Benha Veterinary Medical Journal 37,2 (2019) 42-45

Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

Since 1990

Original Paper

Assessment of bacterial evaluation of imported frozen meat

Hemmat M. Ibrahim[1], Reham A. Amin[1], Mohamed H. Abdelmoaty[2]

[1] *Food Hygiene and Control Dept., Faculty of Veterinary Medicine, Benha Univ.*

[2] *Veterinarian , Armed Forces*

ARTICLE INFO

Keywords

Aeromonas

APC

Imported frozen meat

Psychotrophic

Staphylococci count

ABSTRACT

One hundred random samples of American, Brazilian, Indian and Sudanese imported frozen meat (25 of each) were collected from different shops and supermarkets at Cairo province to evaluate their bacteriological quality. The obtained results indicated that the mean values of APC of the examined imported frozen meat samples were $7.25x10^5$, $1.53x10^6$, $1.54x10^6$, and $2.35x10^6$ in American, Brazilian, Indian and Sudanese, respectively. The mean count of psychotrophic of the examined samples were $1.02x10^6$, $1.42x10^6$, $3.57x10^5$ and $1.50x10^6$, respectively. The mean values of *staphylococci* count in the same samples were $2.41x10^3$, $1.50x10^4$, $1.87x10^4$, and $2.62x10^4$, respectively. For the incidence of *Aeromonas* species in examined frozen meat, *A. hydrophila* was present in 20%, 28 %, 40% and 32% of American, Brazilian, Indian and Sudanese meat, respectively. In the meantime, *A. salmonicida* was present in 21%, 24%, 20% and 20% in the same samples, respectively. On the other hand, *A. puctata* was present in 8%, 16%, 8% and 12% of the examined samples, respectively. *A. sorbia* also present in 8%, 20%, 4% and 8% of the same samples, respectively. Finally, *A. cavia* was present in 8%, 16%, 4% and 4% of the examined samples, respectively.

1. INTRODUCTION

Meat constitutes the most important items of human food, because of its palatability and nutritional value. It is a highly desired food (Hui et al., 2001). Frozen meat is often more heavily contaminated due to the presence of spoilage microorganisms responsible for objectionable changes or pathogens leading to either food infection or intoxication (Tauxe et al., 2002). Although muscles of healthy animals do not contain microorganisms, meat tissues get contamination during the various stages of slaughter and transportation (Ercolini et al., 2006). Total aerobic bacterial count is used as an indicator of bacterial population in meat. It is not a measure of the entire bacterial population, but as its name implies, it is generic test for microorganisms that grow aerobically at mesophilic temperature (APHA, 2001). Storage of meat for long period at low temperature is responsible of several quality problems to meat industry .These problems related to growth of psychotrophic microorganisms (Nasser and Fathi, 1997).

Staphylococcus can be carried on hands, nasal passage or throats. Most food borne illness outbreaks are result of contamination from food handlers and production of heat stable toxins in food. The symptoms of staphylococcal food poisoning are abdominal cramps , nausea , vomiting , sometimes followed by diarrhea (never diarrhea alone). The onset of symptoms remission is observed after 24 hrs (Le Loir, 2003).

Moreover, recent food surveys confirmed that *Aeromonas* species were considered as re-immerging enteric pathogens

which responsible for several food epidemics (Ghenghesh et al., 2008).

A. hydrophila, A. caviae and *A. veronii biovar sorbia* are responsible for 85% of human gastrointestinal disorders. *Aeromonas veronii biovar sorbia* and *A. caviae* provoke enteritis with watery diarrhea and most commonly isolated from so-called travelers' diarrhea cases. Enteritis caused by *A. hydrophila* and *A. jandaei* is characterized by loose stools. *A. caviae* prevails in juvenile diarrheal cases (Parker and Shaw 2010)

A lack of attention to the hygienic design of slaughtering hall and cleaning and sanitation procedures can lead to bacterial contamination of meat, care must be taken to ensure that equipment such as storage chillers, and other product contact surfaces are adequately sanitized (Betts 2014).

Therefore, the goal of this research is assessment of bacterial load especially *Aeromonas species* in imported frozen meat sold at different shops and supermarkets at Cairo province, Egypt.

