samaa Saleh

Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Cairo University, Egypt

Abstract: A total of 170 random samples of imported frozen meat, imported frozen chicken and locally frozen chicken was collected from different markets in Cairo and Giza cities. The samples were transported to the laboratory where they were examined bacteriologically for determination of their sanitary status. Bacteriological examination of the samples revealed that 87, 80 and 72% of imported frozen meat, imported frozen chicken and locally frozen chicken were positive for coliforms. *E. coli* O_{158} was isolated from 8, 6 and 2.86% of locally frozen chicken, imported frozen chicken and imported frozen meat respectively, while *E. coli* O_{86} was isolated from 2 and 1.42% of imported frozen chicken and imported frozen meat respectively. Moreover, *E. coli* O_{44} was isolated only from one sample (2%) of imported frozen chicken. In addition, other *E. coli* species were isolated from 5.71, 2 and 2% of imported frozen meat, imported frozen chicken and locally frozen chicken respectively. *E. coli* O_{157}:H_7 and *Salmonella* species failed to be isolated in all examined samples. *Proteus mirabilis, Pseudomonas, Klebsiella, Enterobacter* and *Providencia stuarti* were isolated at different incidences from the examined samples. The public health hazard of these microorganisms as well as the recommended measures to improve sanitary quality status of imported frozen meat and chicken either imported or locally produced were discussed.

Key words: Frozen Meat · Frozen Chicken · Coliforms · Enteropathogens · *E. coli*

INTRODUCTION

In recent years Egypt imports great amounts of frozen meat every year not only to reflect their consumers local needs but also importation of meat was formed as a main part of the process of greater harmonization of trade in our market [1]. Imported meat is often much more heavily contaminated than home killed meat and contamination rates in excess of 20 % can be expected [2].

Contamination of carcass surfaces by food borne pathogens is a major public health problem, where a healthy animal may harbor pathogenic bacteria on its hide, hair, hooves and intestinal tract [3, 4].

Freezing of meat is based on the retardation of microbial growth to the point where decomposition due to microbial action does not occur [5, 6].

There are two types of enteropathogenic *E. coli* (*EPEC*), typical, which possess the EPEC adherence factor (EAF) plasmid and atypical, which do not possess the EAF plasmid. Currently, the EPEC isolated in industrialized countries are atypical while those from developing countries are typical. Interestingly Cheasty [7] added that the only reservoir for typical EPEC is man, whereas for atypical EPEC both man and animals can be reservoirs. The traditional EPEC serogroups are: O_{26}, O_{55}, O_{86}, O_{111}, O_{114}, O_{119}, O_{125ac}, O_{126}, O_{127}, O_{128ab}, O_{142} and O_{158}. Whereas the new serogroups were: O_{88}, O_{145} and O_{157}. During the past 23 year, a large number of human illness outbreaks have been traced worldwide to consumption of undercooked ground beef and other beef products contaminated with Shiga toxin-producing *Escherichia coli* (STEC). Although several routes exist for human infection with STEC, beef remains a main source [3].

Infections with EPEC are a world-wide public health problem, related to consumption of contaminated ground beef [8]. *E. coli* is a normal and healthy part of the intestinal microflora of many warm blooded animals including humans. However some strains can cause diseases e.g. verocytogenic *E. coli* including serotype O_{157}: H_7 are among group causing chronic and potentially fatal illness, related to their ability to produce one or more toxins known as verotoxin or shiga - like toxin [9].

Corresponding Author: Osama A. Ataala, Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Cairo University, Egypt.

Salmonellosis is a worldwide health problem; Salmonella infections are the second leading cause of bacterial foodborne illness in the United States [10]. Food borne salmonellosis is still the most important food borne infection in man. Such contamination occurs within slaughterhouses and during further processing of meat where abattoirs have been demonstrated as the potential sources of contamination of meat destined for human consumption [11].

The present study was applied to determine the prevalence of *Escherichia coli* and *Salmonella* in imported frozen meat and chicken and locally frozen chicken.

