Kafrelsheikh Vet. Med. J. Vol. 7 No. 2 (2009) (34-44)

PREVALENCE OF AEROMONAS MICROORGANISMS IN FROZEN MINCED MEAT IN ALEXANDRIA GOVERNORATE MARKETS

F. A. El-Shaboury, Amal. F. A. Mansour and Ola, A. M. Basha

Animal Health Research Institute, Alexandria Branch

ABSTRACT

Fifty random samples of frozen minced beef meat were collected from different areas with different sanitation levels at Alexandria city markets and examined for the prevalence of Aeromonas microorganisms. Bacteriological examination revealed that Aeromonas species could be isolated from 11 samples (22%). Aeromonas hydrophila was the most predominant species isolates (9 isolates) followed by Aeromonas cavia (2 isolates). The ability of isolated Aeromonas organisms to produce enterotoxin and haemolysin was investigated. The importance of isolated Aeromonas hydrophila as well as the potential public health significance were recorded and the suggested hygienic measures to improve frozen minced meat had been discussed.

INTRODUCTION

Meat and meat products are liable to be contaminated with different kinds of microorganisms from different sources. Such contaminants may be of public health hazard to consumer or may render the products unmarketable, specially in young countries in which the hygienic measures are still underway, many efforts were done to produce a product free from pathogens of public health hazard and with low microbial content in order to improve its shelf life.

Aeromonas species has long been recognized as a pathogen of fish and frogs ***(Popoff and Veron, 1984, Soh et al., 1997 and Lehane and Rawlin, 2000)***. *Aeromonas hydrophila* occurs widely in nature: in water ***(Picard et al., 1983 and Kombanets et al., 1992)***, in milk ***(Nagah, 1991)***, in Fish ***(Shahat and Hamoda, 2000, Samaha et al., 2004 and Ola Basha, 2007)***, in meat ***(Ozbas et al., 1996 and Amany Shalaby, 2005)*** and in human, Aeromonas causes gastroenteritis with severe human diarrhoea ***(Millership et al., 1983 and Buchanan and Palumbo, 1985)***. In addition, *Aeromonas hydrophila* is widely distributed psychrotrophic bacterium that can produce virulence factors including a relatively heat stable cholera-like enterotoxin and heat labile cytotoxic enterotoxin and is recognized as potential cause of food associated outbreaks of gastroenteritis and as aetiological agent of acute diarrhoea in children ***(El-shenawy and Marth, 1990)***.

Moreover, Aeromonas can produce other human infection, including septicemia, meningitis, wound and eye infection, pneumonia and urinary tract infection ***(Ellison and Mostow, 1984)***.Thus it possesses a highly significant public health problem. The present study was conducted to determine the prevalence of *Aeromonas hydrophila* and other motile aeromonads in commonly consumed frozen minced meat and for detection of Aeromonas producing enterotoxin and haemolysin.

MATERIAL AND METHODS

Sampling:

A total of 50 marketed random samples of frozen minced beef meat were collected from different areas with different sanitation levels at Alexandria city. The Collected samples were transferred to laboratory as rapidly as possible and examined bacteriologically for the presence of Aeromonas organisms.

Bacteriological examination:

Ten grams of each samples were homogenized with 90 ml of peptone water 1% for 2 minutes (using Homogenizer type MPW- 302 poland), then incubated for 24 hours at 30°C. A loopful from peptone water was streaked on Rimler-shotts media, nutrient agar and MacConkey's agar. After 24 hours of incubation at 30 °C, the Rimler-Shotts plates were examined for the presence of yellow orange colonies (the specific colonies of Aeromonas as stated by ***Shotts and Rimler, 1973)***. Colonies from nutrient agar were picked up and transferred to nutrient agar slant for further identification. Gram stain and morphology of colonial appearance were reported, identification by biochemical tests were done according to ***Cruickshank et al. (1975), Lennette et al. (1980) and Krieg and Holt (1984).***

Production of toxin:

The isolated Aeromonas were tested for production of enterotoxin and haemolysin after cultivation at 5°C for 7 days. The enterotoxin activity was examined by the suckling mouse technique, while haemolysin activity was done with rabbit R.B.Cs ***(Majeed et al., 1989a).***

Sensitivity test:

The antibiograms of the isolated *Aeromonas hydrophila* pathogens were done using the disc diffusion method as recorded by ***Bauer et al. (1966)***. The interpretation of inhibition zones were estimated according to limits given by ***Finegold and Martin (1982)*** and ***Bio-Merieux (1984)***. Different antibiotic discs were used such as Oxytetracyclin (30 µg), Ampicillin(40 µg), Streptomycin(10µg), Neomycin(30 µg), Erythromycin (15 µg), Penicillin(10 IU), Chloramphenicol (30 µg) and Sulphamethoxazole (10 µg).

