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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 harbour multidrug resistant *Vibrio* species.

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

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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 trimethoprim are 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, or fluoroquinolone (Tang et al., 2002; Al-Othubi 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	<i>ermB</i>	640	60	Raissy et al., 2012
tetS- F tetS- R	ATCAAGATATTAAGGAC TTCTCTATGTGGTAATC	<i>tetS</i>	590	38	Ture and Boran, 2015
Sul2- F Sul2- R	TGTGCGGATGAAGTCAGCTCC AGGGGGCAGATGTGATCGAC	<i>sulII</i>	625	60	García-Aljaro et al., 2014
strA- F strA- F	TTGATGTGGTGTCCCGCAATGC CCAATCGCAGATAGAAGGCAA	<i>strA</i>	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 30°C 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 final concentration of 1% and incubated at 37°C 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), azitromycin (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 mM Tris-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%) harboured *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.

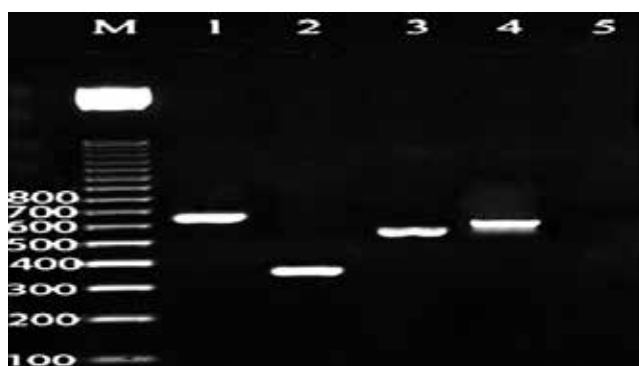


Table 2. Occurrence of *Vibrio* species from giant fresh water prawns (n=50) from Iran

