Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 4, No. 2, p. 47-52, 2014
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Detection of the prevalence of *Aeromonas hyrophila* in shrimp samples by polymerase chain reaction (PCR) and cultural method in the Iran

Faham Khamesipour[1*], Mitra Moradi[2], Esmat Noshadi[2], Manouchehr Momeni Shahraki[2]

[1]*Veterinary Medicine, College of Veterinary Medicine, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran*

[2]*Young Researchers and Elite Club, Islamic Azad University Shahrekord Branch, Shahrekord, Iran*

Article published on February 03, 2014

Key words: PCR, *Aeromonas hydrophila,* shrimp, Iran.

Abstract

Shrimp is one of the most important fishery products of Persian Gulf coastal provinces of Iran. Genus *Aeromonas* has emerged as an important human pathogen because of suspected food-borne outbreaks and the increased incidence of its isolation from patients with traveller's diarrhea. This study was conducted to determine the prevalence rate of A. *hydrophila* in shrimp caught off the south coast of Iran. A total of 36 shrimp were collected in one provinces along Persian Gulf in the south coast of Iran. Samples were collected from September 2013 to May 2013. Using conventional bacteriological techniques, 14 A. *hydrophila* isolates were identified in which 5 strains were confirmed by PCR assay targeting 16S rDNA gene of A. *hydrophila*. Using PCR assays targeting the A. *hydrophila* 16S rDNA gene, 5 (13.888%) isolates were positive. To our knowledge, the present study is the isolation of A. *hydrophila* from shrimp in Iran.

***Corresponding Author:** Faham Khamesipour ✉ dr_faham@yahoo.com

Introduction

Nowadays world is observing the resurgence in the eating of shrimp. Shrimp farms have developed during the past twenty years. In 2008, the manufacture of shrimp in the world was reported 3, 281, 253 metric tons (Khamesipour *et al.,* 2014). Large indication occurs to propose that seafood is high on the list of foods associated by outbreaks of food-borne diseases (Ebrahimzadeh Mousavi *et al.,* 2011; Khamesipour *et al.,* 2014). When researching this, it is worth noting that the microbial status of seafood after catch is closely related to environmental conditions and microbiological quality of the water (Khamesipour *et al.,* 2014).

Aeromonas organisms are straight Gram-negative rods, facultative anaerobic, oxidase positive and glucose fermenting bacteria by occasional filaments. The common are motile by polar flagellae, though, several strain are non-motile. *Aeromonas* species are indigenous to aquatic environments global (Yousr *et al.,* 2007; Niamah, 2012).

Genus *Aeromonas* has emerged as an significant human pathogen since of suspected food-borne outbreaks (Kirov 2003; Ottaviani *et al.,* 2006) and the increased incidence of its isolation from patients by traveller's diarrhea (Parker and Shaw, 2011). Among the 14 species of *Aeromonas* identified to date *A. hydrophila, A. caviae*, and *A. veronii* biotype sobria have most usually been involved in human infections and have been found to produce a variety of virulence factors for example hemolysins, cytotoxins, enterotoxins, proteases, leukocidin, phospholipases, endotoxins, outer membrane proteins, and fimbriae (Chopra and Houston, 1999). 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 (Devlieghere *et al.,* 2000).

The aquatic environment is considered to be the principal reservoir of *Aeromonas* spp. (Swaminathan *et al.,* 2004). *A. hydrophila* infects several species of fish and other terrestrial animals including humans (Panangala *et al.,* 2007). *A. hydrophila* is considered to be the principal cause of bacterial hemorrhagic septicemia in fresh water fish and has been reported in association by numerous ulcerative syndrome and red spot disease. These infections can cause high mortalities in fish hatcheries and in natural waters. *Aeromonas* has been involved in wound infections, sepsis, outbreaks of waterand food-borne gastroenteritis (Swaminathan *et al.,* 2004; Yousr *et al.,* 2007). Specific importance is attached to *A. hydrophila* from a public health perspective by reason of its involvement in foodborne gastroenteritis and various opportunistic infections in immunocompromised human patients (Panangala *et al.,* 2007).

A. hydrophila can be isolated from some sources, for example food, drinking water, sewage, environmental water (soil, fresh and saline water), animal faeces and human clinical samples by a global distribution. These bacteria can develop in refrigeration temperatures and are responsible for food and water-borne diseases, that can cause a range of human diseases that vary in severity from a self-limiting gastroenteritis to potentially fatal septicemia (Niamah, 2012). Too, a number of investigations have shown that members of the genus *Aeromonas* are also widely distributed in many foods for instance meat (Bin Kingombe *et al.,* 2004), sea food (Vivekanandhan *et al.,* 2005; Palu *et al.,* 2006; Illanchezian *et al.,* 2010), and vegetable (Xanthopoulos *et al.,* 2010). Therefore, 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 (2003). The majority (>85%) of gastroenteritis cases are attributed to three*Aeromonas* species, one of them is *A. hydrophila* (Parker and Shaw, 2011).

