Kafkas Univ Vet Fak Derg
20 (5): 691-696, 2014
DOI: 10.9775/kvfd.2014.10892

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

Occurrence of *Aeromonas hydrophila* in Fish, Shrimp, Lobster and Crab in Iran

Ebrahim RAHIMI [1] Mehdi RAISSY [1] Morteza RAZZAGHIMANESH [2]
Asiye AHMADI DASTGERDI [3] Manouchehr MOMENI SHAHRAKI [4]

[1] Department of Food Hygiene, College of Veterinary Medicine, Shahrekord Branch, Islamic Azad University, Shahrekord - IRAN
[2] Graduated Student of Veterinary Medicine, College of Veterinary Medicine, Shahrekord Branch, Islamic Azad University, Shahrekord - IRAN
[3] Department of Food Science and Technology, College of Agriculture, Shahrekord Branch, Islamic Azad University, Shahrekord - IRAN
[4] Young Researchers And Elite Club, Islamic Azad University, Shahrekord Branch, Shahrekord - IRAN

Makale Kodu (Article Code): KVFD-2014-10892

Summary

This study was conducted to determine the prevalence rate and antimicrobial resistance of *Aeromonas hydrophila* in fish, shrimp, lobster and crab caught off the south coast of Iran. A total of 541 samples including, 133 freshly caught fish of 4 different types including *Otollithes ruber, Pamous argenteus, Parastromateus niger* and *Psettodes erumel,* 240 shrimp of 4 different species including *Penaeus monodon, P. semisulcatus, P. indicus,* and *P. merguiensis,* 108 lobster (*Panulirus homarus*) and 60 crab (*Panulirus homarus*) were collected in 3 provinces along Persian Gulf in the south coast of Iran. Samples were collected at the end of each month from September 2011 to May 2012. Using conventional bacteriological techniques, 66 *A. hydrophila* isolates were identified in which 62 strains were confirmed by PCR assay targeting *16S rDNA* gene of *A. hydrophila*. Using PCR assays targeting the *A. hydrophila* cytolytic enterotoxin gene, 57 (10.5%) isolates were positive. The highest prevalence of *A. hydrophila* was found in fish (19.5%), followed by shrimp (9.2%), lobster (9.3%) and crab (6.7%). The highest prevalence of A. hydrophila occurred in summer (21.3%) followed by fall (12.0%), spring (10.8%), and winter (5.6%). To our knowledge, the present study is the first report of the isolation of *A. hydrophila* from fish, shrimp, lobster and crab in Iran.

Keywords: *Aeromonas hydrophila, Seafood, Seasonal variation, Virulence factors, Fish, Shrimp, Lobster, Crab*

İran'da Balık, Karides, Istakoz ve Yengeçlerde *Aeromonas hydrophila* Mevcudiyeti

Özet

Bu çalışma İran'ın güney kıyılarında yakalanan balık, karides, ıstakoz ve yengeçlerde *Aeromonas hydrophila* prevalansını ve etkenin antimikrobiyal dayanıklılığını belirlemek amacıyla yapılmıştır. Çalışmada 4 ayrı türden, *Otollithes ruber, Pamous argenteus, Parastromateus niger* ve *Psettodes erumel,* toplam 133 balık; 4 ayrı türden, *Penaeus monodon, P. semisulcatus, P. indicus,* ve *P. merguiensis,* toplam 240 adet karides; 108 ıstakoz (*Panulirus homarus*) ve 60 yengeç (*Panulirus homarus*) İran'ın güney sahillerinde İran Körfezi boyunca 3 bölgeden olmak üzere toplam 541 örnek toplanmıştır. Örnekler Eylül 2011 ile Mayıs 2012 arası her ayın sonunda toplanmıştır. Rutin bakteriyolojik metot ile 66 *A. hydrophila* izolatı belirlenmiş ve bunların 62'si PCR ile *A. hydrophila 16S rDNA* gen hedefi ile teyit edilmiştir. *A. hydrophila* sitotoksik enterotoksin gen hedefi ile uygulanan PCR metodu ile 57 (%10.5) izolat pozitif olarak tespit edilmiştir. *A. hydrophila* prevalansı en yüksek olarak balıklarda (%19.5), sonrasında ise sırasıyla ıstakozlarda (%9.3), karideslerde (%9,2) ve yengeçlerde (%6.7) belirlenmiştir. Mevsimsel olarak en yüksek *A. hydrophila* prevalansı yaz ayında (%21.3) olurken bunu sırasıyla sonbahar (%12.0), ilkbahar (%10.8) ve kış (%5.6) izledi. Bizim bilgimiz kapsamında bu çalışma İran'da balık, karides, ıstakoz ve yengeçlerde *A. hydrophila* izolasyonunun rapor edildiği ilk çalışmadır.