2. MATERIAL AND METHODS

2.1. Collection of samples

One hundred random samples of different cuts of American, Brazilian, Indian and Sudanese imported frozen meat (25 of

* Corresponding author: Prof. Hemmat M. Ibrahim, Food Hygiene and Control Dept., Faculty of Veterinary Medicine, Benha Univ.

each) were collected from different shops and supermarkets in Cairo province. The collected samples were kept in separate plastic bags and transferred directly to the laboratory in an insulated ice box under complete aseptic conditions without undue delay to evaluate their bacteriological quality.

2.2. Preparation of samples(APHA, 2001)
Under complete aseptic conditions, samples were thawed. Twenty-five grams of the examined samples were removed by sterile scissors and forceps after surface sterilization by hot spatula, transferred to a sterile polyethylene bag, and 225 ml of 0.1 % sterile buffered peptone water were aseptically added to the content of the bag. Each sample was then homogenized in a blender at 2000 rpm for 1-2 minutes to provide a homogenate of 1/10 dilution. One ml from the original dilution was transferred with sterile pipette to another sterile test tube containing 9 ml of sterile buffered peptone water 0.1 % and mixed well to make the next dilution, from which further decimal serial dilutions were prepared. The prepared dilutions were subjected to the following examinations.

2.3 Aerobic plate count (APHA, 2001)
2.4 Psychotrophic bacterial count (ICMSF, 1982)
2.5 Staphylococci count (FDA, 2001)
2.6 Isolation and Identification of Aeromonas species (ICMSF, 1996)
2.6.1 Microscopical examination (A.P.H.A., 1992.)
2.6.2 Biochemical identification (Baron and Finegold, 1990)
2.7. Statistical Analysis
Analysis of Variance (ANOVA) test was applied for statistical evaluation of the obtained results from examined frozen meat samples

3. RESULTS

The obtained results in tables (1-3) indicated that the mean values of APC (CFU/g) of the examined imported frozen meat samples were $7.25x10^5$, $1.53x10^6 \pm S.E$, $1.54x10^6 \pm S.E$, and $2.35x10^6 \pm S.E$, while, in Psychotrophic count were $1.02x10^6$, $1.42x10^6$, $3.57x10^5$and $1.50x10^6$ and *Staphylococci* count in the same were $2.41x10^3$, $1.50x10^4$, $1.87x10^4$, and $2.62x10^4$ in American, Brazilian, Indian and Sudanese samples.
As shown in table (4) results revealed that the incidence of Aeromonas species in examined frozen meat were *A. hydrophila* (20 %, 28 %, 40% and 32%), *A. salmonicida* (21%, 24%, 20% and 20%), *A. puctata* (8%, 16%, 8% and 12%), *A. sorbia* (8%, 20%, 4% and 8%) and finally, *A. cavia* (8%, 16%, 4% and 4%) in American, Brazilian, Indian and Sudanese meat samples, respectively.

4. DISCUSSION

Meat is not only highly susceptible to spoilage, but also frequently implicated in the spread of food borne illness. Contaminated raw meat is one of the main sources of foodborne illness, during slaughter and processing, all potentially edible tissues are subjected to contamination from a variety of sources within and outside animal. In living animals, those surfaces in contact with the environment, harbor a variety of microorganisms. The contaminating organisms are derived mainly from the hide of the animal and also comprise organisms that originate from both faces (Bhandare et al., 2007; Podpecan et al., 2007).

In table (1) The obtained results indicated that the mean values of APC of the examined imported frozen meat samples were $7.25x10^5 \pm 2.38x10^5$, $1.53x10^6 \pm 4.84x10^5$, $1.54x10^6 \pm 4.14x10^5$, and $2.35x10^6 \pm 5.59x10^5$ in American, Brazilian, Indian and Sudanese respectively . The current results were lower than those reported by **Refai et al. (1991)** found that the mean total aerobic bacterial count of the examined frozen meat samples was $2.7x10^6$ (CFU/g) , and higher than that obtained by **Ghazalah (2009)** who detected that the average APC of frozen meat was $13xl0^5$ (CFU/g).