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

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

Identification	Susceptible	Intermediate	Resistance
<i>V. cholera</i>	SXT;AZM;CIP;NAL;NIT	OTC;GEN;ERY;STR	AMP;TET
<i>V. vulnificus</i>	SXT;AZM;CIP;NIT	NAL;TET;ERY	AMP;OTC;GEN;STR
<i>V. vulnificus</i>	SXT;AZM;CIP;NIT	NAL;TET;ERY	AMP;OTC;GEN;STR
<i>V. vulnificus</i>	SXT;AZM;CIP;NIT	TET	NAL;AMP;OTC;GEN;STR;ERY
<i>V. vulnificus</i>	SXT;AZM;CIP;NIT	ERY;TET	NAL;AMP;OTC;GEN;STR
<i>V. vulnificus</i>	SXT;AZM;CIP;NIT;ERY	TET	NAL;AMP;OTC;GEN;STR
<i>V. vulnificus</i>	SXT;AZM;CIP;NIT;ERY;STR	-	TET;NAL;AMP;OTC;GEN
<i>V. vulnificus</i>	SXT;AZM;CIP;NIT;ERY;STR	GEN;NAL	OTC;AMP;TET
<i>V. vulnificus</i>	SXT;AZM;CIP;NIT;ERY;STR	GEN;NAL;TET	OTC;AMP
<i>V. vulnificus</i>	STX;AZM;CIP;NIT	ERY;STR	GEN;NAL;OTC;AMP
<i>V. vulnificus</i>	SXT;AZM;CIP;NIT	ERY;STR;NAL	TET;OTC;GEN;AMP
<i>V. parahaemolyticus</i>	SXT;AZM;NIT	CIP	ERY;STR;NAL;TET;OTC;GEN;AMP
<i>V. parahaemolyticus</i>	SXT;AZM	CIP;NIT	ERY;STR;NAL;TET;OTC;GEN;AMP
<i>V. parahaemolyticus</i>	SXT;AZM	NIT;ERY	CIP;STR;NAL;TET;OTC;GEN;AMP
<i>V. parahaemolyticus</i>	SXT;ATM;NIT;ERY;CIP	GEN;NAL;STR	TET;OTC;AMP
<i>V. parahaemolyticus</i>	SXT;ATM;NIT;ERY;CIP	-	TET;OTC;AMP;GEN;NAL;STR
<i>V. parahaemolyticus</i>	SXT;AZM;NIT;ERY;CIP	STR;GEN;AMP;NAL	TET;OTC
<i>V. parahaemolyticus</i>	SXT;AZM;NIT	CIP;ERY	TET;OTC;STR;GEN;AMP;NAL
<i>V. parahaemolyticus</i>	SXT;AZM;NIT;CIP	-	ERY;TET;OTC;STR;GEN;AMP;NAL
<i>V. parahaemolyticus</i>	SXT;AZM;NIT;CIP	-	ERY;TET;OTC;STR;GEN;AMP;NAL
<i>V. parahaemolyticus</i>	SXT;AZM;NIT;CIP	ERY;STR;NAL	OTC;TET;AMP;GEN
<i>V. parahaemolyticus</i>	SXT;AZM;NIT	CIP;AMP;GEN;ERY;STR	NAL;TET;OTC
<i>V. parahaemolyticus</i>	SXT;AZM;NIT;CIP	STR;ERY	AMP;GEN;NAL;OTC;TET
<i>V. parahaemolyticus</i>	SXT;AZM;NIT;CIP	-	STR;ERY;AMP;GEN;NAL;OTC;TET
<i>V. parahaemolyticus</i>	SXT;AZM;NIT;CIP	GEN;NAL	STR;ERY;AMP;OTC;TET
<i>V. parahaemolyticus</i>	SXT;AZM	CIP;NIT;STR;ERY;NAL	AMP;GEN;OTC;TET
<i>V. parahaemolyticus</i>	SXT;AZM;NIT	CIP	STR;ERY;NAL;AMP;GEN;OTC;TET
<i>V. parahaemolyticus</i>	AZM;NIT	SXT;CIP	STR;ERY;NAL;AMP;GEN;OTC;TET
<i>V. parahaemolyticus</i>	SXT;AZM;NIT;CIP	-	STR;ERY;NAL;AMP;GEN;OTC;TET
<i>V. parahaemolyticus</i>	SXT;AZM	NIT;CIP	STR;ERY;NAL;AMP;GEN;OTC;TET
<i>V. parahaemolyticus</i>	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			
	<i>strA</i>	<i>tetS</i>	<i>ermB</i>	<i>sul2</i>
<i>V. cholera</i>	-	+	-	-
<i>V. vulnificus</i>	+	-	-	-
<i>V. vulnificus</i>	-	+	-	-
<i>V. vulnificus</i>	-	-	-	-
<i>V. vulnificus</i>	+	-	+	-
<i>V. vulnificus</i>	-	-	-	+
<i>V. vulnificus</i>	-	+	-	-
<i>V. vulnificus</i>	-	-	-	-
<i>V. vulnificus</i>	+	-	+	-
<i>V. vulnificus</i>	-	-	+	+
<i>V. vulnificus</i>	-	-	-	-
<i>V. parahaemolyticus</i>	+	+	-	-
<i>V. parahaemolyticus</i>	-	-	+	-
<i>V. parahaemolyticus</i>	-	-	+	+
<i>V. parahaemolyticus</i>	-	-	-	-
<i>V. parahaemolyticus</i>	-	-	+	-
<i>V. parahaemolyticus</i>	-	-	-	-
<i>V. parahaemolyticus</i>	+	+	-	-
<i>V. parahaemolyticus</i>	+	-	-	-
<i>V. parahaemolyticus</i>	+	-	-	-
<i>V. parahaemolyticus</i>	-	-	+	-
<i>V. parahaemolyticus</i>	-	-	-	+
<i>V. parahaemolyticus</i>	+	-	-	-
<i>V. parahaemolyticus</i>	-	-	-	-
<i>V. parahaemolyticus</i>	-	-	-	-
<i>V. parahaemolyticus</i>	-	-	-	-
<i>V. parahaemolyticus</i>	-	+	+	-
<i>V. parahaemolyticus</i>	+	-	-	-
<i>V. parahaemolyticus</i>	-	-	+	-
<i>V. parahaemolyticus</i>	-	+	+	-
<i>V. parahaemolyticus</i>	-	-	-	-

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-Othubi 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 *catI*, *catII*, *catIII*, *catIV* 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 *strA*, *tetS*, *ermB* and *sul2* 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. ■

