Antibiotic resistance pattern of some *Vibrio* strains isolated from seafood

Raissy M.[1, 3*]; Moumeni M.[2]; Ansari M.[3]; Rahimi E.[1]

Received: January 2012 Accepted: May 2012

Abstract

The present study was aimed to evaluate the antimicrobial resistance and the presence of antibiotic resistance genes in *Vibrios* spp. isolated from seafood. A total of 72 isolates of *Vibrio* in 6 species including *V. parahaemolyticus*, *V. vulnificus*, *V. alginolyticus*, *V. harveyi*, *V. mimicus* and *V. cholerae* were examined. The results revealed that all isolates were expressing multiple antibiotic resistances. Of the 72 strains tested, 70 were resistant to ampicillin (97.2%), 60 to gentamycin (83.3%) and 56 to penicillin (77.7%). Eight strains were resistant to 4 antibiotic, 19 resistant to five antibiotics, 10 to six antibiotics, 34 to seven antibiotics and one to eight antibiotics. Results also revealed that 20 *Vibrio* strains (27.7% of total examined strains) contained one to three of the antibiotic resistance genes. *StrB*, *tetS* and *ermB* genes coding for streptomycin, tetracycline and erythromycin resistance were found in 18, 6, 5 isolates, respectively and Sulfamethoxazole resistance gene, *sul2*, was not detected in this study. Detection of resistance genes in *Vibrio* strains obtained from seafood is considered as a potential danger for consumers and also suggests that these resistance determinants might be further disseminated in habitats, thus constituting a serious health risks to human.

Keywords: *Vibrio* spp., Antimicrobial resistance genes, Seafood, Persian Gulf

1 - Department of Food Hygiene and Aquatic Animal Health, Faculty of Veterinary Medicine, Islamic Azad University- Shahrekord Branch, Shahrekord, Iran.
2 - Central Laboratory, Islamic Azad University- Shahrekord Branch, Shahrekord, Iran.
3 - Young Researchers Club, Islamic Azad University- Shahrekord Branch, Shahrekord, Iran.
* Corresponding author's email: mehdi.raissy@iaushk.ac.ir

Introduction

There is a great number of species in *Vibrio* genus. Many of them are pathogenic to human and have been related to food-borne diseases (Chakraborty et al., 1997; Tavakoli, 2012). Part of the natural biota of fish and shellfish is formed by some *Vibrio* species (Ruangpan and Kitao, 1991; Otta et al., 1999) while some species such as *V. anguilarum*, *V. harveyi*, and *V. parahaemolyticus* are related to bacterial infections in fish and aquatic crustaceans (Lightner, 1993; Mohajeri et., 2011). When fish or shrimp are under stress, they seem to be opportunistic pathogens causing disease. There are 12 *Vibrio* species which cause human disease; the most important of them are *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus*. The clinical signs may range from gastroenteritis to wound infection, otitis and septicaemia depending on the bacterial species which cause disease (Ulusarac and Carter, 2004). The main source of *Vibrio* is seafood and there are many reports from all over the world on seafood associated vibriosis outbreaks (Hoi et al., 1998; Daniels and Shafaie, 2000; Nascimento et al., 2001; Morris, 2003; Amirmozafari et al., 2005; Rahimi et al., 2010).

Antimicrobial resistance is one of the most important public health problems that directly relates to disease management and control (Ansari and Raissy, 2010). In treatment of different bacterial diseases, antibiotics such as tetracycline, doxycycline, erythromycin and streptomycin are generally used (Lima, 2001), resistance to which have been reported in many bacteria such as *Vibrio* (Ahmed et al., 2004; Ceccarelli et al., 2006; Ansari and Raissy, 2010). Recently, higher frequency of drug-resistant *Vibrio* has been reported (Ansari and Raissy, 2010, Okoh and Igbinosa, 2010). In this work, we attempted to study antibiotic susceptibility patterns of the *Vibrio* species isolated from seafood. The distribution of antibiotic resistance genes in the isolates is studied as well.