Table 1 Statistical analytical results of APC (CFU/g) of the examined frozen meat samples (n = 25)

Frozen Meat Samples	Min.	Max.	Mean ± S.E
American	$2.3x10^4$	$3.9x10^6$	$7.25x10^5 \pm 2.38x10^{5b}$
Brazilian	$2.3x10^4$	$8.3x10^6$	$1.53x10^6 \pm 4.84x10^{5ab}$
Indian	$2.1x10^4$	$6.6x10^6$	$1.54x10^6 \pm 4.14x10^{5ab}$
Sudanese	$4.3x10^4$	$8.3x10^6$	$2.35x10^6 \pm 5.59x10^{5a}$

Means within a column followed by different letters showed significant difference ($P < 0.05$).

Table 2 Statistical analytical results of psychotrophic count (CFU/g) of the examined frozen meat samples (n = 25)

Type of Sample	Min.	Max.	Mean ± S.E
American	$3.1x10^4$	$5.9x10^6$	$1.02x10^6 \pm 3.64x10^{5b}$
Brazilian	$3.4x10^4$	$4.7x10^6$	$1.42x10^6 \pm 3.57x10^{5ab}$
Indian	$2.9x10^4$	$5.1x10^6$	$1.50x10^6 \pm 3.17x10^{5ab}$
Sudanese	$4.00x10^3$	$8.1x10^6$	$2.42x10^6 \pm 4.86x10^{5a}$

Means within a column followed by different letters showed significant difference ($P < 0.05$).

Table 3 Statistical analytical results of *staphylococci* count (CFU/g) of the examined frozen meat samples (n = 25)

Type of Sample	Min.	Max.	Mean ± S.E
American	< 10	$5.2x10^4$	$2.41x10^3 \pm 2.07x10^{3b}$
Brazilian	< 10	$5.6x10^4$	$1.50x10^4 \pm 4.70x10^{3ab}$
Indian	< 10	$6.5x10^4$	$1.87x10^4 \pm 2.94x10^{3a}$
Sudanian	< 10	$6.7x10^4$	$2.62x10^4 \pm 5.66x10^{3a}$

Means within a column followed by different letters showed significant difference ($P < 0.05$).

Table 4 Incidence of *Aeromonas species* isolated from the examined frozen meat samples (n = 25)

Aeromonasspecies	American		Brazilian		Indian		Sudanese		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
Aeromonas hydrophila	5	20	7	28	10	40	8	32	30	30
Aeromonas salmonicida	3	21	6	24	5	20	5	20	19	19
Aeromonas puctata	2	8	4	16	2	8	3	12	11	11
Aeromonas sorbia	2	8	5	20	1	4	2	8	10	10
Aeromonas caviae	2	8	4	16	1	4	1	4	8	8

% was calculated according to total number of samples

Aerobic plate counts of perishable food, as meat, generally do not relate to food safety hazards, but indicate the level of aerobic microorganisms in the food (Maturin and Peeler, 1998). Its quality, shelf-life and post heat processing contamination (Pamela and Tom, 2008) and the most reliable index for judging the hygienic condition under which it has been produced and stored (Saad, 1976).
Table (2) revealed that the mean values of psychotrophic of the examined imported frozen meat samples were $1.02x10^6 \pm 3.64x10^5$, $1.42x10^6 \pm 3.57x10^5$, $1.50x10^6 \pm 3.17x10^5$ and $2.42x10^6 \pm 4.86x10^5$ in American ,Brazilian, Indian and Sudanese meat, respectively. The obtained results were lower than that obtained by (hafez 1986), who said that the average psychotrophic bacterial count of frozen meat was $11x10^6$ (CFU/g) and higher than that obtained by Tharwat (2008), who said that the mean value was $22.2x10^2$ (CFU/g) Psychotrophic bacterial counts can provide useful information about the keeping quality of meat. Moreover,

there is a general agreement that aerobic spoilage of meat becomes evident when the level of bacteria reaches 10^7 (CFU/g) (off odors) or 10^8 (CFU/g) (slime formation) (Preito et al., 1992).