Anahtar sözcükler: *Aeromonas hydrophila, Deniz ürünü, Mevsimsel varyasyon, Virulans faktörleri, Balık, Karides, Istakoz, Yengeç*

İletişim (Correspondence)
+98 311 6259809
ebrahimrahimi55@yahoo.com

INTRODUCTION

Genus *Aeromonas* has emerged as an important human pathogen because of suspected food-borne outbreaks [1,2] and the increased incidence of its isolation from patients with traveller's diarrhea [3,4]. Among the 14 species of *Aeromonas* known to date *A. hydrophila, A. caviae*, and *A. veronii* biotype sobria have most commonly been involved in human infections and have been found to produce a variety of virulence factors such as hemolysins, cytotoxins, enterotoxins, proteases, leukocidin, phospholipases, endotoxins, outer membrane proteins, and fimbriae [5]. A number of *Aeromonas* spp. are able to grow in raw, cooked, and processed foods, at refrigeration temperature, under modified atmosphere and under modified growing conditions [6,7].

Aeromonas can be found in soil, fresh and saline water, drinking water and animal faeces [8]. Also several investigations have shown that members of the genus *Aeromonas* are also widely distributed in various foods such as meat [9,10], sea food [11-14], and vegetable [15]. Thus, foods have been suggested as a vector in the dissemination of this pathogen. The potential role of *A. hydrophila* in human gastrointestinal infections is noted by Kirov [1]. The majority (>85%) of gastroenteritis cases are attributed to three *Aeromonas* species, one of them is *A. hydrophila* [4].

Currently, there is limited information regarding the prevalence of *A. hydrophila* in seafood in Iran. This study was conducted to determine the prevalence rate and virulence genes of *A. hydrophila* in fish, shrimp, lobster and crab caught off the south coast of Iran.

MATERIAL and METHODS

Sample Collection

A total of 133 freshly caught fish of 4 different species including *Otollithes ruber* (n=39), *Pamous argenteus* (n=37), *Parastromateus niger* (n=28) and *Psettodes erumel* (n=29), 240 freshly caught shrimp of 4 different species including *Penaeus monodon* (n= 60), *P. semisulcatus* (n= 60), *P. indicus* (n= 60), and *P. merguiensis* (n= 60), 108 freshly caught lobster (*Panulirus homarus*) and 60 freshly caught crab *(Porpunus pelagicus)* were collected in 3 provinces (Bushehr, Hormozgan and Khuzestan) along Persian Gulf in the south coast of Iran. Samples were collected at the end of each month from September 2011 to May 2012, placed in separate sterile plastic bags to prevent spilling and cross contamination, and immediately transported to the laboratory in a cooler with ice packs.

