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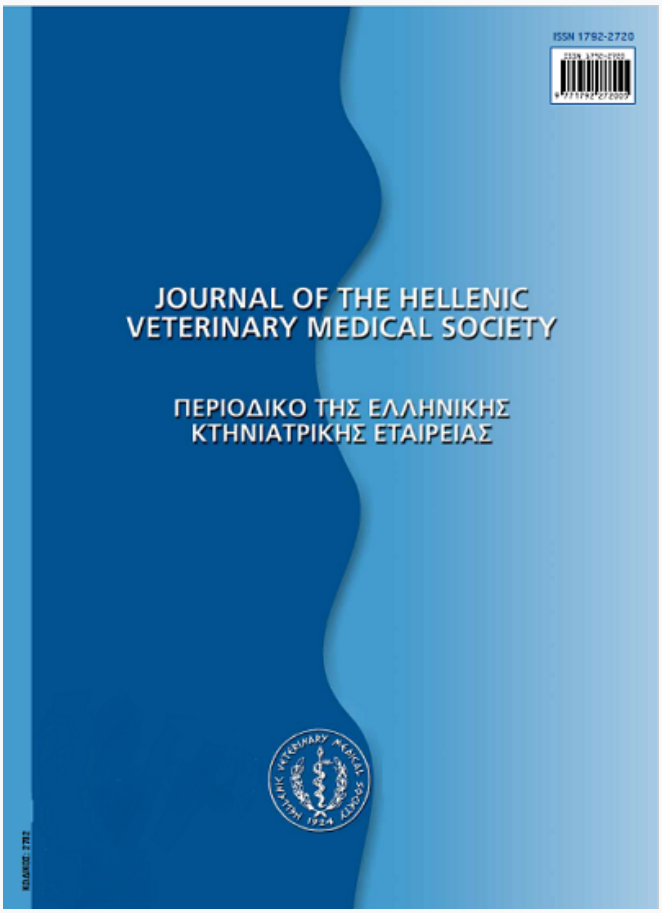
Journal of the Hellenic Veterinary Medical Society

Vol 68, No 1 (2017)



Antimicrobial resistance profile and resistance genes of Vibrio species isolated from giant freshwater prawn (Macrobrachium rosenbergii) raised in Iran

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doi: 10.12681/jhvms.15566

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To cite this article:

SHAKERIAN, A., BARTON, M. D., AKINBOWALE, O. L., & KHAMESIPOUR, F. (2018). Antimicrobial resistance profile and resistance genes of Vibrio species isolated from giant freshwater prawn (Macrobrachium rosenbergii) raised in Iran. *Journal of the Hellenic Veterinary Medical Society*, *68*(1), 79–88. https://doi.org/10.12681/jhvms.15566

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J HELLENIC VET MED SOC 2017, 68(1): 079-088
ΠΕΚΕ 2017, 68(1): 079-088

Research article
Ερευνητικό άρθρο

Antimicrobial resistance profile and resistance genes of Vibrio species isolated from giant freshwater prawn (Macrobrachium rosenbergii) raised in Iran

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ABSTRACT. Because of raising of large-scale high density prawn aquaculture techniques, production of this prawn worldwide is facing a serious threat from fatal diseases caused by nodaviruses and bacteria, particularly from the *Vibrio* species. The development of antibiotic resistance by *Vibrio* represents a potential threat to human health by exchange of resistant genes to human pathogens through food chain. This study aimed to determine antibiotic resistance profile of *Vibrio* isolates from giant fresh water Prawns (*Macrobrachium rosenbergii*) raised in Iran and to detect some antibiotic resistance genes in the isolates. A total of fifty giant fresh water prawns were processed for isolation of *Vibrio* species during February 2015 to August 2015. Identification of *Vibrio* isolates was done following standard biochemical methods. Phenotypic resistance of the isolates as determined by agar dilution method while polymerase chain reaction (PCR) method was used to detect the presence of *erm*, *tetS*, *strA* and *sul2* genes in the isolates. Out of 50 prawns, 31 (62%) isolates of *Vibrio* spp. were reported, of which 20 (40%) were identified as *V. parahaemolyticus,* 10 (20%) were *V. vulnificus* and 1 (2%) were *V. cholera.* Over 90% of the tested strains showed susceptibility to SXT, AZM and NIT. In addition, *strA* and *tetS* genes were detected in all isolated *Vibrio* species. *StrA* gene was identified in 6 *V. parahaemolyticus* strains and also *ermB* and *sul2* genes were not present in the isolate of *V. cholera.* The occurrence of multidrug resistance strains in the environment could be an indication of excessive usage of antibiotics in agriculture and aquaculture fields. This study has shown that giant freshwater prawns raised in Iran habour multidrug resistant *Vibrio* species.