MATERIALS AND METHODS

Sampling: Seventy random samples of frozen meat and fifty random samples of each of locally frozen chicken and imported frozen chicken were collected from different supermarkets in Cairo and Giza cities. At the laboratory each sample was subjected to the bacteriological investigations as follow:

Determination of Coliform Counts (MPN)
It Was Carried out According to APHA [12-14]

Conventional Recovery of *E. coli* Organisms: A loopful from each positive Escherichia coli broth tube (with turbidity and gas formation) was used in determination of fecal coliform bacteria (MPN) was streaked onto plates of Eosin methylene blue (EMB) (Oxoid CM 69) and incubated at 37°C for 24 hours. Typical colonies (Greenish metallic with dark purple center) were picked up and transferred to Nutrient agar slants and incubated at 37°C for 24 hours, for further identification.

Conventional Recovery of Salmonella Organisms [15-19]: From each sample, 25 grams were pre-enriched in 225 ml 1% buffered peptone water and incubated at 37°C for 24 hours. Then one ml of the pre-enriched broth was transferred into a tube containing 10 ml of Rappaports Vassiliadis (Oxoid CM 669) broth and incubated at 40°C for 48 hours. A loopful from the enriched broth was streaked onto Xylose Lysine Desoxycholate (XLD) and S.S agar (Oxoid) and the plates were incubated at 37°C for 24 hours.

The isolated strains were serotyped in the Central laboratories of the Ministry of Health, Cairo, Egypt.

RESULTS AND DISCUSSION

The coliform group of bacteria is a reliable indicator of fecal pollution and general unsanitary condition of meat and meat products. Presence of coliforms in food is indicative of possible presence of enteropathogenic and /or toxigenic microorganisms which could constitute a public health hazard [20].

The results given in Table 1 indicated that imported frozen meat samples were the most frequently contaminated by coliforms (87%), followed by imported frozen chicken (80%) and while locally frozen chicken samples were the least frequently contaminated (72%).

The data given in Table (1) also indicated that the MNP of coliforms ranged from (0.48 to 5.04 $\log_{10}$ CFU/g), (0.47 to 5.04 $\log_{10}$ CFU/g) and (0.6 to 4.04 $\log_{10}$ CFU/g) for imported frozen meat, imported frozen chicken and locally frozen chicken respectively with mean values of 2.4±0.11, 2±0.16 and 1.8±0.11 respectively.

The obtained results are nearly similar to that reported by Varkony [21] who mentioned that the average count of coliforms in quick frozen meat rolls was 2.6 $\log_{10}$ cfu/g, while higher than that average was reported by Morshidy and Roushdy [22] and Chamber *et al.* [23] who mentioned that the mean MPN of coliforms was 3.5 and 2.9 $\log_{10}$ CFU/g respectively.

Regarding the frequency distribution of coliforms in examined samples, the data given in Table (2) indicated that the highest presence of coliforms was in imported frozen meat samples (34%) and locally frozen chicken samples (40%) in the range of 10^2 - 10^3, while in imported frozen chicken (28%) was within the range of 10-10^2.

Considering the total incidence of *E. coli* species isolated from the different types of samples investigated, the results given in Table (3) indicated that 7(10%), 6(12%) and 5(10%) of imported frozen meat, imported frozen chicken and locally frozen chicken respectively were contaminated with *E. coli*. the obtained results are higher than that reported by [24] and [25] who isolated *E. coli* from 1.8 and 2.51% of frozen meat samples examined respectively.

Regarding the chicken samples lower results were reported by Moharum [26]. Mansour [27] recovered *E. coli* with the same serotypes of the present study from (8%) of locally frozen chicken, followed by imported frozen chicken(6%), while imported frozen meat was least contaminated by these strain (2.86 %).

Table 1: Most probable number of coliforms (log_{10} CFU/g) of examined samples

Samples Items		Imported Frozen meat (n=70)	Imported Frozen chicken (n=50)	Locally Frozen chicken (n=50)
Positive samples	No	61	40	36
	%	87%	80%	72%
Min. (CFU/gm)		0.48	0.47	0.6
Max. (CFU/gm)		5.04	5.04	4.04
Mean (CFU/gm)		2.4	2	1.8
S. E.M ±		0.11	0.16	0.11

*S.E.M = Standard Error Mean

Table 2: The frequency distribution of coliforms in examined samples

	Frozen meat (N=70)		Imported Frozen chicken (N=50)		Locally Frozen chicken (N=50)	
Intervals	No	%	No	%	No	%
<10	11	16%	13	26%	17	34%
10-10^2	10	14%	14	28%	5	10%
10^2-10^3	24	34%	9	18%	20	40%
10^3-10^4	15	21%	8	16%	6	12%
10^4-10^5	8	11%	3	6%	2	4%
10^5-10^6	2	3%	3	6%	0	0
Total	70	100%	50	100%	50	100%