Materials and methods

Bacterial isolates

A total of 72 isolates of *Vibrio* species were included in this study. Of these, 10 were *V. parahaemolyticus*, 22 were *V. vulnificus*, 20 were *V. alginolyticus*, 10 were *V. harveyi*, 7 were *V. mimicus* and 3 were *V. cholerae*. These *Vibrio* species were isolated in our previous study from seafood including fish, shrimp, lobster and crab caught off the Persian Gulf. All strains were maintained in Tryptic Soy Broth supplemented 30% glycerol and stored at -70°C after exact identification by PCR.

Antibiotic susceptibility test

Antibiotic susceptibility of the *Vibrio* isolates was studied using the disc diffusion method on Mueller-Hinton agar (Oxoid) according to the instruction of Clinical Laboratory Standards Institute (CLSI, 2007). Discs (Oxoid) contained the following antibiotics: penicillin G (10 U), ampicillin (10 μg), tetracycline (30 μg), doxycycline (30 μg), erythromycin (15 μg), sulfamethoxazole (25 μg), streptomycin (30 μg), gentamicin (30 μg), azitromycin (15 μg), nalidixic acid (30 μg), amikacin (30 μg), ciprofloxacin (5 μg) and norfloxacin (10 µg). The results were recorded as resistant or susceptible by measurement of the inhibition zone diameter according to the standard of CLSI (2007).

DNA Extraction

The genomic DNA was extracted according to the instruction of Ausubel et al. (1987). The isolates were grown overnight at 30 °C in Trypic Soy Broth containing 1% sodium chloride. The bacteria (1.5 ml) was centrifuged for 10 min at 12000g, and the cell pellets were resuspended in 567 µl of Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), followed by addition of 30 µl of 10% (w/v) sodium dodecyl sulfate and 3 µl of proteinase K (Sigma) (20 mg/ml) and incubation at 37 °C for 1 h. The isolates were treated with 100 µl of 5 M NaCl and 80 µl of hexadecyltrimethyl ammonium bromide (CTAB)/NaCl, and incubated at 65 °C for 10 min. The mixture was extracted with an equal volume of phenol-chloroform- isoamyl alcohol (25:24:1, v/v) and DNA was precipitated with 0.6 volume of cold isopropanol and washed with 1 ml of 70% cold ethyl alcohol. The DNA pellet was dried at room temperature for 30 min and resuspended in TE (10 mMTris–HCl, 100 mM EDTA, pH 7.8) buffer and stored at -20 °C. The purity and quantity of genomic DNA was evaluated by measuring optical densities at 260 and 280 nm wavelengths. The DNA concentration of each sample was adjusted to 50 ng/µl for PCR.

PCR assay

Antibiotic resistant genes were identified using polymerase chain reaction (PCR) in the examined *Vibrio* species. Sequence of primers used for detection of *ermB*, *tetS*, *strA* and *sul2* are listed in Table 1. The PCR reaction was performed in a 50 µl reaction system consisting of 2 µl of purified genomic DNA (50 ng/µl), 5 µl of 10× PCR buffer (100 mM Tris–HCl, pH 8.3, 500 mM KCl, 60 mM MgCl2, 0.1% gelatin and 1% Triton X-100), 1 µl each of the primers (50 pmol/µl), 1 µl each of the 10 mM dNTPs, 0.2 µl units Taq DNA polymerase (5 units/µl) and 40 µl of sterile distilled water. Cycling conditions (PTC-100 Eppendorf Thermal cycler) were as follows; initial denaturation at 95°C for 5 min was followed by 30 cycles of 94°C for 1 min, 60°C for 40 seconds and 72°C for 40 seconds with a final extension at 72°C for 7 min and cooling to 4°C. Amplified products were separated by electrophoresis in ethidium bromide stained 1.5% agarose gels at 90 V for 50 min. The product bands on gels were visualized and photographed with a UV transilluminator.