REFERENCES

- Akinbowale OL, Peng H, and Barton MD (2007) Diversity of tetracycline resistance genes in bacteria from aquaculture sources in Australia. *Journal of Applied Microbiology* 103: 2016-2025.
- Akinbowale OL, Peng H, and Barton MD (2006) Antimicrobial resistance in bacteria isolated from aquaculture sources in Australia. *Journal of Applied Microbiology* 100: 1103-1113.
- Al-Othubi SM, Kqueen CY, Mirhosseini H, H adi YA, and Radu S (2014) Antibiotic resistance of *Vibrio parahaemolyticus* Isolated from cockles and shrimp sea food marketed in Selangor, Malaysia. *Clinical Microbiology* 3: 148-154.
- Angulo FJ (2000) Antimicrobial agents in aquaculture: potential impact on health. *APUA Newsletter*. 18: 1-6.
- Ausubel FM, Brent R, Kingston RE, Moore DD, Sideman J, Smith J, and Struhl K (1987) *Current Protocols in Molecular Biology*, USA: Wiley-Blachwell, p. 354.
- Bani S, Mastromarino PN, Ceccarelli D, Le Van A, Salvia AM, Ngo Viet QT, Hai DH, Bacciu D, Cappuccinelli P, and Colombo MM (2007) Molecular characterization of ICEVchVie0 and its disappearance in *Vibrio cholerae* O1 strains isolated in 2003 in Vietnam. *FEMS Microbiology Letters* 266: 42-48.
- Bonami J-R, and Sri WJ (2011) Viral diseases of the giant fresh water prawn *Macrobrachium rosenbergii*: a review. *Journal of Invertebrate Pathology* 106 (1): 131-142.
- Cabello FC, Godfrey HP, Tomova A, Ivanova L, Dölz H, Millanao A, and Buschmann AH (2013) Antimicrobial use in aquaculture re-examined: its relevance to antimicrobial resistance and to animal and human health. *Applied and Environmental Microbiology* 15: 1917-1942.
- CLSI (2010) Performance standards for antimicrobial susceptibility testing: methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria (Approved standard (M45-A)). Wayne, PA: Clinical and Laboratory Standards Institute.
- 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: 1494-1503.
- Dang H, Zhang X, Song L, Chang Y, and Yang G (2007) Molecular determination of oxytetracycline-resistant bacteria and their resistance genes from mariculture environments of China. *Journal of Applied Microbiology* 103: 2580-2592.
- Dang HY, Ren J, Song LS, Sun S, and An LG (2008) Dominant chloramphenicol resistant bacteria and resistance genes in coastal marine water of Jiaozhou Bay, China. *World Journal of Microbiology and Biotechnology* 24: 209-217.
- Daniels NA, MacKinnon L, Bishop R, Altekruze S, Ray B, Hammond RM, Thompson S, Wilson S, Bean NH, Griffin PM, and Slutsker L (2000) *Vibrio parahaemolyticus* infection in the United States, 1973-1998. *The Journal of Infectious Diseases* 181: 1661-1666.
- De Grave S, Cai Y, and Anke rA (2008) Global diversity of shrimps (Crustacea: Decapoda: Caridea) in freshwater. *Hydrobiologia* 595: 287-293.
- Duran GM, and Marshall DL (2005): Ready-to-eat shrimp as international vehicle of antibiotic-resistant bacteria. *Journal of Food Protection* 68: 2395-2401.
- García-Aljaro C, Riera-Heredia J, Blanch AR (2014) Antimicrobial resistance and presence of the SXT mobile element in *Vibrio* spp. isolated from aquaculture facilities. *New Microbiologica* 37: 339-346.
- Goel AK, Jain M, Kumar P, and Jiang SC (2010) Molecular characterization of *Vibrio cholerae* outbreak strains with altered El Tor biotype from southern India. *World Journal of Microbiology and Biotechnology* 26: 281-287.
- Grant MA, Hu J, and Jinneman KC (2006) Multiplex real-time PCR detection of heat labile and heat-stable toxigenes in enterotoxigenic *Escherichia coli*. *Journal of Food Protection* 69: 412-416.
- Guglielmetti E, Korhonen JM, Heikkinen J, Morelli L, and Von Wright A (2009) Transfer of plasmid-mediated resistance to tetracycline in pathogenic bacteria from fish and aquaculture environments. *FEMS Microbiology Letters* 293: 28-34.
- Han F, Walker RD, Janes ME, Prinyawinwatkul W, and Ge B (2007) Antimicrobial susceptibilities of *Vibrio parahaemolyticus* and *Vibrio vulnificus* isolates from Louisiana Gulf and retail raw oysters. *Applied and Environmental Microbiology* 73: 7096-7098.
- Hemmatinezhad B, Khamesipour F, Mohammadi M, Safarpour Dehkordi F, Mashak Z (2015) Microbiological investigation of O-Serogroups, virulence factors and antimicrobial resistance properties of Shiga toxin-producing *Escherichia coli* isolated from ostrich, turkey and quail meats. *Journal of Food Safety* 35: 491-500.
- Khamesipour F, Noshadi E, and Moradi M (2014) Detection of the prevalence of *Vibrio* spp. in shrimp samples by Polymerase Chain Reaction (PCR) and cultural method in the Iran. *AACL BIOFLUX*. 7(1): 1-7.
- Khamesipour F, Doosti A, Koochi A, Chehelgerdi M, Mokhtari-Farsani A, Chengula AA (2015) determination of the presence of Babesia DNA in blood samples of cattle, camel and sheep in Iran by PCR. *Archives of Biological Science Belgrade* 67 (1): 83-90.
- Khodadadi M, Hemmatinezhad B, Doosti A, Khamesipour F, Awosile B (2015) Molecular detection and prevalence of *Chlamydia psittaci* in the blood, liver and muscle tissue of urban pigeons (*Columba livia domestica*) in Iran. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi* 21 (2): 265-269.
- Khuntia CP, Das BK, Samantaray BR, Samal SK, and Mishra BK (2008) Characterization and pathogenicity studies of *Vibrio parahaemolyticus* isolated from diseased freshwater prawn, *Macrobrachium rosenbergii* (de Man). *Aquaculture Research* 39(3): 301-10.
- Kim M, Kwon TH, Jung SM, Cho SH, Jin SY, Park NH, Kim CG, and Kim JS (2013) Antibiotic resistance of bacteria isolated from the internal organs of edible snow crabs. *PLoS ONE* 8: 70887.
- Laganà P, Caruso G, Minutoli E, Zacccone R, and Santi D (2011)