RESULTS

The bacteriological examination revealed that Aeromonas bacterium could be isolated from 11 out of 50 minced frozen beef meat samples (22%). Aeromonas microorganisms were identified as *Aeromonas hydrophila* ,9 isolates (18%) and *Aeromonas caviae* 2 isolates (4%) from the examined minced frozen beef samples (Table, 1). *Aeromonas hydrophila* organisms were Gram negative, motile rod that gave round colonies (2-3 mm in diameter) and yellow-orange in colour on Rimler-Schotts medium.

The morphological and biochemical characteristics of Aeromonas isolates are illustrated in table (2).

Regarding to virulence activity of *Aeromonas hydrophila* isolates it was found that all the examined isolates were produce enterotoxin and produce B-haemolysin on 5% rabbit blood agar (Table ,4) .

The results of drug sensitivity were summarized in table (3). Such table revealed that *Aeromonas hydrophila* were sensitive to Oxytetracycline and Sulphamethoxazole.

Table (1): Incidence of isolated Aeromonas organisms from examined minced frozen beef meat samples (n=50).

Aeromonas isolates	No. of isolates	%
Aeromonas hydrophila	9	18
Aeromonas caviae	2	4
Total	**11**	**22**

Table (2): Morphological and biochemical characteristics of Aeromonas isolates.

Test	Results	
	A.hydrophila	A. caviea
Gram stain	-ve	-ve
Motility	+ve	+ve
Oxidase	+	+
Catalase	+	+
Indol production	+	+
H_2S production	-	-
Nitrate reduction	+	+
Urease production	-	-
Gellatin hydrolysis	+	+
Vogus-proskauer	-	-
Ornithin decarboxylase	-	-
2,3-butandiol dehydrogense	+	-
Fermentation of:		
Glucose	+	-
Mannitol	+	+
Sucrose	+	+

Table (3): Drug sensitivity test of Aeromonas hydrophila isolates.

Drug	Reaction
Oxytetracyclin (30 µg)	+++
Ampicillin (40 µg)	+
Stryptomycin (10 µg)	++
Neomycin (30 µg)	+
Erythromycin (15 µg)	++
Penicillin (10 IU)	+
Chloramphenicol (30 µg)	++
Sulphamethoxazole (10 µg)	+++

Table (4): Production of enterotoxin and haemolycin.

Aeromonas species	Total number of isolates	Enterotoxin positive		Haemolycin positive	
		No	%	No	%
A.hydrophila	9	9	100	9	100
A.caviea	2	0	0	0	0

DISCUSSION

Examination of frozen minced beef samples revealed that aeromonas were found in 22% of examined samples. These results were inagreement with those reported by ***Mackey (1989)***, ***Andrew Hudson and Delac, 1991, Krovacek et al. (1992), Walker and Brooks (1993) and Amany Shalaby (2005)***. The isolation of motile Aeromonas from minced frozen beef meat may be a result of contaminating carcasses in the environment. In addition faecal material may play an indirect role in transmitting Aeromonas organisms from animal source through such factors as handling or the use of contaminated equipment, even from water used in washing the carcasses in the abbatoir as recorded by ***Majeed et al. (1989b)***. Table (1) revealed that *Aeromonas hydrophila* was the most predominant species in the isolated samples 9 (18%) followed by *Aeromonas caviae* 2 isolates (4%).

These results were inagreement with those reported by ***Andrew Hudson and Delacy (1991)***, ***Rajesh and Ashwani (1997), Melas et al. (1999) and Amany Shalaby (2005). Yamamoto et al. (1994) and Akan et al. (1996)*** reported that *Aeromonas caviae* was the least frequently occurring in food of animal origin.

The results of our study showed that *Aeromonas hydrophila* have the ability to produce enterotoxin and with haemolysin activity. Similar results were reported by ***Majeed et al. (1990) and Rajesh and Ashwani (1997)***. Moreover, ***Wakabayashi et al. (1981)*** reported that *Aeromonas hydrophila* produce extra cellular enzymes, cytotoxins and haemolysins. The results of drug sensitivity proved that, the isolated *Aeromonas hydrophila* were highly sensitive to Oxytetracyclin and Sulphamethoxazole. These results were agreement with those reported by ***Neumann and Ploger (1975), Essa et al. (1991) and Ola Basha (2007)***.