Microbiological Analysis

The samples were processed immediately upon arrival using aseptic techniques. All the specimens were rinsed with sterile water to remove the adhering particles. Twenty-five grams of the fish samples were homogenized with 225 mL alkaline peptone-water (APW).The homogenate was incubated for 6 h at 37°C. Whole shrimp, lobster and crab were dipped into screw cap bottles containing APW so as to transfer the bacterial load into APW. Samples were removed from the bottles after dipping for 2 min. After incubation, a loopful of the APW culture was streaked on starch ampicillin agar medium (Himedia, Mumbai, India) and incubated at 37°C for 18-24 h as described by Vivekanandhan et al.[11]. The plates were then flooded with approximately 5 ml of Lugol's iodine solution and amylase positive yellow to honey coloured colonies were isolated. The isolated cultures were then purified by repeated streaking on nutrient agar and maintained in nutrient agar slants. Tubes with alkaline slant and acid butt after 24 h at 37°C were considered as presumptive positive for *A. hydrophila*. The presumptive isolates were confirmed as *A. hydrophila* based on the following reactions: motile, Gram-negative, cytochrome oxidase positive, glucose fermentation positive, arginine dihydrolase positive, ornithine decarboxylase negative, ONPG positive, H_2S from cystein, acetoin from glucose, gas from glucose, l-arabinose utilization and fermentation of salicin [11]. We have used a type strain of *A. hydrophila* (ATCC 7966), as reference strain to compare the results.

Detection of A. hydrophila from Pure Culture

One milliliter pure culture of *A. hydrophila*, identified by biochemical tests, was centrifuged at 13.000 g for 5 min at room temperature. Purification of DNA was achieved using a Genomic DNA purification kit (Fermentas, GmbH, Germany) according to the manufacturer's instructions and the total DNA was measured at 260 nm optical density according to the method described by Sambrook and Russell [16].

The PCR procedures used in this study have been described previously [17]. Two genes selected for the identification of the *A. hydrophila* and *A.* hydrophila cytolytic enterotoxin gene as a multivirulence gene causing lethality in mice, haemolysis, cytotoxicity and enterotoxigenicity were the 16S rDNA gene [18], and the Aero gene [19], respectively. The sequences of the three sets of primers used for gene amplification are presented in *Table 1*. All oligonucleotide primers were obtained from

Table 1. *Primer sequences and predicted lengths of PCR amplification products*
Tablo 1. *Primer sekansları ve tahmini PCR amlifikasyon ürün boyutları*

Primer	Oligonucleotide Sequence (5-3)	Fragment Size (pb)	Reference
16S rDNA1	GAAAGGTTGATGCCTAATACGTA	462	[5]
16S rDNA1	CGTGCTGGCAACAAAGGACAG		
Aero1	CTCAGTCCGTGCGACCGACT	685	[16]
Aero2	GATCTCCAGCCTCAGGCCTT		

a commercial source (Cinna Gen, Iran). PCR amplification was performed using a DNA thermal cycler (Master Cycler Gradiant, Eppendrof, Germany) in a total volume of 50 µl. The reaction mixture consisted of 5 µl of template DNA, 5 µl 10x PCR buffer (+$MgCl_2$) (Roche Applied Science, Germany), 4 µl of deoxyribonucleoside triphosphates (2.5 mmol L-1 each of dATP, dTTP, dGTP and dCTP), 0.5 µl of each primer, and 0.25 µl (0.5 U µl-1) of Taq DNA polymerase (Roche Applied Science, Germany), with 50 µl sterile water added. Thirty PCR cycles were run under the following conditions; denaturation at 94°C for 2 min, primer annealing at 56°C for 2 min, and DNA extension at 72°C for 2 min in each cycle.

Detection of A. hydrophila form Enrichment Broth

One millimeter enrichment broth from each shrimp sample was centrifuged at 13.000 g for 5 min at room temperature. The cell pellets were subjected to DNA extraction as described above. A 5 µl aliquot of each sample was used for PCR amplification. All reactions were performed in triplicate. The PCR-amplified products were examined by electrophoresis in a 1.5% agarose gel, stained with a 1% solution of ethidium bromide, and examined under UV illumination. In the present study, *A. hydrophila* (ATCC 7966) were used as the positive control and DNase free water was used as the negative control, respectively.