***Keywords*:** *antibiotic resistance genes, antimicrobial susceptibility, giant freshwater prawns, Macrobrachium rosenbergii, Vibrio species*

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Date of initial submission: 23-10-2015
Date of revised submission: 7-1-2015
Date of acceptance: 24-1-2016

INTRODUCTION

The giant freshwater prawn, *Macrobrachium rosenbergii* (locally known as 'udang galah'), belongs to the genus *Macrobrachium*, which is the largest genus of the family Palaemonidae (De Grave et al., 2008). They are found in most inland freshwater areas, including lakes, rivers, swamps, estuarine areas, ponds, canals as well as in irrigation duct (New, 2002). However, with the rise of large-scale high density prawn aquaculture techniques, production of this prawn worldwide is facing a serious threat from fatal diseases caused by nodaviruses and bacteria, particularly from the *Vibrio* species (Tonguthai, 1995; Bonami et al., 2011). The emergence of these pathogens has had a detrimental impact on the *M. rosenbergii* farming industry, causing considerable economic losses.

Vibrio is a Gram-negative halophilic bacterium found abundantly in marine and estuarine environments (Ramesh et al., 1990; Thompson et al., 2004; Khamesipour et al., 2014; Raissy et al., 2014). Among the different species, *Vibrio parahaemolyticus* has emerged as an important pathogen for *M. rosenbergii* (Khuntia et al., 2008). Severe *V. parahaemolyticus* infection in prawns leads to a disease known as 'Vibriosis' (Roque et al., 1991; Xu et al., 1991). *M. rosenbergii* suffering from vibriosis may appear black in colour on the carapace, with red discolouration of the exoskeleton and loss of appendages within six days, leading to an 80% mortality rate (Khuntia et al., 2008).

Recently, polymerase chain reaction (PCR) showed to be a valuable and rapid tool for diagnosis of animal and human diseases (Rahimi et al., 2012; Hemmatinezhad et al., 2015; Khamesipour et al., 2015; Khodadadi et al., 2015; Solati et al., 2015; Tajbakhsh et al., 2015). The polymerase chain reaction (PCR) assays is one of the molecular techniques that is widely used to detect the presences of pathogenic *V. parahaemolyticus* strain in food and environment (Panicker et al., 2004; Yamamoto et al., 2008; Paydar et al., 2013; Malcolm et al., 2015). PCR primers can be multiplexed in a single reaction to increase the detection limit or tailored as real-time PCR analysis to provide more rapid results (Grant et al., 2006; Zhang et al., 2014).

Vibrio spp. usually are said to be highly susceptible to most clinically used antibiotics (Mala et al., 2014; Shaw et al., 2014). However, over the years, antibiotic resistance strains have emerged into the environment due the excessive use of antibiotics and other chemotherapeutic agents in human, agriculture, and aquaculture fields (Cabello et al., 2013). In the aquaculture field, antimicrobials are used not to promote growth but rather to prevent (prophylactic use) and treat (therapeutic use) bacterial infections on fish and other invertebrates (Cabello et al., 2013). Oxytetracycline, tetracycline, quinolone, sulphonamides, and trimethoprimare among the antibiotics allowed and used in the Asian aquaculture industry to ensure continuous production of sea food (Rico et al., 2012; Yano et al., 2014).

There are many clinically used antibiotics as a choice of treatment for *Vibrio* spp. infections including cephalothin (first generation cephalosporins), cefuroxime (second generation cephalosporin), cefotaxime and ceftazidime (third generation cephalosporins), tetracycline, doxycycline, orfluoroquinolone (Tang et al., 2002; Al-Othrubi et al., 2014). The use of antimicrobials in the aquaculture has caused the development of antibiotic resistant bacteria and antibiotic resistant genes.