Table 3: Incidence of *E. coli* species in examined samples

	Imported frozen meat (N=70)		Imported frozen chicken (N=50)		Locally frozen chicken (N=50)	
Serotypes isolated	No	%	No	%	No	%
E. coli (O_{158})	2	2.86%	3	6%	4	8%
E. coli ($O_{86:}K_{61}$)	1	1.42%	1	2%	0	0
E. coli ($O_{44:}K_{74}$)	0	0	1	2%	0	0
Untypical *E. coli* species	4	5.71%	1	2%	1	2%
Total incidence of *E. coli* species	7	10%	6	12%	5	10%

The obtained results also indicated that *E. coli* (O_{157}:H_7) had been failed to be isolated from all the investigated samples. Similar results were reported by several investigators [24, 28-29] while Kihal and Barka [30] isolated one strain of *E. coli* (O157:H7) from 251 imported frozen meat samples with an incidence of 0.44%. Regarding the isolation of Salmonella during the present investigation, the obtained results proved that all the different types of samples weren't contaminated with salmonellae; however Moharum [28] Isolated one strain of Salmonella from 1082 samples of frozen boneless beef and Vanderlinde [31] reported that 3 out of 787 frozen meat samples were contaminated with salmonellae.

During the trials for isolation of *E. coli* and salmonella the following organisms Proteus mirabilis, Pseudomonas, Klebsiella, Enterobacter and Providencia stuarti were recovered at different incidences from the examined samples as shown in Table (4).

These results are higher than those detected by Rashwan [32] and lower than those recorded by Nassar and Fathi [33] and Banwart [34].

Proteus mirabilis causes 90% of proteus infection and can be considered a community- acquired infection, both proteus and pseudomonas organisms are Gram negative bacilli and can cause endotoxin induced sepsis, resulting in systemic inflammatory response syndrome (SIRS) [35].

Klebsiella and Enterobacter species are considered as potential health hazard members of the coliform group and can cause food spoilage [36, 37].

From these results presence of coliforms and *E. coli* species in chicken and meat products might be indicative of defective techniques applied during preparation, handling, processing and storage which may lead to economic loses [29]. From the given results in Table 5 it is observed that 70% of the samples of imported frozen meat were unaccepted according to the limits of codex almintares while 50 % and 58 % of the samples of imported and locally frozen chicken examined during this investigation should be considered unaccepted samples on the other hand E.S.S. (1991) revealed no limites about presence of coliform counts.

Table 4: Incidence of other pathogenic microorganisms isolated from examined samples

	Imported frozen meat (N=70)		Imported frozen chicken (N=50)		Locally frozen chicken (N=50)	
Isolates	No	%	No	%	No	%
Proteus Mirabilis	6	9%	5	10%	1	2%
Pseudomonas	4	6%	3	6%	2	4%
Klebsiella spp.	1	1%	0	0	2	4%
Enterobacter	0	0	0	0	1	2%
Providencia stuarti	1	1 %	0	0	0	0

Table 5: The acceptability of the examined imported frozen meat, imported frozen and locally frozen chicken based on the coliform limit of Codex Almintares (n=170)

	Accepted samples		Unaccepted samples	
Samples	Number	%	Number	%
Imported Frozen meat (n=70)	21	30%	49	70%
Imported frozen chicken (n=50)	26	52%	24	48%
locally frozen chicken (n=50)	25	50%	25	50%
Total number of samples	72	42%	98	58%

*Acceptable limits of coliforms according to Codex Alimentarius [18] should not exceed than 10^2 /g

* There is no limit mentioned to coliforms in the E.S.S. [19]

CONCLUSION

The study displayed that high percent of imported frozen meat, imported frozen chicken and locally frozen chicken were positive for coliforms. *Proteus mirabilis, Pseudomonas, Klebsiella, Enterobacter* and *Providencia stuarti* were isolated at different incidences from the examined samples. Such contamination occurs within slaughterhouses and during further processing of meat where abattoirs have been demonstrated as the potential sources of contamination. Therefore, it is advisable to improve sanitary quality status of imported frozen meat and chicken either imported or locally produced.

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