Statistical Analysis

Data were transferred to Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA, USA) for analysis. Using SPSS 18.0 statistical software (SPSS Inc., Chicago, IL, USA), chi-square test and fisher's exact two-tailed test analysis were performed and differences were considered significant at values of $P<0.05$.

RESULTS

Using conventional bacteriological techniques, 66 *A. hydrophila* isolates were identified. A PCR assay targeting *16S rDNA* gene of *A. hydrophila* confirmed 62 strains as *A. hydrophila* (*Fig. 1*). Using PCR assays targeting the *A. hydrophila* cytolytic enterotoxin gene, 57 (91.9%) isolates were positive (*Fig. 2*). The PCR assays were performed in triplicates and no variability in the results was present. The highest prevalence of *A. hydrophila* was found in fish (19.5%), followed by shrimp (9.2%), lobster (9.3%) and crab (6.7%) (*Table 2*). There were significant differences ($P<0.05$) in the level of contamination with *A. hydrophila* between different types of seafood samples; however, no significant differences ($P>0.05$) were found between different shrimp and fish species. No significant differences in the prevalence rates ($P>0.05$) were observed between seafood isolated in Bushehr, Hormozgan and Khuzestan.

The PCR assays performed on enrichment broth from each sample gave positive results for *A. hydrophila* in 73 (13.5%) samples. *A. hydrophila* cytolytic enterotoxin gene

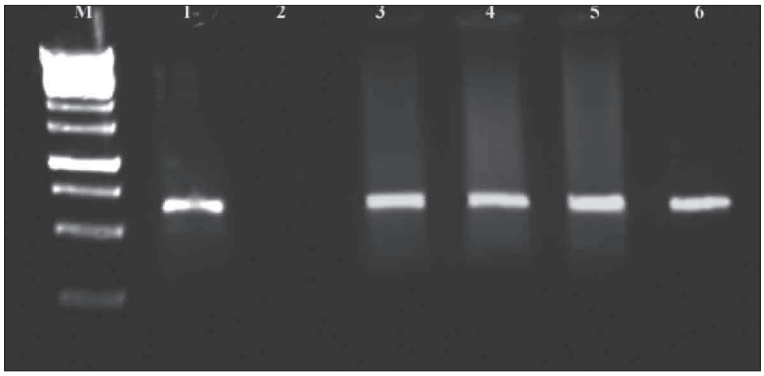

Fig 1. Electropherogram of the amplification products of the polymerase chain reaction (PCR) assay. M, 100 bp DNA ladder; lane1, positive control; lane 2, negative control; lanes 3 to 6, *Aeromonas hydrophila* positive samples from fish, shrimp, lobster and crab

Şekil 1. Polimeraz zincir reaksiyonu ile amplifiye edilen ürünlerin elektroferogramı. M, 100 bp DNA merdiveni; sütun1, pozitif kontrol; sütun 2, negatif kontrol; sütun 3-6, balık, karides, ıstakoz ve yengeçlerde *Aeromonas hydrophila* pozitif örnekler