Antibiotic-resistant bacteria may represent a potential threat to human health due to direct transmission through the food chain (Duran and Marshall, 2005) or by transferring the acquired antimicrobial resistance to human pathogens by mobile genetic elements (Angulo, 2000; Serrano, 2005; Guglielmetti et al., 2009). Although several investigations have been conducted in different countries regarding antibiotic resistance in *Vibrio* spp. isolated from aquaculture (Roque et al., 2001; Dang et al., 2006; Akinbowale et al., 2007; Laganà et al., 2011; Raissy et al., 2012), and little research focused on *Vibrio* spp. isolated from gilthead sea bream (Snoussi et al, 2006) there is no research has been conducted on the distribution and antimicrobial susceptibility pattern of *Vibrio* species isolated from Giant Freshwater Prawn in Iran. Therefore, the present study aimed to determine the antibiotic resistance profile of *Vibrio* spp., isolated from giant fresh water prawns (*Macrobrachium rosenbergii*) reared in Iran and study the distribution of antibiotic resistance genes encoding resistance to some commonly used antibiotics in the isolates.

MATERIALS AND METHODS

Sample collection

Fifty giant fresh water prawns (*Macrobrachium*

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

Primer	Sequence (5'-3')	Target gene	Amplicon size (bp)	Annealing temperature (°C)	Reference
ermB- F ermB- R	AGACACCTCGTCTAACCTTCGCTC TCCATGTACTACCATGCCACAGG	*ermB*	640	60	Raissy et al., 2012
tetS- F tetS- R	ATCAAGATATTAAGGAC TTCTCTATGTGGTAATC	*tetS*	590	38	Ture and Boran, 2015
Sul2- F Sul2- R	TGTGCGGATGAAGTCAGCTCC AGGGGGCAGATGTGATCGAC	*sulII*	625	60	García-Aljaro et al., 2014
strA- F strA- F	TTGATGTGGTGTCCCGCAATGC CCAATCGCAGATAGAAGGCAA	*strA*	383	57	Goel et al., 2010

rosenbergii) purchased from the supermarkets and local fish markets in Iran from during February to August 2015. Straight away after collection, the prawns samples were kept in cool boxes with an internal temperature of 2°C to 4°C and aseptically transported and processed within 1 hour of collection in the laboratory.

Isolation of *Vibrio* species

Each prawn was homogenized in 225 ml alkaline saline peptone water (ASPW) pH 8.5 (Oxoid CM1028 Hampshire, UK) using a stomacher (Bagmixer 400W, Interscience, St Nom, France) at 11000 rev min-1 for 3 min.

The homogenates were incubated overnight at 30°C and then cultured onto selective media Thiosulphate citrate bile salt sucrose (TCBS) agar (Oxoid CM0333), and CHROM agar *Vibrio* (Oxoid CM1050). The inoculated plates were incubated overnight at 30oC for 24-48 h.

Bacterial characterization

Isolates with green, blue green or yellow green, 2-3mm in diameter colonies, on TCBS agar and those with mauve 2-3mm in diameter colonies on CHROM agar plates, were presumptively taken as presumptively *Vibrio* spp. The colonies were subcultured onto nutrient agar plates (Oxoid, Hampshire, UK) supplemented with 5 g/l NaCl to a a final concentration of 1% and incubated at 37oC for 24 h according to ISO/TS(28). Presumptive *Vibrio* spp., were further identified to species level using MICROBACT 24E identification kits (Oxoid Ltd.). Then, identified isolates were stored at -80°C until needed for further analysis.

