PREVALENCE OF AEROMONAS HYDROPHILA AND YERSINIA ENTEROCOLITICA IN SOME SEAFOOD'S SOLD IN SHARKIA GOVERNORATE MARKETS AND THE EFFECTS OF HEAT TREATMENTS ON THEIR VIABILITIES

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ABSTRACT

A total of 120 samples of fresh mullet, frozen tilapia fillet and chilled shrimps (40 each) were collected from the markets of Sharkia Governorate for surveying the organisms of Aeromonas hydrophila and Yersinia enterocolitica in their flesh. Aeromonas organisms. Were detected in 34 (85%), 12 (30%) and 28 (70%) of samples, with the mean levels of total colony count were 4.91±0.247, 4.383±0.463 and 4.44±0.125 log CFU/g respectively. Such organisms were identified as Aeromonas hydrophila, A. caviae and A. sobria with acommon prevalence for A. hydrophila.

Whilst Yersinia organisms recognized in 26 (65%), 6 (15%) and 20 (50%) samples of the examined samples, with the mean value of a total colony count of 5.142 ±0.353, 4.284±0.671 and 3.06±0.221 log CFU/g respectively. These organisms were identified into, Y. enterocolitica, Y. fredrediksenii, Y. Intermedia and Y. Kristensenii with a highest prevalence for Y. enterocolitica .

The present investigation revealed also that both of A. hydrophila and Y. enterocolitica were sensitive to heat treatment. Thus, good ripening of sea foods is adequate to control of these microorganisms.

INTRODUCTION

Fish and other aquatic foods are important sources of high quality, easily digestible protein and often low fat **(Salem, 2003)**. Moreover, it contained high levels of some essential minerals and trace elements as phosphorus, iodine and zinc. Because these foods have gained popularity among international consumers; thus, the studying of these microbiological contaminations is significant for the public health of consumer.

Aeromonas hydrophila is a common contaminant of fish and seafood. They also are ubiquitous in the water environment **(Hänninen et al, 1997)**. It occurs widely in coastal waters aud wastewater. The prevalence of Aeromonas spp. in the aquatic envi-

ronment has been reeognized as a potential health risk, and some countries have adopted aeromonas eounts as an additional indicator of water quality **(Kong et al, 1999)**. Moreover, Aeromonas hydrophila is a psyehrotrophie spoilage bacterium and potential pathogen which has been isolated from a variety of refrigerated foods of animal origin as fish, shrimps and other fish products **(James et al, 1997)**. From the public health point of view, there is now evidence that some strains of Aeromonas species are enteropathogens. Such strains possess virulence properties, such as the ability to produce enterotoxins, cytotoxins, haemolysins and/or the ability to invade epithelial cells. Strains with these properties are common contaminants of drinking water and a wide range of foods. Contact or consumption of contaminated water, especially in summer, is a major risk factor in Aeromonas-associated gastroenteritis **(Kirov, 1993)**.

Yersinia enterocolitica is a foodborne pathogen and it contaminates refrigerator foods due to its psychrotrophic nature **(Vishnubhatla et al, 2000)**. So; fish, shrimps and fish products which often sold in chilled or frozen condition may be exposed to contamination with Yersinia enterocolitica which responsible for the most human illnesses by Yersinia spp. Infection with Y. enteroeolitica can cause a variety of symptoms depending on the age of the person infeeted. Infection with Y. enterocolitica occurs most often in young children. Common symptoms in children are fever, abdominal pain, and diarrhea, wbich is often bloody. Symptoms typically develop 4 to 7 days after exposure and may last 1 to 3 weeks or longer. In older children and adults, right-sided abdominal pain and fever may be the predominant symptoms, and may be confused with appendicitis. In a small proportion of eases, complications such as skin rash, joint pains, or spread of bacteria to the blood stream can occur **(C.D.C 2009)**.

The aim of the present study is to determine the prevalenee of Aeromonas hydrophila and Yersinia enterocolitica in some sea foods in Sharkia Governorate and study the effect of heat treatment on their viabilities.