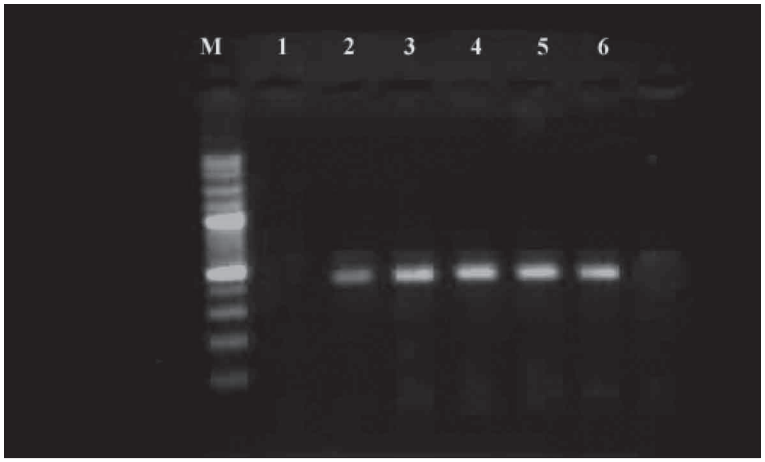

Fig 2. Electropherogram of the amplification products of the polymerase chain reaction (PCR) assay. M, 100 bp DNA ladder; lane1, negative control; lane 2, positive control; lanes 3 to 6, *A. hydrophila* cytolytic enterotoxin gene positive samples

Şekil 2. Polimeraz zincir reaksiyonu ile amplifiye edilen ürünlerin elektroferogramı. M, 100 bp DNA merdiveni; sütun1, negatif kontrol; sütun 2, pozitif kontrol; sütun 3 - 6, *A. hydrophila* sitolitik enterotoksin gen pozitif örnekler

Table 2. *Distribution of the 16S rDNA, and cytolytic enterotoxin genes possessing A. hydrophila isolates detected by PCR in different fish, shrimp, lobster and crab species*
Tablo 2. *Balık, karides, ıstakoz ve yengeçlerde PCR ile tespit edilen A. hydrophila 16S rDNA ve sitolitik enterotoksin gen dağılımı*

Sample	No. of Samples	Pure Culture (%)		Enrichment Broth (%)	
		16S rDNA	Cytolytic Enterotoxin	16S rDNA	Cytolytic Enterotoxin
Fish					
Otollithes ruber	39	7 (17.9%)	7 (17.9%)	9 (23.1%)	9 (23.1%)
Pamous argenteus	37	3 (8.1%)	3 (8.1%)	5 (13.5%)	4 (10.8%)
Parastromateus niger	28	5 (17.9%)	5 (17.9%)	7 (25.0%)	7 (25.0%)
Psettodes erumel	29	11 (37.9%)	11 (37.9%)	12 (41.4%)	12 (41.4%)
Shrimp					
Penaeus monodon	60	9 (15.0%)	7 (11.7%)	9 (15.0%)	7 (11.7%)
Penaeus semisulcatus	60	3 (5.0%)	3 (5.0%)	4 (6.7%)	4 (6.7%)
Penaeus indicus	60	5 (8.3%)	5 (8.3%)	6 (10.0%)	6 (10.0%)
Penaeus merguiensis	60	5 (8.3%)	4 (6.7%)	7 (11.7%)	6 (10.0%)
Lobster					
Panulirus homarus	108	10 (9.3%)	8 (7.4%)	10 (9.3%)	8 (7.4%)
Crab					
Porpunus pelagicus	60	4 (6.7%)	4 (6.7%)	4 (6.7%)	4 (6.7%)
Total	541	62 (11.5%)	57 (10.5%)	73 (13.5%)	67 (12.4%)

Table 3. *Prevalence of Aeromonas hydrophila isolated from fish, shrimp, lobster and crab*
Tablo 3. *Balık, karides, ıstakoz ve yengeçlerde Aeromonas hydrophila prevalansı*

Season	Seafood Sample				Total
	Fish	Shrimp	Lobster	Crab	
Winter	4/41(9.8)*	2/60 (3.3)	0/27 (0.0)	0/14 (0.0)	6/142 (4.2)
Spring	5/31 (16.1)	5/60 (8.3)	3/29 (10.3)	2/18 (11.1)	15/138 (10.9)
Summer	12/37 (32.4)	10/60 (16.7)	5/24 (20.8)	2/15 (13.3)	29/136 (21.3)
Fall	5/24 (20.8)	5/60 (8.3)	2/28 (7.1)	0/13 (0.0)	12/125 (9.6)

** Results expressed as the number of Aeromonas-positive samples/number of samples analyzed (%)*

was detected in 67 (91.8%) isolates. Overall, the observed difference in the frequency of detection of the *A. hydrophila* cytolytic enterotoxin genes from the pure culture versus the enrichment broth was not statistically significant.