Polymerase chain reaction (PCR) technique was used to assay for the detection of *aero* and *hlyA* genes in *Aeromonas* spp. isolated from environmental and shellfish sources (Niamah, 2012). Also, PCR have

been developed for detection of *Aeromonas* spp. from water samples and food products (Swaminathan *et al.,* 2004).

The objective of this study was to detect the prevalence of *A. hydrophila* in farmed shrimp by PCR and cultural method in the Iran.

Materials and methods

Sample collection

A total of 36 freshly shrimp were collected randomly from shrimp farms in one province (Bushehr) along Persian Gulf in the south coast of Iran. Samples were collected from October 2013 to November 2013, 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 by aseptic methods. All the specimens were rinsed by sterile water to remove the adhering particles. Total shrimp were dipped into screw cap bottles containing alkaline peptone-water (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 via Vivekanandhan *et al.* (2005). The plates were then flooded with approximately 5 ml of Lugol's iodine solution and amylase positive yellow to honey coloured colonies were isolated. Tubes by 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, H2S from cystein, acetoin from glucose, gas from glucose, l-arabinose utilization and fermentation of salicin (Vivekanandhan *et al.,* 2005). We have used a type strain of *A. hydrophila* (ATCC 7966), as reference strain to compare the results.

Detection of A. hydrophila from pur culture

One milliliter pure culture of *A. hydrophila*, identified via biochemical tests, was centrifuged at 13000g 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 following to the method described via Sambrook and Russell (2001).

The PCR procedures used in this study have been described previously (Chu and Lu, 2005). one genes selected for the identification of the *A. hydrophila* were the 16S rDNA gene (Dorsch *et al.*, 1994). All oligonucleotide primers were obtained from 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 (+MgCl2) (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 13000g 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 controls 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) analysis was performed.

Results and discussion

A total number of 36 shrimps were studied and 5 samples (13.888%) contained *A. hydrophila*. Using conventional bacteriological techniques, 14 *A. hydrophila* isolates were identified. A PCR assay targeting *16S rDNA* gene of *A. hydrophila* confirmed 5 strains as *A. hydrophila*. The PCR assays were performed in triplicates and no variability in the results was present.

The results showed a high presence of *A. hydrophila* DNA in shrimps specimens. These results indicated that this infection is an important agent to increase economical costs. These finding suggested that control and eradication programs for *A. hydrophila* infection are necessary in Iran. Our findings support the power of PCR test for *A. hydrophila* detection in shrimps samples and could be easily used for routine diagnosis.

During the last several decades, the researchers reported the food-borne infection cases in humans which were caused by consuming the contaminated fresh-raw shellfish, shrimp and other seafood (Radu *et al.,* 2003; Colakoglu *et al.,* 2006; Panangala *et al.,* 2007; Khamesipour *et al.,* 2013; Khamesipour *et al.,* 2014).

The findings of the current study are comparable by those reported from Malaysia (Radu *et al.,* 2003),Taiwan (Tsai and Chen, 1996), New Zealand (Hudson and Delacy, 1991); India (Vivekanadhan *et al.,* 2005) and of Switzerland (Gobat and Jemmi, 1993).

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 (Vivekanadhan *et al.,* 2005). Also this is in agreement with findings of Tasi and Chen (1996) and Colakoglu *et al.* (2006).

The prevalence of cytolytic enterotoxin gene carrying *A. hydrophila* isolates reported in our study are comparable with those reported from Malaysia (Bin Kingombe *et al.,* 2004; Illanchezian *et al.,* 2010). 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 methods employed, seasonal effects and/or laboratory methodologies employed in different studies (bacteriological and biochemical testing vs. PCR assays) (Chu and Lu, 2005; Vivekanandhan *et al.,* 2005). Additionally, 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.

Conclusions

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 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 results of the current study revealed that the shrimp from the studied region is contaminated with

A. hydrophila. Although the source of the bacteria is typically aquatic environment, secondary contamination during catching, handling and transportation may also give to their distribution. It should be consider that occurrence of the *A. hydrophila* in human is highly depends on the nutritional habits. Iranian cooking processes include a high degree of boiling and roasting which might drop this organism from the seafood, although the toxin may stay in the food stuff.

Acknowledgments

The authors would like to express their deep sense of gratitude and sincere thanks to the staff of the Biotechnology Research Center of Islamic Azad University of Shahrekord Branch in Iran.

References

Bin Kingombe CI, Huys G, Howald D, Luthi E, Swings J, Jemmi T. 2004. The usefulness of molecular techniques to assess the presence of *Aeromonas* spp. harboring virulence markers in foods. International Journal of Food Microbiology **94**, 113-121. http://dx.doi.org/10.1016/S0168-1605(03)00105-3

Chopra AK, Houston CW. 1999. Enterotoxins in *Aeromonas* associated gastroenteritis. Microbes and Infection **1**, 1129-1137. http://dx.doi.org/10.1016/S1286-4579(99)00202-6

Chu WH, Lu CP. 2005. Multiplex PCR assay for the detection of pathogenic *Aeromonas hydrophila*. Journal of Fish Diseases **28**, 437-441.