MATERIAL AND METHODS

Collection of samples :

A total of 120 samples of fresh mullet, frozen tilapia fillet and chilled shrimps (40 each) were eolleeted from the markets of Sharkia Governorate. Each sample was packaged in a sterile plastic bag. The collected samples were transferred directly to the laboratory under aseptie condition with a minimum of delay, wherein they were subjected to bacteriological examination.

Determination of Aeromonas organisms count :

The count of Aeromonas organisms was determined by using the surfaee sprcad platc technique, where 10 gm from each sample were aseptically transferred to 90 ml of peptone water 0.1% and blended for 2 min. The prepared samples were serially diluted up to 10-6 using 0.1% peptone water, and the count was earried out on the aforementioned dilutions as rccommended **(Palumbo et al, 1985)** using MacConkey manitol ampicillin agar. The number of colonies which showed red color in a eountable plate was enumerated as Aeromonas organisms.

Isolation of Aeromonas species:

a- Enrichment procedure: This was done according to the technique was adopted **(Palumbo et al, 1989)**.

b- Isolation and identification techniques: The technique adopted was that used as previously described **(Okrend et al, 1987)** and **(Koneman et al, 1994)** .

Isolation and Identification of Yersinia enterocolitica

a- Enrichment procedure

1 ml of rinse solution of fish meat was placed aseptically into enrichment broth (Trypticase soy broth) and incubated at 37°C for 24 hours **(Greenwood and Hooper 1989)**.

b- Isolation technique

A loopful of the incubated broth was streaked directly onto Cefsuldin Irgasan Novovbiocin (CIN) as previously described **(Schiemann, D. A. 1979)** and then incubated at 37°C for 24 hours. The presumptive colonies were identified **(Schiemann, and Devenish 1982)**.

Bacterial culture (inoculum)

The culture of microorganism was maintained on brain heart infusion agar (BHI) slants inoculated was incubated into BHI broth and inoculated overnight at 37°C. Serial dilution of fresh culture was carried out onto 0.1% peptone water to obtain a target level of microorganism of 107 CFU/ ml when 0.1 ml of inoculum was applied to each side of product.

Sample inoculation

Sixty negative sub samples of both Aeromonas hydrophila and Yearsinia enterocolitica resulted from fish fillet meat samples were grounded. Thirty ground samples were mixed with Aeromonas hydrophila and another 30 ground samples were mixed with Yersinia enterocolitiea at a ratio of 1 ml of culture per 100 gm of meat sample. The inoculation level for the both examined bacteria was about 107 CFU /g (7 log). Inoculated fish meat samples were kept at 4°C for 30 min to allow bacterial cells attachment to meat.

Heat treatment:

Thirty of the inoculated samples with Aeromonas hydrophila were treated by submersion in thermostatically - controlled water bath at 30- 50°C, 50- 70°C and 70- 90°C for one minute (10 samples for each temperature). Another 30 inoculated samples with Yersinia entcrocolitica were exposed to the same heat treatments as previously mentioned with Aeromonas hydrophila. After the conducting of the heat treatments, the examined samples were cooled immediately in an ice water bath. All samples were tested microbiologieally for obtaining the count of Aeromonas hydrophila and Yersinia enterocolitica after the mentioned heat treatment.

RESULTS AND DISCUSSION

Results achieved in Table (1) revealed that Aeromonas organisms. were detected in 34 (85%), 12 (30%) and 28 (70%) of mullet, frozen tilapia fillet and shrimp samples with mean logarithmic levels 4.91 ± 0.247, 4.383 ± 0.463 and 4.44 ± 0.125 log/g respectively. These results were higher in both the incidence and count than those previously reported **(Abd El- Daym 1999)** and **(Abo - EL-Alla 2000)** On the other hand, another study in

Finland detected higher incidence of Aeromonas spp. than those in the present study in fish and shrimps (93% and 100%, respectively) **(Hänninen et al, 1997)** .