Table 3 shows the seasonal prevalence of *A. hydrophila* in fish, shrimp, lobster and crab caught off the south coast of Iran. Overall, the highest prevalence of *A. hydrophila* in seafood samples occurred in summer (21.3%) which was significantly ($P<0.05$) higher than spring (10.9%), fall (9.6%) and winter (4.2%); however, the difference in the prevalence rates of *A. hydrophila* between fall and spring was not statistically significant.

DISCUSSION

To our knowledge, the present study is the first report of the isolation of *A. hydrophila* from four different types of fish, four species of shrimp including *P. monodon*, *P. semisulcatus*, *P. indicus*, and *P. merguiensis*, lobster and crab caught off the south coast of Iran. Out of 133 fishes analysed, 26 (19.5%) fishes were found to be contaminated with *A. hydrophila*. These findings are comparable with those reported from Malaysia [20] and Taiwan [21], New Zealand [22] and Turkey [23]; however, are higher than the prevalence reported from India [11] and of Switzerland [24].

Overall, 9.9% (22 of 240) of all shrimp samples were contaminated with *A. hydrophila*. The prevalence of *A. hydrophyla* in different shrimp species such as *P. monodon*, *P. semisulcatus*, *P. indicus*, and *P. merguiensis* was found to be 15%, 5%, 8.3% and 8.3% respectively. The prevalence of different shrimp species in different shrimp species observed in this study is similar to a recent report in different shrimp species that showed a prevalence of different shrimp species of 16.58%, 13.20% and 25.52% in *P. indicus*, *P. monodon*, and *P. semisulcatus*, respectively [10].

Also this is in agreement with findings of Tsai and Chen [21] and Colakoglu et al.[25]. In another study conducted in coastal South India, *A. hydrophila* was identified in 35.6% of shrimp samples [26]. No previous report could be found on the occurrence of *A. hydrophila* on the lobster and crab.

The prevalence of cytolytic enterotoxin gene carrying *A. hydrophila* isolates reported in our study are comparable with those reported from Malaysia [9,13]. However, our results are higher than the results of a study conducted in India [27].

Variation in the prevalence of *A. hydrophila* isolates from raw fish and shrimp, samples reported in different studies may be a result of different sampling techniques employed, seasonal effects and/or laboratory methodologies employed in different studies (bacteriological and biochemical testing vs. PCR assays) [11,16]. Furthermore, a higher prevalence rate of *A. hydrophila*-positive in seafood could be due to cross-contamination during manual processing or insufficient hygiene during storage and transport in the seafood markets.

The overall prevalence levels in shrimp, lobster and crab were much lower than those recorded in fishes ($P<0.05$). This is in agreement with findings of Tsai and Chen [21] and Vivekanandhan et al.[11]. The chitinous shell of the prawns may not be that conducive for proliferation of the *A. hydrophila*, as the moisture rich body surface of fish [11].

The prevalence of *A. hydrophila* isolated from fish, shrimp, lobster and crab samples in this study was significantly ($P<0.05$) higher in summer (21.3%) than spring, fall and winter. This finding is in agreement with other studies that reported peak prevalence rate of *A. hydrophila* in seafood in the warmer months [10,25]. This could be due to the increased coastal water pollution resulting from land run off, municipal sewage outflows and storm water surge during the monsoon season [11]. However, in some studies no apparent pattern in the seasonality of *A. hydrophila* prevalence was observed in shrimp samples [2].

In this study, *A. hydrophila* was more detected by the PCR assays than the cultural method. This could be due the higher analytical and diagnostic sensitivities of the PCR assays. PCR is capable of detecting culturable and also non-culturable but viable cells, which increases its sensitivity as a detection method. The high throughput and cost-effective m-PCR system developed in this study could provide a powerful addition to conventional methods for more accurate risk assessment and monitoring of pathogenic strains of the *A. hydrophila*. The PCR method identified potential pathogenic *A. hydrophila* strains in <8 h. In addition, the method had advantages in terms of its specificity, easy of use and cost, compared to biochemical and DNA hybridization methods [17].