- Susceptibility to antibiotics of *Vibrio* spp. and *Photobacterium damsela* ssp. *Piscicida* strains isolated from Italian aquaculture farms. *New microbiologica* 34: 53-63.
- Lajnef R, Snoussi M, Romalde JL, Nozha C, and Hassen A (2012) Comparative study on the antibiotic susceptibility and plasmid profiles of *Vibrio alginolyticus* strains isolated from four Tunisian marine biotopes. *World Journal of Microbiology and Biotechnology* 28: 3345-3363.
- Macauley JJ, Adams CD, and Mormile MR (2007) Diversity of tet resistance genes in tetracycline resistant bacteria isolated from a swine lagoon with low antibiotic impact. *Canadian Journal of Microbiology* 53: 1307-1315.
- Mala E, Oberoi A, and Alexander VS (2014) *Vibrio* isolates from cases of acute diarrhea and their antimicrobial susceptibility pattern in a tertiary care hospital. *International Journal of Basic and Applied Sciences* 3: 35-37.
- Malcolm TTH, Cheah YK, Radzi CWJWM, Kasim FA, Kantilal HK, John TYH, Martinez-Urtazaf J, Nakaguchig Y, Nishibuchig M, and Son R (2015) Detection and quantification of pathogenic *Vibrio parahaemolyticus* in shellfish by using multiplex PCR and loop-mediated isothermal amplification assay. *Food Control* 47: 664-671.
- Manjusha S, and Sarita GB (2011) Plasmid associated antibiotic resistance in *Vibrio* isolated from coastal waters of Kerala. *International Food Research Journal* 18: 1171-1181.
- New MB (2002) Farming freshwater prawns: a manual for the culture of the giant river prawn (*Macrobrachium rosenbergii*): Food and Agriculture Organization of the United Nations 148: 1-11.
- Okuda J, Ishibashi M, Hayakawa E, Nishino T, Takeda Y, Mukhopadhyay AK, Garg S, Bhattacharya SK, Nair GB, and Nishibuchi M (1997) Emergence of a unique O3:K6 clone of *Vibrio parahaemolyticus* in Calcutta, India and isolation of strains from the same clonal group from Southeast Asian travelers arriving in Japan. *Journal of Clinical Microbiology* 35: 3150-3155.
- Panicker G, Myers ML, and Bej AK (2004) Rapid detection of *Vibrio vulnificus* in shell fish and Gulf of Mexico water by real-time PCR. *Applied and Environmental Microbiology* 70: 498-507.
- Paydar M, Teh CSJ, and Thong KL (2013) Prevalence and characterization of potentially virulent *Vibrio parahaemolyticus* in seafood in Malaysia using conventional methods, PCR and REP-PCR. *Food Control* 32: 13-18.
- Rahimi E, Khamesipour F, Yazdi F, Momtaz H (2012) Isolation and characterization of enterohaemorrhagic *Escherichia coli* O157:H7 and EHEC O157:NM from raw bovine, camel, water buffalo, caprine and ovine milk in Iran. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi* 18: 559-64.
- Raissy M, Khamesipour F, Rahimi E, and Khodadoostan A (2014) Occurrence of *Vibrio*, *Aeromonas hydrophila*, *E. coli* and *Campylobacter* in crayfish (*Astacus leptodactylus*) from Iran. *Iranian Journal of Fisheries Sciences* 13(4): 944- 954.
- Raissy M, Moumeni M, Ansari M, and Rahimi E (2012) Antibiotic resistance pattern of some *Vibrio* strains isolated from seafood. *Iranian Journal of Fisheries Sciences* 11(3): 618-626.