Table (2) showed that three strains of Aeromonas Spp. were detected, A. hydrophyla was detected in 30, 10 and 20 samples of mullet, frozen tilapia fillet and shrimp respectively, the same examined sea foods contained A. caviae in 3, 1 and 5 samples respectively. Meanwhile, A. sobria was detected in 1, 1 and 3 of the previously mentioned samples respectively. It is evident from these data that A. hydrophyla was the most common species from the examined samples followed by A. caviae and A. sobria, this result coincided with those obtained in Egypt **(Abo - EL- Alla 2000)**.

Concerning the effects of heat treatment on the Aeromonas Spp. count, Table 3 revealed that the temperature range from 30- 50°C for 1 minute redueed the Aeromonas Spp. count from 7 log/ CFU (the initial count) to 4.709 log/ CFU, while; 50- 70°C for one minute redueed the initial number to 2.269 log/ CFU. Furthermore, the initial number of the tested microorganism reached to zero when exposed to 70-90°C for one minute. Thus, the obtained results revealed that the Aeromonas Spp. was highly sensitive to thermal treatment. Our results coincided with another study recorded that Aeromonas Spp. was obviously sensitive to heat treatment than E coli O157 H7, Staphylococcus aureus, and Salmonella typhimurium and it was killed within 2 minutes at 55°C. **(Nishikawa et al, 1993)**.

Regarding Yersinia spp., Table (4) showed that it was detected in 26 (65%), 6 (15%) and 20 (50%) of the examined mullet fish, frozen tilapia fillet and shrimp samples respectively, with the mean total colony counts of 5.142 ± 0.353, 4.284 ± 0.671 and 3.06 ± 0.221 log/ CFU respectively in the same mentioned sea foods. These incidences were higher than those detected in meat products in Egypt **(Saleh, and Salah El- Dien, 2005)**, while; another Egyptian study could not be detect Yersinia spp. in all the examined fish samples in both rural and urban areas **(Soliman et al, 2002)**. On contrary, our figures reeoded lower incidence of Yersinia spp. than those reported in fish in India **(Khare et al, 1996)**.

Table (5) showed that four strains of Yersinia spp. were detected in the examined samples. Y. enterocolitica was isolated from 14, 5 and 15 samples of mullet fish, frozen tilapia fillet and shrimp respeetively. Y. fredrediksenii was detected in 4, 1 and 3 samples of the mentioned examined sea foods respectively. On the other hand, Y. Intermedia was found in 5 mullet fish and 2 shrimp samples, while; Y. Kristensenii was detected only in 3 mullet samples. The obtained results showed that, Y. enterocolitica was the most predominant isolate, this result agreed with another Egyptian study **(Bahout, and Moustafa, 2004)**. On contrast, the most predominant isolates in the examined fish samples in a previously Indian study were Y. intermedia and Y. pestis **(Khare et al, 1996)**.

Table (6) indicates that the temperature ranged from 30- 50°C for 1 minute reduced the total count of Y. enterocolitiea from 7 log/ CFU (the initial eount) to 4.414 log/ CFU. Also, the exposure to 50- 70°C for 1 minute

reduced the count of the examined microorganism from 7 log/ CFU to 3.255 log/ CFU. Meanwhile, the exposure to temperature ranged between 70- 90°C for 1 minute was suffielent to reduce the initial number of Y. enterocolitica from 7 log / CFU to zero. This finding revealed a relatively high sensitivity of Y. enterocolitica to heat treatment, and it was highly expected due to the psychotropic nature of this microorganism. The obtained results coincided with those reported in Spain **(Pagán et al, 1996)** and U.S.A. **(Porto-Fett et al, 2009)**.

Concerning the fitness of the examined sea foods for the human consumption, Aeromonas Spp. and Yersinia spp. were not judged by "Egyptian Organization for Standardization and Quality Control" **(EOSQC 2005)**.

From aforementioned results, it is evident that the examined sea foods suffered from relativcly high incidence and levels of both Aeromonas spp. and Yersinia spp. especially A. hydrophila and Y. enterocolitica. These results explained by lack of hygienic supervision during handing and transformation of these sea foods. Moreover, because the examined sea foods were displayed in frozen or chilling state, the psychotropic character of the tested microorganisms plays an important role to elevate its incidence and count **(Vishnubhatla et al, 2000)** and **(Abo - EL- Alla 2000)**. Fortunately, the obtained results showed that both A. hydrophila and Y. enterocolitica were sensitive to heat treatment. This result can threw a light upon the suggested solutions of food contamination problem with these mentioned microorganisms.