This study shows the importance of fish, shrimp, lobster and crab as potential sources of *A. hydrophila* infection in people. *Aeromonas* spp. is being considered as a pathogen of emerging importance due to its special features such as ubiquitous presence in the aquatic environment, multiplicity of virulence factors and psychrotrophic nature. Though the occurrence of foodborne infections due to *Aeromonas* has not been recognized in Iran, it has been suggested in other countries in association with consumption of various foods. In Iran, fish and other sea foods is usually eaten after being cooked, and therefore, sea food may be a low risk food, even if contaminated with *Aeromonas* species, although the toxin remain in foodstuff. On the other hand, in recent years, the trend of consuming ready-to-eat under cooked seafoods in public places is getting popular, and thus, there is always the possibility of cross-contamination at the processing, food preparation and service steps. Most of modern approaches to control levels of contamination with microorganisms are effective against *A. hydrophila*.

REFERENCES

1. Kirov SM: *Aeromonas* species. **In,** Hocking AD (Ed): Foodborne Microorganisms of Public Health Significance. 553-575, AIFST Inc., 2003.

2. Ottaviani D, Santarelli S, Bacchiocchi S, Masini L, Ghittino C, Bacchiocchi I: Occurrence and characterization of *Aeromonas* spp. in mussels from the Adriatic Sea. *Food Microbiol*, 23, 418-422, 2006.

3. Hanninen ML, Salmi S, Mattila L, Taipalinen R, Siitonen A: Association of *Aeromonas* spp. with travellers' diarrhoea in Finland. *J Med Microbiol*, 42, 26-31, 1995.

4. Parker JL, Shaw JG: *Aeromonas* spp. clinical microbiology and disease. *J Infect*, 62, 109-118, 2011.

5. Chopra AK, Houston CW: Enterotoxins in *Aeromonas* associated gastroenteritis. *Microbes Infect*, 1, 1129-1137, 1999.

6. Pin C, Benito Y, Garcia ML, Selgas D, Tormos J, Casas C: Influence of temperature, pH, sodium chloride, and sodium nitrite on the growth of clinical and food motile *Aeromonas* spp. strains. *Arch Lebensmittelhygiene*, 47, 35-56, 1996.

7. Devlieghere F, Lefevere I, Magnin A, Debevere J: Growth of *Aeromonas hydrophila* in modified-atmospherepacked cooked meat products. *Food Microbiol*, 17, 185-196, 2000.

8. Fiorentini C, Barbieri E, Falzano L, Matarrese P, Baffone W, Pianetti A, Katouli M, Kuhn I, Mollby R, Bruscolini F, Casiere A, Donelli G: Occurrence, diversity and pathogenicity of mesophilic *Aeromonas* in estuarine waters of the Italian coast of the Adriatic Sea. *J Appl Bacteriol*, 85, 501-511, 1998.

9. Bin Kingombe CI, Huys G, Howald D, Luthi E, Swings J, Jemmi T: The usefulness of molecular techniques to assess the presence of *Aeromonas* spp. harboring virulence markers in foods. *Int J Food Microbiol*, 94, 113-121, 2004.

10. Kariptas E, Erdem B, Görgülü Ö: Protein profiles in different strains of *Aeromonas hydrophila* isolated from retail foods. *Kafkas Univ Vet Fak Derg*, 15(6), 885-890, 2009.

11. Vivekanandhan G, Hatha AAM, Lakshmanaperumalsamy P: Prevalence of *Aeromonas hydrophila* in fish and prawns from the seafood market of Coimbatore, South India. *Food Microbiol*, 22, 133-137, 2005.