Results

Antibiogram profile

The susceptibilities of 72 *Vibrio* strains including *V. vulnificus* (22 strains); *V. alginolyticus* (20 strains); *V. parahaemolyticus* (10 strains); *V. harveyi* (10 strains); *V. mimicus* (7 strains) and *V. cholerae* (3 strains) to 13 different antibiotics was examined. Of the 72 strains tested, 70 were resistant to ampicillin (97.2%), 60 to gentamycin (83.3%), 56 to penicillin (77.7%), 18 to streptomycin (25.0%) and five to erythromycin (6.9%) and 13 to tetracycline (18.1%). No isolate was resistant to sulfamethoxazole (Table

2). Eight strains (13.3%) were resistant to four antibiotic, 19 resistant to five antibiotics (30.0%), ten to six antibiotics (30.0%), 34 to seven antibiotics (6.7%), and one to eight antibiotics (3.3%).

The antibiotic resistance genes of Vibrio species

In order to finding a relationship between the multidrug-resistance phenotypes of *Vibrio* species and the presence of antibiotic resistance genes, polymerase chain reaction tests were carried out using specific primers. The obtained results revealed that 20 *Vibrio* strains (27.7% of total examined strains) contained one to three of the antibiotic resistance genes (Table 2). *StrB*, *tetS* and *ermB* genes coding for streptomycin, tetracycline and erythromycin resistance were found in 18, 6 and 5 isolates, respectively and sulfamethoxazole resistance gene, *sul2*, was not detected in this study.

Table 1: Sequence of primers used for detection of antibiotics resistance genes

Primer	Sequence(5'------ 3')	Target gene	Amplicon size	Reference
ermB-F	AGACACCTCGTCTAACCTTCGCTC	*ermB*	640	Sutcliffe et al., 1996
ermB-R	TCCATGTACTACCATGCCACAGG			
tetS-F	ATCAAGATATTAAGGAC	*tetS*	590	Charpentier et al., 1993
tetS-R	TTCTCTATGTGGTAATC			
SUL2-F	AGGGGGCAGATGTGATCGAC	*Sul2*	271	Hochhut et al., 2001
SUL2-R	TGTGCGGATGAAGTCAGCTCC			
strA-F	TTGATGTGGTGTCCCGCAATGC	*strA*	267	Hochhut et al., 2001
strA-R	CCAATCGCAGATAGAAGGCAA			

Table 2: Phenotypic and genotypic characterization of *Vibrio* strains and their antibiotics resistance genes