- Ramesh A, Loganathan B, Venkateswaran K (1990) Ecological dynamics of marine luminous bacteria. *Journal of Basic Microbiology* 30(9): 689-703.
- Rico A, Satapornvanit K, Haque MM, Min J, Nguyen PT, Telfer T, and van den Brink PJ (2012) Use of chemicals and biological products in Asian aquaculture and their potential environmental risks: a critical review. *Reviews in Aquaculture* 4: 75-93.
- 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: 383-387.
- Rosser SJ, and Young HK (1999) Identification and characterization of class I integrons in bacteria from an aquatic environment. *Journal of Antimicrobial Chemotherapy* 44: 11-18.
- Ruangpan L, Kitao T (1991) *Vibrio* bacteria isolated from black tiger shrimp, *Penaeus monodon* Fabricius. *Journal of Fish Diseases* 14(3): 383-388.
- Serrano PH (2005) Responsible use of antibiotics in aquaculture. In: Food and agriculture organization (FAO). Fisheries Technical Paper 469, Roma, 97.
- Shaw KS, Rosenberg GRE, He X, Jacobs JM, Crump BC, and Sapkota AR (2014) Antimicrobial susceptibility of *Vibrio vulnificus* and *Vibrio parahaemolyticus* recovered from recreational and commercial areas of Chesapeake Bay and Maryland coastal bay. *PLoS ONE* 9: e89616.
- Snoussi M, Chaieb K, Rouabhia M, and Bakhrouf A (2006) Quantitative study, identification and antibiotics sensitivity of some *Vibrio* naceae associated to a marine fish hatchery. *Annals of Microbiology* 56: 289-293.
- Snoussi M, Hajlaoui H, Noumi E, Zanetti S, and Bakhrouf A (2008) Phenotypic and molecular characterization of *Vibrio alginolyticus* strains recovered from juveniles and older *Sparus aurata* reared in a Tunisian marine farm. *Annals of Microbiology* 58: 141-146.
- Snoussi M, Noumi E, Lajnef R, Belila A, Yazidi N, and Bakhrouf A (2011) Phenotypic characterization and enterobacterial repetitive intergenic consensus polymerase chain reaction (eric-pcr) of *Aeromonas* spp. and *Vibrio* spp. strains isolated from *Sparus aurata* fish farm (Khenis, Tunisia). *African Journal of Microbiology Research* 5: 2920-2928.
- Solati SM, Tajbakhsh E., Khamesipour F, Gugnani HC (2015) Prevalence of virulence genes of biofilm producing strains of *Staphylococcus epidermidis* isolated from clinical samples in Iran. *AMB Express* 5, 47.
- Srinivasan V, Nam HM, Nguyen LT, Tamilselvam B, Murinda SE, and Oliver SP (2005) Prevalence of antimicrobial resistance genes in *Listeria monocytogenes* isolated from dairy farms. *Foodborne Pathogens and Disease* 2: 201-211.
- Sudha S, Mridula C, Silvester R, and Hatha AAM (2014) Prevalence and antibiotic resistance of pathogenic *Vibrios* in shellfishes from Cochin market. *Indian Journal of Geo-Marine Sciences* 43: 815-824.
- Tajbakhsh E, Tajbakhsh S, Khamesipour F (2015) Isolation and molecular detection of Gram negative bacteria causing urinary tract infection in patients referred to Shahrekord hospitals, Iran. *Iranian Red Crescent Medical Journal* 17(5): e24779.
- Taneja N, Samanta P, Mishra A, and Sharma M (2010) Emergence of tetracycline resistance in *Vibrio cholerae* O1 biotype El Tor serotype Ogawa from north India. *Indian Journal of Pathology*