Antimicrobial susceptibility test

Antimicrobial resistance/susceptibility of the isolates were determined using agar dilution method according to Clinical and Laboratory Standards Institute (CLSI) (2012) guidelines. Eleven antibiotics which included: ampicillin (10 µg), ciprofloxacin (5 µg), tetracycline (30 µg), nitrofurantion (300 µg), gentamicin (10 µg), nalidixic acid (30 µg), oxytetracycline (30 µg), erythromycin (15 µg), azitromycine (15 µg), sulfamethoxazole (25 µg) and streptomycin (30 µg) were used. Doubling dilutions of the antibiotic stock solutions were incorporated into Mueller–Hinton agar plates with final concentrations ranging from 0.25 µg/mL to 128 µg/mL (512 µg/mL in the case of sulfonamides). Plates were inoculated with bacteria emulsified in 0.85% NaCl to a turbidity equivalent to a 0.5 McFarland turbidity standard (equivalent to 1 x 108 cfu/ml) using a multipoint inoculator. The inoculated plates were incubated overnight at 30°C. The results were recorded as resistant or susceptible by measuring the inhibition zone diameter according to the CLSI (2010) criteria. Antibiotic sensitivity test were done for each triplicate samples.

DNA Extraction

The genomic DNA was extracted following the method described by Ausubel et al., 1987. The isolates were grown overnight at 30°C in Tryptic Soy Broth containing 1% sodium chloride. The bacteria (1.5 ml) were 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 hexadecyl trimethyl 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 amplification of resistance genes

Antibiotic resistance genes in the isolates were identified using polymerase chain reaction (PCR). 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. PCR reactions were performed in a thermal cycler (Eppendorf, Mastercycler Gradient). 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

Prevalence of Vibrio species in giant freshwater prawns

Overall, 50 giant fresh water prawns samples were collected in Iran, 31 (62%) isolates of *Vibrio* spp. were reported during February 2015 to August 2015, of which 20 (40%) were identified as *V. parahaemolyticus,* 10 (20%) were *V. vulnificus* and 1 (2%) were *V. cholera* (Table 2).

Phenotypic resistance profile of Vibrio isolates from giant freshwater prawns

The susceptibility of 31 *Vibrio* strains was assessed against 11 different antibiotics. Out of 31 isolates, 29 (93.5%) were resistant to ampicillin, 1 (3.2%) to ciprofloxacin, 24 (77.4%) to tetracycline, 23 (74.2%) to gentamicin, 20 (64.5%) to nalidixic acid, 30 (96.8%) to oxytetracycline, 11 (35.5%) to erythromycin and 18 (58.1%) to streptomycin. None of the isolates was resistant to nitrofurantoin, azithromycin and sulphamethoxazole (Table 3) while over 90% of the showed susceptibility to SXT, AZM and NIT.

Almost all of the *Vibrio* isolates were susceptible to SXT and AZM. Multiple resistance was observed in all identified *V. parahaemolyticus* strains (resistant to 7 antibiotics). In strains with multiple resistance the most frequent antibiotic combination was STR, ERY, NAL, AMP, GEN, OTC and TET. Analysis of the antimicrobial resistance profiles revealed 31 resistance patterns, of which the most frequent resistance pattern was TET and GEN. Table 3 reports the number of the susceptible, intermediate and resistant strains of *Vibrio* spp. isolated from giant fresh water prawns according to the breakpoints proposed by NCCLS and CLSI.

Prevalence of antibiotic resistance genes in Vibrio isolates from giant freshwater prawns

All *Vibrio* spp. isolates (*n*=31) were screened for *ermB*, *tetS*, *strA* and *sul2* resistant genes. Electrophoresis of PCR products for detection of *ermB*, *strA*, *tetS* and *sul2*, genes encoding factor are shown in Figure 1. The PCR result showed *strA* and *tetS* gene were detected in all isolated *Vibrio* species. In addition, *strA* gene was identified in 6 *V. parahaemolyticus* strains and also *ermB* and *sul2* genes were not present in the isolate of *V. cholera*. Out of 31 isolates, 9 (29%) were positive for *strA* gene, 7 (22.6%) for *tetS* gene, 10 (32.3%) for *ermB* and 4 (12.9%) for *sul2* (Table 4). Of the 31 ARG-positive strains, 2 (6.5%) haboured *strA*+*ermB*, *ermB*+*sul2*, *strA*+*tetS* and *teS*+*ermB* genes.