REFERENCES

- ***Akan,D.;Diker,K.S.; Kocak, C.; Yildirin, M. and Bozkurt, S. (1996):*** Isolation of motile Aeromonads from raw milk. Gida, 21: 383-386.
- ***Amany, M. Shalaby (2005):*** Study on prevalence of Aeromonas hydrophila in some foods of animal origin. J. Egyp. Vet. Med. Assoc., 65: 55-62.
- ***Andrew Hudson, J. and Delacy, M. (1991):*** Incidence of motile aeromonads in Newzeeland retail foods. Journal of food protection, 54(9): 696-699.
- ***Bauer, A.W.; Kurby, W.M.M.; Sherris, J.C. and Turk, M. (1966):*** Antibiotic susceptibility testing by a standardized single disc method. Am. J. Clin. 45: 493-496.
- ***Bio-Merieus (1984):*** Laboratory reagents and products. Bacteriology. Ma Cly. L. Etoile 69260. Charbonmiees Les-Bains. France.
- ***Buchanan, R.L. and Palumbo, S.A. (1985):*** Aeromonas hydrophila and Aeromonas sorbia as potential food poisoning species: a review. J. Food safety. 7: 15-29.
- ***Cruickshank, R,; Duguid, J.P.; Marmian, B.P. and Swain, R.H.A. (1975):*** Medical Microbiol. Vol. 2, the practice of medical microbial. 12th Ed., Churchill Livingstone, London.
- ***Ellison, R.T. and Mostow, S.R. (1984):*** Pyogenic meningitis manifestation during therapy for Aeromonas hydrophila species. Arch. Inter. Med., 144: 2078-2079.
- ***El-Shenawy, A. and Marth, E.H. (1990):*** Aeromonas hydrophila in foods. Egyptian. J. Dairy Sci., 18: 219-234.

- ***Essa, M.; Badran, A. and Moustafa, M. (1991):*** An outbreak of Redmouth disease among cultured fresh water fishes in Ismailia Governorate Alex. J. Vet. Sci., 6(7): 109-118.
- ***Finegold, F.R and Martin, O.K. (1982):*** Serological characterization of a typical strains of Enterobactereacea isolation from seabreams. Gyobyo-Kenkyu. Fish Pathology. 33: 4, 265-274.
- ***Kombanets, E.V.; Isaeva, N.M. and Balakhnin, L.A. (1992):*** Bacteria of the genus Aeromonas and their role in aquea culture. Mikrobial, Z.H. 54(4): 89-99.
- ***Krieg, N.R. and Holt, J.G. (1984):*** Genus III Aeromonas, Kluver and Van Niel 1936, 398al, p. 545-548 (Ed.) Bergy's Manual of systemic Bacteriology, Vol.1. Williams and Wilkins Co., Baltimore.
- ***Krovacek, K.; Faris, A.; Baloda, S.B.; Peterz, M.; Lindberg, T. and Mansoon, I. (1992):*** Prevalence and characterization of Aeromonas spp.Isolated from foods in Uppsala, Sweden.Food microbial.9: 29-36.
- ***Lehane, I. and Rawlin, G.T. (2000):*** Topically acquired bacterial zoonoses. Med. Of Asut. Sep., 173(5): 256-259.
- ***Lennette, E.H.; Balows, A.; Hausler, Wand Traunt, J. (1980):*** Manual of clinical Microbiology. Am. Soc. Microbiology Washington.
- ***Mackey, B. (1989):*** The incidence of food poisoning bacteria on red meat and poultry in the united kingdom, food science and technology. Today, 3(4): 246-250.
- ***Majeed, N., Egan, A.F. and Macrae, I.C. (1989a):*** Enterotoxigenic Aeromonads on retail Lamb meat and offal. J. App. Bacteriol. 67: 165-170.

- ***Majeed, N., Egan, A.F. and Macrae, J.C. (1989b):*** Incidence of Aeromonads in samples from an abattoir processing lamb. Journal of applied bacteriology 67: 597-604.
- ***Majeed,N.,Egan,F.F.and Macrae I.C.(1990):***Production of exotoxins by Aeromonas species at 5°C. J. App. Bacteriol, 69: 332-337.
- ***Melas, D. Papageorgiou, and Martis, A.I (1999):*** Enumeration and confirmation of Aeromonas hydrophila, A. caviae and A. sobria isolated from raw milk and other milk products in Northern Greece. J. Food Prot. 62: 463-466.
- ***Millership,S.E.;Curnow, S.R. and Chattopadhyay, B. (1983):*** Faecal carriage rate of Aeromonas hydrophila. J. Clin. Path.; 36(8): 920-923.
- ***Nagah, S. (1991):*** Occurrence of Aeromonas hydrophila in raw milk. Assiut. Vet. Med. J. 25(50): 98-102.
- ***Neumann, W. and Ploger, W. (1975):*** Antibiotic sensitivity tests on strains of Aeromonas isolated from carp. Deutsche. Tirrarziliche Wochenshrift, 82(2): 80-83.
- ***Ola.A.Basha (2007):*** Bacteriological studies on Aeromonas infections of Nile Tilapia (Oreochromas niloticus) in fish farms in Alexandria Governorate. Assiut Vet. Med. J. 53: 81-90.
- ***Ozbas, Z.Y.; Vural, H. and Aytac, S.A. (1996):*** Combined effect of gamma-irradiation and conventional cooking on Aeromonas hydrophila in meat. Bull. Zlebensm Unters Forsch, 202(1): 60-62.
- ***Picard,B.;Arlet,G.and Goullet,P.(1983):*** Water origin of Aeromonas hydrophila hospital infections. Press Med., 12 (11): 700-702.