CONCLUSION AND RECOMMENDATIONS

1- Maintaining clean water supply and instruments used in ice produetion for chilling of sea foods.
2- Transportation, storage and displaying of sea foods should be under hygienic procedures.
3- Continuous monitoring of the hygienic state of different sea foods is highly recommended.
4- Sea foods must be exposed to adequate temperature during its ripening to destruct the microorganisms under the present investigation.

Table (1): Occurrence and intensity of Aeromonas organisms in surveyed seafood samples. *(n* = 40 each)*

Type of samples	Numbers and percentages of Aeromonas-contaminated samples	Logarithmic levels of Aeromonas organisms (Log) in contaminated samples		
		Min.	Max.	Mean± S.E.
Mullet	34 (85 %)	2.26	6.857	4.91 ±0.247
Frozen tilapia fillet	12 (30%)	1.973	6.50	4.383 ±0.463
Shrimp	28 (70%)	3.437	5.623	4.44 ±0.125

n* = number of examined samples.

Table (2): Serotyping of *Aeromonas spp* in the examined samples (n=40 for each).

Type of samples	No. of total isolated strains	*A. hydrophyla*	*A. caviae*	*A. sobria*
Mullet	34	30	3	1
Frozen tilapia fillet	12	10	1	1
Shrimp	28	20	5	3
Total (120 samples)	**74 strains (100%)**	**60 strain (81.08)**	**9 strains (12.16%)**	**9 strains (12.16%)**

Table (3): Effects of 3 heat treatments on the mean logarithmic counts ±S.E. of *Aeromonas hydrophila* in fish fillet (n = 10 for each treatment).

Initial population of Aeromonas hydrophila	mean logarithmic levels of *Aeromonas hydrophila* (log/ CFU)		
	After heating at 30- 50 °C for one min.	After heating at 50- 70 °C for one min.	After heating at 70 - 90°C for one min
7 log/g	4.709 log/g	2.269 log/g	0.0 log/g

Table (4): Occurrence and intensity of *Yersinia* organisms in surveyed seafood samples. *(n* = 40 each)*

Type of samples	Numbers and percentages of *Yersinia*-contaminated samples	Logarithmic levels of *Yersinia* organisms (Log) in contaminated samples		
		Min.	Max.	Mean± S.E.
Mullet	26 (65%)	2.3	7.6	5.142 ±0.353
Frozen tilapia fillet	6 (15%)	1.079	5.434	4.284 ±0.671
Shrimp	20 (50)	1.778	4.698	3.06 ±0.221

n* = number of examined samples.

Table (5): Serotyping of *Yersinia Spp.* In the examined samples (n=40 for each).

Type of samples	No. of total isolated strains	*Y. enterocolitica*	*Y. fredrediksenii*	*Y. Intermedia*	*Y. Kristensenii*
Mullet	26	14	4	5	3
Frozen tilapia fillet	6	5	1	0.0	0.0
Shrimp	20	15	3	2	0.0
Total (120 samples)	**52 strains (100 %)**	**34 strains (65.38%)**	**8 strains (15.38%)**	**7 strains (13.46%)**	**3 strains (5.77%)**

Table (6): Effects of 3 heat treatments on the mean logarithmic counts ±S.E. *of Yersinia enterocolitica* in fish fillet (n = 10 for each treatment).

Initial population of *Yersinia enterocolitica*	Mean logarithmic levels of Yersinia enterocolitica (log/ CFU)		
	After heating at 30- 50 °C for one min.	After heating at 50- 70 °C for one min.	After heating at 70 - 90°C for one min
7 log/g	4.414 log/g	3.255 log/g	0.0 log/g

REFERENCES

Abd El- Daym, W. F. A. (1999) : Microbiological aspects of smoked fishes at retail outlets. Master D. Thesis, Vet. Med. Sci. (Meat Hygiene) Zagazig Univ.