DISCUSSION

Apart from viral diseases, *Vibrio* infections causing Vibriosis is another factor hindering the

Figure 1. Electrophoresis of PCR products for detection of ermB, strA, tetS and sul2, genes encoding factor. M: 100bp ladder, 1-4: Positive samples, 5: Negative control.

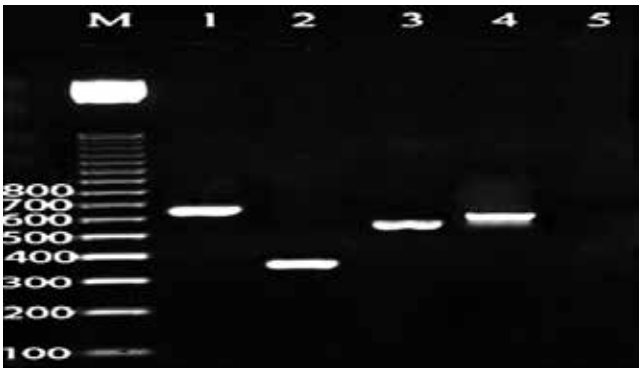

Table 2. Occurrence of Vibrio species from giant fresh water prawns (n=50) from Iran

Vibrio	Isolation rate (%)
V. parahaemolyticus	20 (40)
V. vulnificus	10 (20)
V. cholera	1 (2)

Table 3. Resistance profile of Vibrio spp. isolated from fresh giant prawns

Identification	Susceptible	Intermediate	Resistance
V. cholera	SXT;AZM;CIP;NAL;NIT	OTC;GEN;ERY;STR	AMP;TET
V. vulnificus	SXT;AZM;CIP;NIT	NAL;TET;ERY	AMP;OTC;GEN;STR
V. vulnificus	SXT;AZM;CIP;NIT	NAL;TET;ERY	AMP;OTC;GEN;STR
V. vulnificus	SXT;AZM;CIP;NIT	TET	NAL;AMP;OTC;GEN;STR;ERY
V. vulnificus	SXT;AZM;CIP;NIT	ERY;TET	NAL;AMP;OTC;GEN;STR
V. vulnificus	SXT;AZM;CIP;NIT;ERY	TET	NAL;AMP;OTC;GEN;STR
V. vulnificus	SXT;AZM;CIP;NIT;ERY;STR	-	TET;NAL;AMP;OTC;GEN
V. vulnificus	SXT;AZM;CIP;NIT;ERY;STR	GEN;NAL	OTC;AMP;TET
V. vulnificus	SXT;AZM;CIP;NIT;ERY;STR	GEN;NAL;TET	OTC;AMP
V. vulnificus	STX;AZM;CIP;NIT	ERY;STR	GEN;NAL;OTC;AMP
V. vulnificus	SXT;AZM;CIP;NIT	ERY;STR;NAL	TET;OTC;GEN;AMP
V. parahaemolyticus	SXT;AZM;NIT	CIP	ERY;STR;NAL;TET;OTC;GEN;AMP
V. parahaemolyticus	SXT;AZM	CIP;NIT	ERY;STR;NAL;TET;OTC;GEN;AMP
V. parahaemolyticus	SXT;AZM	NIT;ERY	CIP;STR;NAL;TET;OTC;GEN;AMP
V. parahaemolyticus	SXT;ATM;NIT;ERY;CIP	GEN;NAL;STR	TET;OTC;AMP
V. parahaemolyticus	SXT;ATM;NIT;ERY;CIP	-	TET;OTC;AMP;GEN;NAL;STR
V. parahaemolyticus	SXT;AZM;NIT;ERY;CIP	STR;GEN;AMP;NAL	TET;OTC
V. parahaemolyticus	SXT;AZM;NIT	CIP;ERY	TET;OTC;STR;GEN;AMP;NAL
V. parahaemolyticus	SXT;AZM;NIT;CIP	-	ERY;TET;OTC;STR;GEN;AMP;NAL
V. parahaemolyticus	SXT;AZM;NIT;CIP	-	ERY;TET;OTC;STR;GEN;AMP;NAL
V. parahaemolyticus	SXT;AZM;NIT;CIP	ERY;STR;NAL	OTC;TET;AMP;GEN
V. parahaemolyticus	SXT;AZM;NIT	CIP;AMP;GEN;ERY;STR	NAL;TET;OTC
V. parahaemolyticus	SXT;AZM;NIT;CIP	STR;ERY	AMP;GEN;NAL;OTC;TET
V. parahaemolyticus	SXT;AZM;NIT;CIP	-	STR;ERY;AMP;GEN;NAL;OTC;TET
V. parahaemolyticus	SXT;AZM;NIT;CIP	GEN;NAL	STR;ERY;AMP;OTC;TET
V. parahaemolyticus	SXT;AZM	CIP;NIT;STR;ERY;NAL	AMP;GEN;OTC;TET
V. parahaemolyticus	SXT;AZM;NIT	CIP	STR;ERY;NAL;AMP;GEN;OTC;TET
V. parahaemolyticus	AZM;NIT	SXT;CIP	STR;ERY;NAL;AMP;GEN;OTC;TET
V. parahaemolyticus	SXT;AZM;NIT;CIP	-	STR;ERY;NAL;AMP;GEN;OTC;TET
V. parahaemolyticus	SXT;AZM	NIT;CIP	STR;ERY;NAL;AMP;GEN;OTC;TET
V. parahaemolyticus	SXT;CIP	AZM;NIP;ERY;STR	NAL;AMP;GEN;OTC;TET