Name of species	Antibiotic resistance pattern	Strain(s) showing presence of gene encoding			
		strA	*tetS*	*ermB*	*sul2*
Vibrio vulnificus	6	+	-	-	-
Vibrio vulnificus	1	+	+	+	-
Vibrio vulnificus	5	-	-	-	-
Vibrio vulnificus	5	-	-	-	-
Vibrio vulnificus	7	-	-	-	-
Vibrio vulnificus	3	+	+	+	-
Vibrio vulnificus	5	-	-	-	-
Vibrio vulnificus	5	-	-	-	-
Vibrio vulnificus	8	-	-	-	-
Vibrio vulnificus	5	-	-	-	-
Vibrio vulnificus	6	+	-	-	-
Vibrio vulnificus	7	-	-	-	-
Vibrio vulnificus	2	-	+	+	-
Vibrio vulnificus	6	+	-	-	-
Vibrio vulnificus	5	-	-	-	-
Vibrio vulnificus	5	-	-	-	-
Vibrio vulnificus	5	-	-	-	-
Vibrio vulnificus	7	-	-	-	-
Vibrio vulnificus	5	-	-	-	-
Vibrio vulnificus	6	+	-	-	-
Vibrio vulnificus	7	-	-	-	-
Vibrio vulnificus	7	-	-	-	-
Vibrio alginolyticus	5	-	-	-	-
Vibrio alginolyticus	5	-	-	-	-
Vibrio alginolyticus	8	+	-	-	-
Vibrio alginolyticus	2	-	+	+	-
Vibrio alginolyticus	7	-	-	-	-
Vibrio alginolyticus	6	+	-	-	-
Vibrio alginolyticus	5	-	-	-	-
Vibrio alginolyticus	8	+			
Vibrio alginolyticus	5	-	-	-	-
Vibrio alginolyticus	5	-	-	-	-
Vibrio alginolyticus	5	-	-	-	-
Vibrio alginolyticus	5	-	-	-	-
Vibrio alginolyticus	7	-	-	-	-
Vibrio alginolyticus	7	-	-	-	-
Vibrio alginolyticus	8	+	-	-	-
Vibrio alginolyticus	7	-	-	-	-
Vibrio alginolyticus	7	-	-	-	-
Vibrio alginolyticus	5	-	-	-	-
Vibrio alginolyticus	7	-	-	-	-
Vibrio alginolyticus	5	-	-	-	-
Vibrio parahaemolyticus	5	-	-	-	-
Vibrio parahaemolyticus	3	+	+	+	-
Vibrio parahaemolyticus	7	-	-	-	-
Vibrio parahaemolyticus	5	-	-	-	-
Vibrio parahaemolyticus	6	+	-	-	-
Vibrio parahaemolyticus	5	-	-	-	-
Vibrio parahaemolyticus	7	-	-	-	-
Vibrio parahaemolyticus	5	-	-	-	-
Vibrio parahaemolyticus	5	-	-	-	-
Vibrio parahaemolyticus	5	-	-	-	-
Vibrio mimicus	6	+	-	-	-
Vibrio mimicus	7	-	-	-	-
Vibrio mimicus	7	-	-	-	-
Vibrio mimicus	6	+	-	-	-
Vibrio mimicus	5	-	-	-	-
Vibrio mimicus	5	-	-	-	-
Vibrio mimicus	5	-	-	-	-
Vibrio harveyi	7	-	-	-	-
Vibrio harveyi	4	+	+	-	-
Vibrio harveyi	7	-	-	-	-
Vibrio harveyi	5	-	-	-	-
Vibrio harveyi	5	-	-	-	-
Vibrio harveyi	8	+	-	-	-
Vibrio harveyi	7	-	-	-	-
Vibrio harveyi	5	-	-	-	-
Vibrio harveyi	6	+	-	-	-
Vibrio harveyi	7	-	-	-	-
Vibrio cholerae	8	+	-	-	-
Vibrio cholerae	5	-	-	-	-
Vibrio cholerae	5	-	-	--	-

Legend:1- AMP, DOX, STR, GEN, TET, ERY, NOR, PEN.; 2- AMP, TET, ERY, NOR, PEN, GEN, NAL.; 3- PEN, NAL, STR, TET, DOX, ERY.; 4- TET, AZT, AMP, DOX, NOR, STR.;5- PEN, NOR, DOX, AMP, AK, CIP, GEN.;6- STR, DOX, AZT, AMP, NOR, AK.7- DOX, AMP, PEN, GEN, CIP.; 8- STR, AMP, AK, GEN, TET.
AMP, ampicillin; SUL, sulfamethoxazole; AZT, azitromycin; DOX, doxycycline; GEN, gentamicin; NAL, nalidixic acid; NOR, norfloxacin; STR, streptomycin; TET, tetracycline; ERY, erythromycin; PEN, penicillin G; CIP, ciprofloxacin; AK, amikacin.

Discussion

In this study, resistance to ampicillin was observed in 97.2% of the analyzed isolates, in other studies similar percentages have been reported, ranging from 44.4% to 100% in vibrios from different sources (Radu et al., 1998; Lesmana et al., 2001). French et al. (1989) reported similar antibiotics susceptibility profile for *V. parahaemolyticus*. Antibiotic resistance of *V. harveyi* strains isolated from shrimp and water to ampicillin has been reported as well (Teo et al., 2000). Roque et al. (2000) found out that all the *Vibrio* isolates isolated from seawater were also ampicillin resistant.