Abo - EL-Alla (2000) : Studies on Aeromonas hydrophila in fresh water fish (Oreochromis niloticus and Labeo Niloticus) and smoked fishes (Herrings) in Assiut Governorate.

Ahmed, M. Sh.; Zaitoun, A. M. and Ali, H. S. (1991) : Motile Aeromonas septicaemia (MAS) in Mormyrus Kannume at Assiut Governorate. Assiut Vet. Med. J., 25: 145-150.

Bahout, A. A. and Moustafa, A. H. (2004) : Occurrence of Yersinia species in raw and pasteurized milk in Dakahlia Governorate. Assiut Vet. Med. J. Vol. 50 No.101: 57-63.

Bastawrows, A. F. and Mohammed, A. A. (1999) : Some microbiological investigations on Aeromonas hydrophila group in Oreochromis niloticus and Clarias lazera in Assiut Governorate. Assiut Vet. Med. J.,40:197-206.

C. D. C. Central of disease control and prevention (2009) : Yersinia enterocolitica. On web site: http.//www.cdc.gov/index.htm

Egyptian Organization for standardization and Quality Control (2005) : The permissible limits for fish. 1-889/2005.

Greenwood, M. H. and Hooper, W. L. (1989) : Improved methods for the isolation of Yersinia species from milk and foods. Food Microbiol., 41(3): 99-104.

Hänninen, M, L.; Oivanen, P. and Hirvelä-kosk, V. I. (1997) : Aeromonas species in fish, fish-eggs, shrimp and freshwater. Intern. J. of Food Microbiol. 34(1) : 17-26.

James, S D.; Sheldon, Brian W. and Foegeding, P. M. (1997) : Thermal resistance of Aeromonas hydrophila in liquid whole egg. J. of Food Prot. 60(3): 231-236.

Khare S. S.; Kamat, A. S.; Doctor T. R. Nair P. M. (1996) : Incidenee of Yersinia enterocolitica and related species in some fish, meat and meat products in India. Journal of the science of food and agriculture vol. 72, (2), pp. 187-195.

Kirov, S. M. (1993) : The public health significance of Aeromonas spp. in foods. Intern. J. of Food Microbiol. 20(4): 179-198.

Koneman, E. W.; Allen, S. D.; Janda, W. M.; Schreckenberger, P. C. and Winn, W. C. Jr. (1994) : Interoduction to Diagnostic Microbiology J.B. Lippincott Company pp. 117-123.

Kong, R. Y. C. A.; Pelling, C. L. and R. S. S. Wu (1999) : Identification of oligonucleotide primers targeted at the 16S–23S rDNA Intergenic spacers for genus- and species-specific detection of aeromonads. Marine Pollution Bulletin 38(9): 802-808

Krend, A. J. G.; Rose, B. E. and Bennett, B. (1987) : Incidence and toxigenicity of Aeromonas species in retailed poultry, beef and pork. J. Food Port., 50: 509-513.

Nishikawa, Y.; Ogasawara, J. and Kimura, T. (1993) : Heat and acid sensitivity of motile Aeromonas: a comparison with other food-poisoning bacteria. International journal of food microbiology. 18(4):271-8.

Pagán, R.; Mañas, P.; Raso, J.; and Sala Trepat, F.J.(1999): Heat resistanee of Yersinia enterocolitica grown at different temperatures and heated in different media. Intern. J. of Food Microbiol. 47(1-2):59 -66.

Palumbo, S. A.; Maxino, F.; Williams, A. C.; Buchanaaaaon, R. L. and Thayer, D. W. (1985) : Starch - ampicillin agar for the quantitative detection of Acromonas hydrophila. Appl. Environ. Microbiol., 50 : 1027-1030.

Palumbo, S. A.; Bencivengo, M. M.; Delcarral, F.; Williams, A. C. and Buchanan, R. L. (1989) : Charaeterization of Aeromonas hydrophil group isolated from retailed food of animal origin. J. Clin. Microbiol. 27: 854-859.