In table (3) The mean values of *staphylococci* of the examined imported frozen meat samples were in $2.41x10^3 \pm 2.07x10^3$, in $1.50x10^4 \pm 4.70x10^3$, in $1.87x10^4 \pm 2.94x10^3$, and $2.62x10^4 \pm 5.66x10^3$ in American, Brazilian, Indian and Sudanese meat, respectively

the achieved results were nearly similar to that obtained by Habeel (1999), who collected 90 samples from Alexandria markets and found that the incidence of staphylococcal was 53.33% and higher than that found by Phillips et al. (2001), who analyzed 990 samples of imported frozen beef meat recorded that coagulase positive *staphylococci* was 17.5% of the analyzed samples

The presence of *staphylococci* may be due to contamination during dressing and evisceration in the slaughter house, contaminated equipment, butchers, hand with abrasions and wounds, slaughter of animal beside dressed one in the same area in the slaughter hall and contaminated air from workers with their aerosol which contaminate air with *staphylococci* (Bennett, 2005).

Also, exposure of Imported frozen meat to thawing and refreezing in market shops and street vendors, yielding an abundant supply of water and form an excellent media for bacterial growth and multiplication (Jay et al., 2015)

Table (4) declare the incidence of *Aeromonas* species in examined frozen meat where A. hydrophila present in 20 %, 28 %,40% and 32% of American, Brazilian, Indian and Sudanese meat respectively. While *A. salmonicida* present in 21%,24%,20% and 20% in the same samples respectively on the other hand *A. puctata* were present in 8%,16%,8% and 12% of the examined samples respectively . *A. sorbia* also present in 8%,20%,4% and 8% of the same samples respectively. Finally, *A. cavia* present in 8%,16%,4% and 4% of the examined samples respectively the obtained results were nearly similar to that reported by (Stratev et al. 2012)

The motile mesophilic aeromonads consisting of *A. hydrophilia, A. sobria* and *A. cavie* , are considered causative agents of human gastroenteritis , wound infections and septicemia (Isonhood and Drake, 2001)

4. CONCULOSION

High hygienic precaution should be followed to improve the bacteriological quality of Imported frozen meat and to prevent growth and multiplication of bacteria that may cause a serious public health hazards. From the obtained results, we found that the Sudanese frozen meat was the most contaminated samples as it had record the highest APC $2.35x10^6 \pm 5.59x10^5$ (CFU/g), the highest psychotrophic count $2.42x10^6 \pm 4.86x10^5$ (CFU/g), also the highest staphylococcal count $2.62x10^4 \pm 5.66x10^3$ (CFU/g). A. hydrophilia was the most isolated microorganism from most of the frozen meat samples as it was isolated from 10 samples (40%) from the Indian meat.