- ***Popoff, M. and Veron, M. (1984):*** Aeromonas, P.545-584. INN.R. Krieg (ed). Bergy's manual of systemic bacteriology, Vol.1. The Williams and Wilkings Co. Baltimore.
- ***Rajesh, K and Ashwani, K. (1997):*** Prevalence of motile aeromonads in foods of animal origin. Journal of science and technology, India, 34(3): 228-229.
- ***Samaha, H.A.; Haggag, Y.N. and Nadia Mahfouz. (2004):*** Brackish and marine water fish as a source of certain bacterial pathogens to human beings. J. Alex. Vet. Sci., 21: 238-248.
- ***Shahat, A.A. and Hamoda, E.E. (2000):*** Aeromonas septicaemia in Tilapia nilotica cultured farm. J. Egypt. Vet. Med. Ass., 60: 139-145.
- ***Shotts, E.B.J.R. and Rimler, R. (1973):*** Medium for the isolation of Aeromonas hydrophila. App. Microbiol. 26(4): 550-553.
- ***Son,R.;Rusul,G.;Sahilab,A.M.;Zainuri,A.;Raha, A.R. and Salmuh, I (1997):*** Antibiotic resistance and plasmid profile of Aeromonas hydrophila isolates from cultured fish (Tilapia Mossambica). Lett. Appl. Micro. 24: 479-482.
- ***Wakabayashi, H.; Kani, K.; Hus, T.U. and Egusa, S. (1981):*** Pathogenic activities of Aeromonas hydrophila biovar hydrophila (chester). Fish. Pathol. 15(3/4): 319-325.
- ***Walker, S.J. and Brooks, J. (1993):*** Survey of the incidence of Aeromonas and Yersinia species in retail foods. Food control, 4(1): 34-40.
- ***Yamamoto, K.; Nagamine, S.; Kamiya, T.; Murakmi, R.; Kikuchi, J. and Sato, H. (1994):*** Identificaiton to the genus species levels of Aeromonas strains isolated from raw meat and carp, and evaluation of their biological activities. J. of food hygienic-society of Japan. 35(2): 187-194.

مدى تواجد ميكروبات الايروموناس فى اللحوم المفرومة المجمدة فى أسواق محافظة الإسكندرية

د. فتح الله على الشابورى ، د. آمال فهمى على منصور، د. علا عبد العزيز محمد باشا

معهد بحوث صحة الحيوان – فرع الإسكندرية

أجريت هذه الدراسة على خمسون عينة لحوم بقرية مفرومة مجمدة تم تجميعها من أسواق محافظة الإسكندرية من مستويات معيشية مختلفة وذلك لعزل ميكروبات الايروموناس والتى تتسبب فى حدوث اضطرابات ونزلات معوية للإنسان نتيجة لإفرازها توكسينات فى الطعام. ولقد تم عزل عدد 11 معزولة من ميكروبات الايروموناس وتلاحظ وجود عدد 9 معزولات من الايروموناس هيدروفيلا وعدد أثنين معزولة من الايروموناس كافى.

ولقد أجريت دراسة بعض عوامل الضراوة لعترات الايروموناس هيدروفيلا المعزوله مثل إفراز أنزيم تحلل الدم وكذلك إفراز التوكسينات مع دراسة حساسية هذه المعزولات البكتيرية للمضادات الحيوية المختلفة وأفضل الطرق التشخيصية من العزل البكتريولوجى والفحوص البيوكيميائية لتعريف الميكروب المعزول. هذا وقد تمت دراسة الأهمية الصحية والاقتصادية والمقترحات الواجب اتباعها للمحافظة على اللحوم المفرومة من التلوث بهذه الميكروبات.