There is an agreement between the results that show high individual and multiple antibiotics resistance among all examined *Vibrio* strains, and other researches (Ansari and Raissy, 2010, Okoh and Igbinosa, 2010). One study revealed that all *Vibrio* strains were found to harbor antibiotics resistant genes and showed resistances to ampicillin, furazolidone, nalidixic acid, streptomycin, trimethoprim-sulfamethoxazole and trimethoprim (Ramachandran et al., 2007). Thungapathra et al. (2002) indicated that in a total number of 94 isolates of *V. cholera*, 43 strains contained R-plasmids and exhibited resistances to ampicillin, neomycin, tetracycline, gentamicin, streptomycin, sulfonamide, furazolidone and chloramphenicol.

In spite of the fact that in some previous studies streptomycin and tetracycline were considered to be effective against *Vibrio* species (Li et al., 2003), we found resistances to both antibiotics in the examined *Vibrio* isolates. In this study, resistance to tetracycline was found in 13 *Vibrio* isolates (18.1%). Another study indicated that 43.0% of *Vibrio* isolates from shrimp are resistant to this antibiotic (Roque et al., 2000). The results showed that 20 *Vibrio* strains had one or more resistance genes. In 18, 6, 5 isolates, *StrB*, *tetS* and *ermB* genes were found respectively coding for streptomycin, tetracycline and erythromycin resistance. Sulfamethoxazole resistance gene, *sul2*, was not found in this study.

Falbo et al. (1999) formerly detected the *strB* gene for aminoglycoside resistance (streptomycin) in Albania and Italy in 1994, and Thungapathra et al. (2002) found it in India from 1997 to 1998. Okoh and Igbinosa (2010) have detected it in South Africa in 2010. Previously, Li et al. (1999) have detected tetracycline resistance gene in *V. alginolyticus* and *V. vulnificus* isolated from cultured sea bream in Hong Kong. In this study, some of the studied strains did not contain *tetS* gene, but they were resistant to tetracycline which may be due to the presence of other genes encoding resistance to tetracycline such as *tetA*, *tetB*, *tetM* and *tetK*. This finding is similar to the results of Dang et al. (2006).

The results revealed that multi-drug resistant *Vibrio* spp. present in seafood, obtain antibiotic resistance via plasmids and they can transfer the resistance via transformation, conjugation and other mobile elements such as integrons. Moreover, *Vibrio* species are capable of transferring the plasmid-encoded resistance into other bacterial genera, which can be transferred to human either directly or indirectly. To our knowledge, this is the first report available on the chromosomal antibacterial resistance in *Vibrio* spp. from Iran. Regarding the strange ability of acquired drug resistance determinants in *Vibrio* spp., frequent assessment of antibacterial susceptibility profile either chromosomal or plasmid mediated may lead to a better knowledge.

References

Ahmed, AM., Nakagawa, T., Arakawa, E., Ramamurthy, T., Shinoda, S. and Shimamoto, T., 2004. New aminoglycoside acetyltransferase gene, *aac(3)-Id*, in a class 1 integron from a multiresistant strain of *Vibrio fluvialis* isolated from an infant aged 6 months**.** *Journal of Antimicrobial Chemotherapy*, 53(**6**), 947-951.

Amirmozafari, N., Forohesh, H. and Halakoo, A., 2005. Occurrence of Pathogenic Vibrios in Coastal Areas of Golestan Province in Iran. *Archives of Razi Institute*, 60 (**1**), 33-44.

Ansari, M. and Raissy, M., 2010. In vitro susceptibility of commonly used antibiotics against *Vibrio* spp. isolated from Lobster (*Panulirus homarus*). *African Journal of Microbiology Research*, 4(**23**), 2629-2631.

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Sideman, J., Smith, J. and Struhl, K., 1987. Current Protocols in Molecular Biology, USA: Wiley-Blachwell, 354p.