- and Microbiology 53: 865-866.
- Tang HJ, Chang MC, Ko WC, Huang KY, Lee CL, and Chuang YC (2002) In vitro and in vivo activities of newer fluoroquinolones against *Vibrio vulnificus*. *Antimicrobial Agents and Chemotherapy* 46: 3580-3584.
- Tendencia E, and de la Pena L (2001) Antibiotic resistance of bacteria from shrimp ponds. *Aquaculture* 195: 193-204.
- Thompson FL, Iida T, and Swings J (2004) Biodiversity of vibrios. *Microbiology and Molecular Biology Reviews* 68(3): 403-431.
- Tonguthai K (1995) Diseases of the freshwater prawn, *Macrobrachium rosenbergii*. *The Aquatic Animal Health Research Institute* 4: 1-4.
- Ture M, and Boran H (2015) Phenotypic and genotypic antimicrobial resistance of *Lactococcus* sp. strains isolated from rainbow trout (*Oncorhynchus mykiss*). *Bulletin of the Veterinary Institute in Pulawy* 59: 37-42.
- Vaseeharan B, Ramasamy P, Murugan T, and Chen JC (2005) In vitro susceptibility of antibiotics against *Vibrio* spp. And *Aeromonas* spp. isolated from *Penaeus monodon* hatcheries and ponds. *International Journal of Antimicrobial Agents* 26: 285-291.
- Xu B, Xu H, Ji W, and Shi J (1994) Pathogens and pathogenicity to *Penaeus orientalis* Kishinouye. *Acta Oceanologica Sinica* 13(2): 297-304.
- Yamamoto A, Iwahori J, Vuddhakul V, Charernjiratrak W, Vose D, Osaka K, Shigematsu M, Toyofuku H, Yamamoto S, Nishibuchi M, and Kasuga F (2008) Quantitative modeling for risk assessment of *Vibrio parahaemolyticus* in bloody clams in southern Thailand. *International Journal of Food Microbiology* 124: 70-78.
- Yano Y, Hamano K, Satomi M, Tsutsui I, Ban M, and Aue-umneoy D (2014) Prevalence and antimicrobial susceptibility of *Vibrio* species related to food safety isolated from shrimp cultured in tin land ponds in Thailand. *Food Control* 38: 30-45.
- Zhang XX, Zhang T, and Fang HHP (2009) Antibiotic resistance genes in water environment. *Applied Microbiology and Biotechnology* 82: 397-414.
- Zhang Z, Xiao L, Lou Y, Jin M, Liao C, Malakar PK, Pana Y, AND Zhao Y (2014) Development of a multiplex real-time PCR method for simultaneous detection of *Vibrio parahaemolyticus*, *Listeria monocytogenes* and *Salmonella* spp. In raw shrimp. *Food Control* 51: 31-36.