AMP= ampicillin 10μg, AZM= azitromycine 15μg, CIP= ciprofloxacin 5μg, ERY= erythromycin 15μg GEN= gentamicin 10 μg, NAL= nalidixic acid 30μg, NIT= Nitrofurantion 300 μg, OTC= oxytetracycline 30μg, STR= streptomycin 30μg, SXT= sulfamethoxazole 25μg, TET= tetracycline 30μg

Table 4. Resistance genes for antibiotics resistant Vibrio spp. isolated from fresh giant prawns

Species of Isolated	Strain(s) showing presence of gene encoding			
	***str*A**	***tet*S**	***erm*B**	***sul*2**
V. cholera	-	+	-	-
V. vulnificus	+	-	-	-
V. vulnificus	-	+	-	-
V. vulnificus	-	-	-	-
V. vulnificus	+	-	+	-
V. vulnificus	-	-	-	+
V. vulnificus	-	+	-	-
V. vulnificus	-	-	-	-
V. vulnificus	+	-	+	-
V. vulnificus	-	-	+	+
V. vulnificus	-	-	-	-
V. parahaemolyticus	+	+	-	-
V. parahaemolyticus	-	-	+	-
V. parahaemolyticus	-	-	+	+
V. parahaemolyticus	-	-	-	-
V. parahaemolyticus	-	-	+	-
V. parahaemolyticus	-	-	-	-
V. parahaemolyticus	+	+	-	-
V. parahaemolyticus	+	-	-	-
V. parahaemolyticus	+	-	-	-
V. parahaemolyticus	-	-	+	-
V. parahaemolyticus	-	-	-	+
V. parahaemolyticus	+	-	-	-
V. parahaemolyticus	-	-	-	-
V. parahaemolyticus	-	-	-	-
V. parahaemolyticus	-	-	-	-
V. parahaemolyticus	-	+	+	-
V. parahaemolyticus	+	-	-	-
V. parahaemolyticus	-	-	+	-
V. parahaemolyticus	-	+	+	-
V. parahaemolyticus	-	-	-	-

shrimp aquaculture industry worldwide (Tonguthai, 1995). Knowledge about the interaction between *M. rosenbergii* and *Vibrio* species is in its infancy, and in-depth study is urgently needed to address this issue. The data analyses obtained in this study clearly showed a significant impact of *Vibrio* spp. infection on the *M. rosenbergii* transcriptome. In addition, the emergence and the spread of resistance to antibiotics among Gram-negative organisms have been increasing rapidly in recent years. The epidemiological importance of preventing these drug resistant strains from spreading in the community has become a global problem (Taneja et al., 2010).