Ceccarelli, D., Salvia, A. M., Sami, J., Cappuccinelli, P. and Maria, M., 2006. Colombo New Cluster of Plasmid-Located Class 1 Integrons in *Vibrio cholerae* O1 and a dfrA15 Cassette-Containing Integron in *Vibrio parahaemolyticus* isolated in Angola. *Antimicrobial Agents and Chemotherapy*, 50(**7**), 2493-2499.

Chakraborty, S., Nair, G.B. and Shinoda, S., 1997. Pathogenic *Vibrios* in the natural aquatic environment. *Review of Environmental Health*, 12(**2**), 347-351.

Charpentier, E., Gerbaud, G. and Courvalin, P., 1993. Characterization of a new class of tetracycline-resistance gene *tet(S)* in *Listeria monocytogenes* BM4210. Gene, 131(**1**), 27-34.

CLSI, Clinical and Laboratory Standards Institute, 2007. Performance Standards for Antimicrobial Susceptibility Testing; Fifteenth Informational Supplement. CLSI document M100-S15. Clinical and Laboratory Standards Institute. Wayne, Pennsylvania.

Dang, H., Zhang, X., Song, L., Chang, Y. and Yang, G., 2006. Molecular characterizations of oxytetracycline resistant bacteria and their resistance genes from mariculture waters of China. *Marine Pollution Bulletin*, 52(**11**), 1494-1503.

Daniels, N.S. and Shafaie, A., 2000. A review of pathogenic *Vibrio* infections for clinicians. *Infections in Medicine*, 17(**10**), 665- 685.

Falbo, V., Carattoli, A., Tosini, F., Pezzella, C., Dionisi, A.M. and

Luzzi, I., 1999. Antibiotic resistance conferred by a conjugative plasmid and a class I integron in *Vibrio cholerae* O1 El Tor strains isolated in Albania and Italy**.** *Antimicrobial Agents and Chemotherapy*, 43(**3**), 693-696.

French, G. L., Woo, M. L., Hui, Y. W. and Chan, K. Y., 1989. Antimicrobial susceptibility of halophilic vibrios**.** *Journal of Antimicrobial Chemotherapy*, 24(**2**), 183-194.

Hoi, L., Larsen, J. L., Dalsgaard, I. and Dalsgaard, A., 1998. Occurrence of *Vibrio vulnificus* in Danish marine environments. *Applied and Environmental Microbiology,* 64(**10**), 7-13.

Lesmana, M., Subekti, D., Simanjuntak, C. H., Tjaniadi, P., Campbell, J. R. and Oyofo, B. A., 2001. *Vibrio parahaemolyticus* associated with cholera-like diarrhea among patients in North Jakarta, Indonesia. *Diagnostic Microbiology and Infectious Disease*, 39(**2**), 71-75.

Li, J., Yie, J., Foo, R. W. T., Ling, J. M. L., Xu, H. S. and Woo, N. Y. S., 1999. Antibiotic resistance and plasmid profiles of *Vibrio* isolates from cultured Silver Sea Bream, *Sparus sarba*. *Marine Pollution Bulletin,* 39(**1-12**), 245-249.

Lightner, D. V., 1993. Diseases of cultured penaeid shrimps. In J.P. Mc Vey (Ed.), CRC Handbook of Mariculture. 2nd ed, CRC Press, Boca Raton, pp. 393- 486.

Lima, A. A., 2001. Tropical diarrhea: New developments in traveler's diarrhea. *Current Opinion in Infectious Disease,* 14(**5**), 547-552.

Lin, X. T., 2006. Epidemiological investigation of 94 food poisoning cases caused by *Vibrio parahaemolyticus*. *Shanghai Journal of Preventive Medicine,* 18(**2**), 141-143.