Porto-Fett, A. C.; Juneja, V. K.; Tamplin, M. L. and Luchansky, J. B. (2009) : Validation of cooking times and temperatures for thermal inactivation of Yersinia pestis strains KIM5 and CDC-A1122 in irradiated ground beef.. J Food Prot. 72(3):564-71.

Saleh, M. A. and Salah El-Dien, W. M. (2005): Microbiological studies on some meat products At Sharkia Governorate Markets. Zag. Vet. J. Vol. 33 (3): 141-151.

Salem D. A. (2003) : Survey of some environmental pollution in freshwater fishes Assiut Governorate. Egypt. Ass. Univ. Bull. Environ. Res. Vol.6 (2): 15- 36.

Schiemann, D. A. (1979) : Synthesis of a selective agar medium for Y. enterocolitica. Canadian J. of Microbiol., 25: 1298-1304.

Schiemann, D. A. and Devenish, J. A. (1982) : Relationship of Hela cell infectivity to biochemical, serological and virulence characteristics of Y. enterocolitica. Infect. Immun., 35: 497-506.

Soliman, M. R.; Abo El-Monem, K. H. M. and Saad, S. M. (2002) : Mierobiological quality of ready to eat meat products and fishes in urban and rural areas. J. Egypt. Vet. Med. Ass. 62,No. 6a: 39-51.

Vishnubhatla, A.; Fung, D. Y. C.; Obrat, R. D.; Hays M. P.; Nagaraja, T. G. and Flood, S. J. A. (2000) : Rapied 5' Nuclease (Toq Man) Assay for detection of virulent strain oe Yersinia enteroeolitica. Appl. Environ. Microbiol. 66 (9): 4131-4135.

الملخص العربى

مدى تواجد جراثيم إيروموناس هيدروفيلا واليارسينيا انتروكلوتيكا فى بعض المأكولات البحرية المباعة فى أسواق محافظة الشرقية وتأثير المعالجات الحرارية على حيويتها

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أجريت هذه الدراسة لاستبيان مدى تواجد ميكروبى إيروموناس هيدروفيلا ويارسينيا أنتروكلوتيكا فى بعض المأكولات البحرية بمحافظة الشرقية، وكذلك لدراسة أثر المعالجة الحرارية على تلك الميكروبات.

تم تجميع عدد ١٢٠ عينة من أسماك البورى الطازج المبرد، وفيليه البلطى المجمد والجمبرى المبرد (٤٠ عينة من كل نوع).

وقد أسفرت الدراسة عن وجود جراثيم إيروموناس فى ٣٤ (٨٥٪)، ١٢ (٣٠٪)، ٢٨ (٧٠٪) من عينات البورى، فيليه البلطى المجمد والجمبرى المبرد على التوالى وقد كان لوغاريتم متوسط أعداد هذه الميكروبات ٤٫٩١، ٤٫٣٨٢، ٤٫٤٢ خلية/جم على التوالى، وقد تم تصنيف ٣ عترات من الإيروموناس وهى إيروموناس سوبريا، إيروموناس كافى وإيروموانس هيدروفيلا وقد وجد أن الأخيرة هى العترة الغالبة.

أما جراثيم يارسينيا فقد أسفرت الدراسة عن وجود ذلك الميكروب فى ٢٦ (٦٥٪)، ٦(١٥٪)، ٢٠ (٥٠٪) عينة من عينات البورى، فيليه البلطى المجمد والجمبرى المبرد على التوالى وذلك بلوغاريتم متوسط قدره ١٤٢٫٥، ٢٨٤٫٤، ٣٫٠٦ خلية/جم على التوالى، وقد تم عزل يارسينيا فريدريكسينا، يارسينيا أنترميديا، يارسينيا كريستنسنا ويارسينيا أنتركلوتيكا وقد وجد أن العترة الأخيرة هى الغالبة فى معظم العينات.

من ناحية أخرى أسفرت نتائج الدراسة عن حساسية الميكروبات المختبرة للحرارة وهذه الخاصية توضح أن التعرض لحرارة النضج كافية لجعل المأكولات البحرية آمنة من الميكروبات محل الدراسة.