5. REFERENCES

1. American Public Health Association (A.P.H.A.) 1992. Compendium of Methods for the Microbiological Examination of Foods 3rd Ed Washington, D.C, USA.
2. American Public Health Association "APHA" 2001. Compendium of methods for microbiological examination of foods. 4th Edition 365-366. 800. 1st, NW Washington DC 2000 1-3710.
3. Baron, E.J., Finegold, S.M. 1999. Diagnostic Microbiology. The C. V. Mosby co., 18130. Westline industrial Drive, St. Louis, Missouri 63146, USA.
4. Bennett, R. W. 2005. Staphylococcal enterotoxin and its rapid identification in food by enzyme-linked immunosorbent assay-based methodology. J. Food Protec. 68(6): 1264-1270.
5. Betts, R.2014. Microbial yeast and moulds . International Food Hygiene 24(4): 10-11
6. Bhandare, S. G., Sherikarv, A. T. ; Paturkar, A. M.; Waskar, V.S. and Zende, R. J. 2007. A comparison of microbial contamination of sheep/goat carcasses in a modern Indian abattoir and traditional meat shops. Food. Contr. 18: 854-868.
7. EL-Atall, Sohair 1987. Microbiological status of frozen meat. M. V. Sc. Thesis (Meat Hygiene), Fac. Vet. Med., Cairo University.
8. Ercolini D.; Russo, F.;Torrieri,E.; Masi,P. and Villani,F. 2006. Changes in the spoilage-related microbiota of beef during refrigerated storage under different packaging conditions. Appl Environ Microbiol. 72 (7): 4663-4671 .
9. Food and Drug Administration "FDA" 2001. Evaluation and definition of potentially hazardous foods. Analysis of microbial hazards related to time / temperature control of food for safety. Department of Health and Human Services. Food and Drug Administration Chapter 4:1-19.
10. Food Safety and Inspection Service "FSIS". United States Department of Agriculture 2003. Meat preparation: Beef from farm to table. Washington. DC. 20250-3700.
11. Ghenghesh K. S.; Ahmed S. F.; El-Khalek R. A.; Al-Gendy, A. and Klena, J. 2008. Aeromonas-associaXeA infections in developing countries. J. Infect. Developing Countries, 2: 81-98.
12. Gill, C. O. and Newton, K. G. 1982. The effect of lactic acid concentration on the growth of meat Gram negative psychotrophs from a meat workers. Appl. Environ. Microbial., 43: 284-288.
13. Ghazalah, S. 2009. Studies on the microbiological quality of frozen meat. M. V. Sc. Thesis (Meat Hygiene), Fac. Vet. Med., Alex. University.
14. Habeel, W. B. 1999. Studies on the effect of freezing on the keeping quality of frozen meat and its products. Ph.D. Thesis (Meat Hygiene), Alex. University.
15. Hafez, A.F. 1986. Studies on the sanitary condition of frozen meat marketed in Sharkia province. Ph.D. Thesis. Fac. Vet. Med. Zag. Univ.
16. Hui, Y. H.; Nip, W. R; Rogers, R. W. and Youn, O. A. 2001. Meat science and application. Marcel Bekker, New York.
17. International Commission on Microbiological specification for food "ICMSF" 1982. Microbial ecology of Food. Academic Press. New York.
18. International Commission on Microbiological specification for food "ICMSF" 1996. Microorganisms in foods, characteristics of food microbial pathogens, Listeria monocytogenes. Blackie Academic Professional, London, Pp. 141-182.
19. Isonhood, J.; Drake, M. 2001. Aremonas Species in Food. Journal of Food Protection 65 (3): 557-582 .
20. Jay, J.M.; Loessner, M.J. and Golden, D.A. 2005. Modern food microbiology. 7th ed. New York: Springer Sci and Business Media
21. Le Loir, Y.; Baron, F. and Gautier, M. 2003. S. aureus and food poisoning. Genetics and Molecular Research, 2(1): 63-76.
22. Maturin, L. J. and Peeler, J. T. 1998. Aerobic plate count. Chapter 3. In: Food and Drug Administration Bacteriological Analytical Manual, 8th Ed. (revision A), (CD-ROM version). R.L. Merker (Ed.). AOAC International, Gaithersburg, MD.
23. Nasser, A. and Fathi, S. H. 1997. Prevalence of psychrotrophic microorganisms in imported frozen meat. Fleischwirtschaft, 87:25.27.
24. Parker, J. and Shaw J. 2010. Aeromonas spp. clinical microbiology and disease. J. Infection 20:1-10.
25. Philips, D. X.; Summer, J.; Alexander, J. F. and Dutton, K. M. 2001. Microbiological quality of Australian beef. J. Food Protec., 64(5): 692-696.
26. Prieto, M.; Garcia, M. R.; Garcia, M. L; Alonso, C. and Otero, A. 1992. Species of Pseudomonas obtained at 7 °C and 30 °C

during aerobic storage of lamb carcasses. J. Appl. Bacteriol. 73: 317-323.
27. Refaie, R.S.; Seham, M.A.; Thabet, A.R. and El-Timawy, A.M. 1991. Microbiological quality of frozen meat in Assiut. Assiut. Vet. Med. J. 24 (48): 198-163
28. Saad, S. M. 1976. Studies on sanitary condition for locally manufactured pasterma. M. V. Sc. Thesis (Meat Hygiene), Fac. Vet. Med., Zag, University.
29. Stratev, D.; Vashin, I. and Rusev, V. 2012. Prevalence and survival of Aeromonas spp. in foods. A Review. Revue Med. Vet., 163(10).486- 494.
30. Tauxe, R. V. 2002. Emerging food borne pathogens. Int. J. Food Microbiol. 78(l-2): 31-41.
31. Tharwat, A. 2008. Microbiological evaluation of frozen meat in EL-Sharkia Province. M. V. Sc. Thesis (Meat Hygiene), Fac. Vet. Med., Zagazig University.