Mohajeri, J., Afsharnasab, M., Jalali, B., Kakoolaki, S., Sharifrohani, M. and Haghighi, A., 2011. Immunological and histopathological changes in penaeus semisulcatus challenged with vibrio harveyi. *Iranian Journal of Fisheries Sciences,* 10 (**2**)**,** 254-265.

Morris Jr., J. G., 2003. Cholera and other types of vibriosis: a story of human pandemics and oysters on the half shell. *Clinical Infectious Diseases,* 37(**2**), 272- 280.

Nascimento, S. M. M., Vieira, R. H. S. F., Theophilo, G. N. D., Rodrigues, D. P. and Vieira, G.H.F., 2001. *Vibrio vulnificus* as a health hazard for shrimp consumers. *Revista do Instituto de Medicina Tropical de Sao Paulo,* 43(**5**), 263- 266.

Okoh, A. I. and Igbinosa, E. O., 2010. Antibiotic susceptibility profiles of some *Vibrio* strains isolated from wastewater final effluents in a rural community of the Eastern Cape Province of South Africa. *BMC Microbiology,* 10(**143**), 1-6.

Otta, S. K., Karunasagar, I. and Karunasagar, I., 1999. Bacterial flora associated with shrimp culture ponds growing *Penaeus mondon* in India. *Journal of Aquaculture in Tropics,* 14(**4**), 309- 318.

Radu, S., Elhadi, N., Hassan, Z., Rusul, G., Lihan, S., Fifadara, N., Yuherman, N. and Purwati, E., 1998. Characterization of *Vibrio* *vulnificus* isolated from cockles (*Anadara granosa*): antimicrobial resistance, plasmid profiles and random amplification of polymorphic DNA analysis. *FEMS Microbiology Letters*, 165(**1**), 139-143.

Rahimi, E., Ameri, M., Doosti, A. and Gholampour, A. R., 2010. Occurrence of toxigenic *Vibrio parahaemolyticus* strains in shrimp in Iran. *Foodborne Pathogens and Diseases*, 7(**9**), 1107-11.

Ramachandran, D., Bhanumathi, R. and Singh, D. V., 2007. Multiplex PCR for detection of antibiotic resistance genes and the SXT element: application in the characterization of *Vibrio cholerae*. *Journal of Medical Microbiology*, 56(**3**), 346-351.

Roque, A., Molina-Aja, A., Bolan-Mejia, C. and Gomez-Gil, B., 2001. In vitro susceptibility to 15 antibiotics of vibrios isolated from penaeid shrimps in Northwestern Mexico. *International Journal of Antimicrobial Agents*, 17(**5**), 383-7.

Ruangpan, L. and Kitao, T., 1991. *Vibrio* bacteria isolated from black tiger shrimp, *Penaeus monodon* Fabricius. *Journal of Fish Diseases*, 14(**3**), 383– 388.

Son, R., Nasreldine, E. H., Zaiton, H., Samuel, L., Rusul, G. and Nimita, F., 1998. Characterization of *Vibrio vulnificus* isolated from cockles (*Anadara granosa*): antimicrobial resistance, plasmid profile and random amplification of polymorphic DNA analysis. *FEMS Microbiology Letters*, 165(**1**), 139-143.

Sutcliffe, J., Grebe, T., Tait-Kamradt, A. and Wondrack, L., 1996. Detection of erythromycin-resistant determinants by PCR. *Antimicrobial Agents and Chemotherapy*, 40(**11**), 2562-6

Tavakoli H., Soltani M., Bahonar A., 2012. Isolation of some human pathogens from fresh and smoked shad. *Iranian Journal Fisheries Sciences*, 11(**2**), 424-429**.**

Teo, J. W. P., Suwanto, A. and Poh, C. L., 2000. Novel β-lactamase genes from two environmental isolates of *Vibrio harveyi*. *Antimicrobial Agents and Chemotherapy*, 44(**5**), 1309-14.