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

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

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

Ebrahimzadeh Mousavi HA, Akhondzadeh Basti A, Mirzargar SS, Soltani M, Taheri Mirghaed A, Esmaeili H, Firouzbakhsh F. 2011. *Vibro parahaemolyticus* in shrimps and their environment in South Iran cultured. International Journal of Veterinary Research **5(3),** 149-150.

Gobat PF, Jemmi T. 1993. Distribution of mesophilic *Aeromonas* species in raw and ready-to-eat fish and meat products in Switzerland. International Journal of Food Microbiology **20**, 117-120. DOI:10.1016/0168-1605(93)90099-3.

Hudson JA, De Lacey KM. 1991. Incidence of motile aeromonads in New Zealand retail foods. Journal of Food Protection **54**, 696-699.

Illanchezian S, Jayaraman S, Manoharan MS, Valsalam S. 2010. Virulance and cytotoxicity of seafood borne *Aeromonas hydrophila*. Brazilian Journal of Microbiology **41**, 978-983. http://dx.doi.org/10.1590/S1517-83822010000400016

Khamesipour F, Khodadoustan Shahraki A, Moumeni M, Khadivi Boroujeni R, Yadegari M. 2013. Prevalence of Listeria monocytogenes in the crayfish (Astacus leptodactylus) by polymerase chain reaction in Iran. International Journal of Biosciences **3(10),** 160-169. http://dx.doi.org/10.12692/ijb/3.10.160-169

Khamesipour F, Noshadi E, Moradi M. 2014 Detection of *Vibrio* spp. in shrimp from aquaculture sites in Iran using polymerase chain reaction (PCR). AACL Bioflux **7 (1),** 1-7.

Kirov SM. 2003. *Aeromonas* species, 553–575. In: Hocking AD. (ed.), Foodborne Microorganisms of Public Health Significance. AIFST Inc., 752.

Niamah AK. 2012. Detected of *aero* gene in *Aeromonas hydrophila* isolates from shrimp and peeled shrimp samples in local markets. Journal of Microbiology, Biotechnology and Food Sciences **2 (2)**, 634-639.

Ottaviani D, Santarelli S, Bacchiocchi S, Masini L, Ghittino C, Bacchiocchi I. 2006. Occurrence and characterization of *Aeromonas* spp. in mussels from the Adriatic Sea. Food Microbiology **23**, 418-422. http://dx.doi.org/10.1016/j.fm.2005.08.001

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

Panangala VS, Shoemaker CA, van Santen VL, Dybvig K, Klesius PH. 2007. Multiplex-PCR for simultaneous detection of 3 bacterial fish pathogens, *Flavobacterium columnare, Edwardsiella ictaluri*, and *Aeromonas hydrophila*. Diseases of aquatic organisms **74**, 199-208. doi:10.3354/dao074199.

Parker JL, Shaw JG. 2011. *Aeromonas* spp. clinical microbiology and disease. Journal of Infection **62**, 109-118. http://dx.doi.org/10.1016/j.jinf.2010.12.003

Radu S, Ahmad N, Ling FH, Reezal A. 2003. Prevalence and resistance to antibiotics for *Aeromonas* species from retail fish in Malaysia. International Journal of Food Microbiology **81**, 261-266. http://dx.doi.org/10.1016/S0168-1605(02)00228-3

Sambrook J, Russell DW. 2001. Molecular Cloning: A Laboratory Manual. 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. 517.

Swaminathan TR, Rathore G, Abidi R, Kapoor D. 2004. Detection of *Aeromonas hydrophila* by polymerase chain reaction. Indian J. Fish **51(2),** 251-254.

Tsai GJ, Chen TW. 1996. Incidence and toxigenicity of *Aeromonas hydrophila* in seafood. International Journal of Food Microbiology **31**, 121-131.

Vivekanandhan G, Hatha AAM, Lakshmanaperumalsamy P. 2005. Prevalence of *Aeromonas hydrophila* in fish and prawns from the seafood market of Coimbatore, South India. Food Microbiology **22**, 133-137. http://dx.doi.org/10.1016/j.fm.2004.01.015

Xanthopoulos V, Tzanetakis N, Litopoulou-Tzanetaki E. 2010. Occurrence and characterization of *Aeromonas hydrophila* and *Yersinia enterocolitica* in minimally processed fresh vegetable salads. Food Control **21**, 393-398. http://dx.doi.org/10.1016/j.foodcont.2009.06.021

Yousr AH, Napis S, Rusul GRA, Son R. 2007. Detection of Aerolysin and Hemolysin Genes in *Aeromonas* spp. Isolated from Environmental and Shellfish Sources by Polymerase Chain Reaction. ASEAN Food Journal **14 (2),** 115-122.