One of the main findings in this study was the wide spread of antimicrobial resistance among *Vibrio* spp. isolated from giant fresh water prawns samples. Resistance to ampicillin was prevalent, which was in agreement with the previous studies (Roque et al., 2001; Akinbowale et al., 2006). The result is also in agreement with other studies that reported *V. parahaemolyticus* isolated from seafood samples are commonly resistance to ampicillin (Okuda et al., 1997; Han et al., 2007; Al-Othrubi et al., 2014). The ampicillin-resistant pattern could be due to the fact that first generation antibiotics, including ampicillin, is misused in the environment thus reducing the susceptibility and efficiency of ampicillin in the treatment of *Vibrio* infection (Sudha et al., 2014). This is not surprising because this antibiotic is naturally produced and dispersed in the environment, and thus readily select for the resistance determinants or resistant bacterial strains (Rosser and Young, 1999; Bani et al., 2007).

In the present study the susceptibility of 31 *Vibrio* strains was assessed against 11 different antibiotics. All 11 antibiotics used in this study are among the antibiotics recommended by Centre for Disease Control and Prevention (CDC) for the treatment of *Vibrio* spp. infections that includes fluoroquinolones (levofloxacin), cephalosporins (cefotaxime and ceftazidime), aminoglycosides (amikacin and gentamicin), and folate pathway inhibitors (trimethoprim- sulfamethoxazole) (Daniels et al., 2000; Shaw et al., 2014).

In the present study the resistance rate to tetracycline is high, which was consistent with former reports (Tendencia and de la Pena, 2001; Vaseeharan et al., 2005). Antibiotics in this family, particularly oxytetracycline, are commonly used in agriculture,

and aquaculture fields and this could be an explanation for the resistance dissemination observed. In the macrolides class, erythromycin demonstrated little efficacy. Similar findings were also observed in *Vibrio* strains isolated in Tunisian and Malaysian aquaculture (Snoussi et al., 2008; Snoussi et al., 2011; Lajnef et al., 2012).

Numerous antibiotic resistant genes can be found in bacteria and environments as β-lactam and penicillin resistant genes *penA* and *blaTEM-1* (Srinivasan et al., 2005; Zhang et al., 2009), chloramphenicol resistant genes *cat*I, *cat*II, *cat*III, *cat*IV and *floR* (Dang et al., 2007, 2008), tetracycline resistant genes *tatA, tatB, tatC, tatD, tatE, tatG, tatH, tatJ, tatY, tatZ*, and many more (Macauley et al., 2007; Zhang et al., 2009; Kim et al., 2013). These antibiotic resistant genes can be transfer among different bacteria via conjugation, transduction, or transformation (Manjusha and Sarita, 2011).

In the present study, the *str*A, *tet*S, *erm*B and *sul*2 resistance genes were detected in the identified *Vibrio* isolates. Several *tet* genes [e.g. *tet* (A), *tet* (B) and *tet* (D) genes encoding active efflux pumps] have been identified previously in *Vibrio* spp. from a maricultural environment (Dang et al., 2006, 2007). Further studies are required to elucidate the mechanisms underlying tetracycline resistance and other antibiotic resistance of the isolates in our collection.

CONCLUDING REMARKS

Our study has shown that *Vibrio* spp. is present all year round in Giant Freshwater Prawn (*Macrobrachium rosenbergii*) in Iran. Antimicrobial resistance has attained the importance of a global public-health problem. The increase in the magnitude of bacterial species resistant to multiple antimicrobial agents relies on various factors apart from the environmental stresses which the organism is facing over the years. Our results indicate the circulation of multidrug-resistant *Vibrio* spp. harboring mobile genetic elements in Giant Freshwater Prawn (*Macrobrachium rosenbergii*) and confirm the wide diversity of resistance mechanisms mediating antimicrobial resistance among the pathogens. The association of antimicrobial resistance determinants with transferable genetic elements may promote the rapid dissemination of antimicrobial resistance among *Vibrio* spp. and other aquatic bacteria. The extent of the antimicrobial resistance and the threats caused by environmental contamination of resistant bacteria are of particular concern. This could be the result of the intrinsic resistance of microorganisms, horizontal gene transfer or antibiotic pressure.

CONFLICT OF INTEREST STATEMENT

None of the authors of this article has any conflict of interest.

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