Thungapathra, M., Amita Sinha, K. K., Chaudhuri, S. R., Garg, P., Ramamurty, T., Nair, G. B. and Ghosh, A., 2002. Occurrence of antibiotic resistance gene cassettes aac(6')-Ib, dfrA5, dfrA12, and ereA2 in class 1 integrons in non-O1, non-O139 *Vibrio cholerae* strains in India**.** *Antimicrobial Agents and Chemotherapy*, 46(**9**), 2948-55.

Ulusarac, O. and Carter, E., 2004. Varied clinical presentations of *Vibrio vulnificus* infections: a report of four unusual cases and review of the literature. *South Eastern Asian Medical Journal*, 97(**2**), 163-168.

Zulkifli, Y., Alitheen, N. B., Raha, A. R., Yeap, S. K., Marlina, Son, R. and Nishibuchi, M., 2009. Antibiotic resistance and plasmid profiling of *Vibrio parahaemolyticus* isolated from cockles in Padang, Indonesia. *International Food Research Journal*, 16(**1**), 53-58.

الگوی مقاومت ضد میکروبی برخی سویه های ویبریو جدا شده از فراورده های دریائی

مهدی رئیسی*[1]، منوچهر مومنی[2]، مهسا انصاری[3] و ابراهیم رحیمی[1]

چکیده

مطالعه حاضر با هدف بررسی مقاومت ضد میکروبی و حضور ژنهای مقاومت ضد میکروبی در گونه های ویبریو جدا شده از فرآورده های دریائی انجام شد. تعداد ۷۲ جدایه ویبریو از ۶ گونه شامل *V. parahaemolyticus*، *V. vulnificus*، *V. alginolyticus*، *V. harveyi*، *V. cholerae* و *V. mimicus* مورد بررسی قرار گرفتند. نتایج نشان می دهد که همه جدایه ها مقاومت ضد میکروبی چندگانه دارند. از ۷۲ جدایه بررسی شده، ۷۰ مورد به آمپی سیلین (۹۷/۲٪)، ۶۰ مورد به جنتامایسین (۸۳/۳٪) و ۵۶ مورد به پنی سیلین (۷۷/۷٪) مقاوم بودند. هشت سویه به چهار آنتی بیوتیک، ۱۹ سویه به ۵ آنتی بیوتیک، ۱۰ سویه به ۶ آنتی بیوتیک، ۳۴ سویه به ۷ آنتی بیوتیک و یک سویه به ۸ آنتی بیوتیک مقاوم بودند. نتایج همچنین نشان می دهد که ۲۰ سویه (۲۷/۷ درصد از موارد بررسی شده) دارای ۳-۱ ژن از ژنهای مقاومت ضد میکروبی است. *Str*B، *tet*S و *erm*B ژنهای کد کننده مقاومت به استرپتومایسین، تتراسیکلین و اریترومایسین به ترتیب در ۱۸، ۶ و ۵ جدایه یافت شدند و ژن مقاومت به سولفامتوکسازول (*Sul2*) در این مطالعه یافت نشد. یافتن ژنهای مقاومت در سویه های ویبریو جدا شده از فراورده های دریائی به عنوان یک خطر بالقوه برای مصرف کننده تلقی می شود و بیان کننده این است که ژنهای مقاومت ممکن است بشکل بیشتری در طبیعت گسترش یابند که دربرگیرنده خطرات جدی برای سلامت انسان می باشد.

واژگان کلیدی: ویبریو، ژنهای مقاومت ضد میکروبی، فرآورده های دریائی، خلیج فارس

۱-گروه بهداشت مواد غذائی و بهداشت و بیماریهای آبزیان، دانشکده دامپزشکی، دانشگاه آزا داسلامی واحد شهرکرد.، ایران.
۲-آزمایشگاه مرکزی، دانشگاه آزا داسلامی واحد شهرکرد
۳-باشگاه پژوهشگران جوان، دانشگاه آزاد اسلامی واحد شهرکرد
*پست الکترونیکی نویسنده مسئول : mehdi.raissy@iaushk.ac.ir