12. Palu´ AP, Gomes LM, Miguel MAL, Balassiano IT, Queiroz MLP, Freitas-Almeida AC, de Oliveir SS: Antimicrobial resistance in food and clinical *Aeromonas* isolates. *Food Microbiol*, 23, 504-509, 2006.

13. Illanchezian S, Jayaraman S, Manoharan MS, Valsalam S: Virulance and cytotoxicity of seafood borne *Aeromonas hydrophila*. *Braz J Microbiol*, 41, 978-983, 2010.

14. Avsever ML, Polat SH, Türk N, Metin DY: *Saprolegnia* sp. and *Aeromonas hydrophila* isolation from freshwater-crayfish *(Astacus leptodactylus). Kafkas Univ Vet Fak Derg,* 17(5), 873-875, 2011.

15. Xanthopoulos V, Tzanetakis N, Litopoulou-Tzanetaki E: Occurrence and characterization of *Aeromonas hydrophila* and *Yersinia enterocolitica* in minimally processed fresh vegetable salads. *Food Control*, 21, 393-398, 2010.

16. Sambrook J, Russell DW: Molecular Cloning: A Laboratory Manual. 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2001.

17. Chu WH, Lu CP: Multiplex PCR assay for the detection of pathogenic *Aeromonas hydrophila*. *J Fish Dis*, 28, 437-441, 2005.

18. Dorsch M, Ashbolt NJ, Cox PT, Goodman AE: Rapid identification of *Aeromonas* species using 16S rDNA targeted oligonucleotide primers: A molecular approach based on screening of environmental isolates. *J Appl Bacteriol*, 77, 722-726, 1994.

19. Chopra AK, Houston CW, Peterson JW, Jin GF: Cloning, expression, and sequence analysis of a cytolytic enterotoxin gene from *Aeromonas hydrophila*. *Can J Microbiol*, 39, 513-523, 1993.

20. Radu S, Ahmad N, Ling FH, Reezal A: Prevalence and resistance to antibiotics for *Aeromonas* species from retail fish in Malaysia. *Int J Food Microbiol*, 81, 261-266, 2003.

21. Tsai GJ, Chen TW: Incidence and toxigenicity of *Aeromonas hydrophila* in seafood. *Int J Food Microbiol*, 31, 121-131, 1996.

22. Hudson JA, De Lacey KM: Incidence of motile aeromonads in New Zealand retail foods. *J Food Prot*, 54, 696-699, 1991.

23. Durmaz Y, Türk N: Isolation of motil aeromonases and to determine antimicrobial susceptibility from farms of trout. *Kafkas Univ Vet Fak Derg*, 15(3), 357-361, 2009.

24. Gobat PF, Jemmi T: Distribution of mesophilic *Aeromonas* species in raw and ready-to-eat fish and meat products in Switzerland. *Int J Food Microbiol*, 20, 117- 120, 1993.

25. Colakoglu FA, Sarmasik A, Koseoglu B: Occurrence of *Vibrio* spp. and *Aeromonas* spp. in shellfish harvested off Dardanelles of Turkey. *Food Control*, 17, 648-652, 2006.

26. Thayumanavan T, Vivekanandhan G, Savithamani K, Subashkumar R, Lakshmanaperumalsamy P: Incidence of haemolysin-positive and drug-resistant *Aeromonas hydrophila* in freshly caught finfish and prawn collected from major commercial fishes of coastal South India. *FEMS Immunol Med Microbiol,* 36, 41-45, 2003.

27. Yogananth N, Bhakyaraj R, Chanthuru A, Anbalagan T, Mullai Nila K: Detection of virulence gene in *Aeromonas hydrophila* isolated from fish samples using PCR technique. *Global J Biotechnol Biochem,* 4, 51-53, 2009.

28. Wang C, Silva JL: Prevalence and characterization of *Aeromonas* species isolated from processed channel catfish. *J Food Prot*, 62, 30-34, 1999.