Antibiotic resistance pattern of some Vibrio strains isolated from seafood

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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 Sulamethoxazole 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

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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 al., 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 Shafae, 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 Igbinos, 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 antibiotics, 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>
<thead>
<tr>
<th>Primer</th>
<th>Sequence(5'------ 3')</th>
<th>Target gene</th>
<th>Amplicon size</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ermB-F</td>
<td>AGACACCTCGTCTAACCTTCGCTC</td>
<td>ermB</td>
<td>640</td>
<td>Sutcliffe et al., 1996</td>
</tr>
<tr>
<td>ermB-R</td>
<td>TCCATGTACTACCATGCCACAGG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tetS-F</td>
<td>ATCAAGATATTTAAGGAC</td>
<td>tetS</td>
<td>590</td>
<td>Charpentier et al., 1993</td>
</tr>
<tr>
<td>tetS-R</td>
<td>TTCTCTATGTGTTAATC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SUL2-F</td>
<td>AGGGGCGCATGTGTGATCGAC</td>
<td>Sul2</td>
<td>271</td>
<td>Hochhut et al., 2001</td>
</tr>
<tr>
<td>SUL2-R</td>
<td>TGTGCGGATGAGTCAGCTCC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>strA-F</td>
<td>TTGATGTGTTGTTCCCGCAATGC</td>
<td>strA</td>
<td>267</td>
<td>Hochhut et al., 2001</td>
</tr>
<tr>
<td>strA-R</td>
<td>CCAATCGCAGATAGAAGGCAA</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2: Phenotypic and genotypic characterization of *Vibrio* strains and their antibiotics resistance genes

<table>
<thead>
<tr>
<th>Name of species</th>
<th>Antibiotic resistance pattern</th>
<th>Strain(s) showing presence of gene encoding strA</th>
<th>tetS</th>
<th>ermB</th>
<th>sul2</th>
</tr>
</thead>
</table>
| *Vibrio vulnificus* | 6 + - - - | vibrio vulnificus 1 + + + + | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnificus 5 - - - - | vibrio vulnifica...
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).

The results revealed that multi-drug resistant Vibrio spp. present in seafood, obtain antibiotic resistance via plasmids 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


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

مهدی رئیسی ۱، منوچهر مومی ۲، مهسا انصاری ۳ و ابراهیم رحیمی ۱

چکیده
مطالعه حاضر با هدف بررسی مقاومت ضد میکروبی و حضور زندهای مقاومت ضد میکروبی در گونه های ویبریو جدا شده از V. vulnificus , V. parahaemolyticus , V. mimicus و V. cholerae , V. harveyi , V. alginolyticus فراورده های دریایی انجام شد. تعداد ۷۳ جدایی ویبریو از ۶ گونه شامل مورد بررسی قرار گرفتند. نتایج نشان می دهد که همه جدایی ها مقاومت ضد میکروبی چندگانه دارند. از ۷۳ جدایی بررسی شده، ۷۰ مورد به آمی سیلین (۹۴/۷%)، ۶۰ مورد به جناتامایسین (۸۳/۳%) و ۵۴ مورد به پنی سیلین (۷۸/۴%) مقاوم بودند. هشت سویه به چهار آنتی بیوتیک، ۱۹ سویه به ۵ آنتی بیوتیک، ۱۰ سویه به ۶ آنتی بیوتیک، ۴۴ سویه به ۷ آنتی بیوتیک و یک سویه به ۸ آنتی بیوتیک مقاوم بودند. نتایج همگانی نشان می دهد که در ۶۹ جدایی کد ۷۵/۷ درصد از موارد بررسی شده (دایر ۱) زندهای مقاومت ضد میکروبی است. در سایر جدایی های دیگر، مقاومت به استریتامایسین، تتراسیکلین و اریترامایسین به ترتیب در ۱۸، ۶ و ۵ جدایی یافت شدند و زن مقاومت به سولفامیتکاژول (Sul ۲) در این مطالعه یافته نشد. بافت زنده مقاوم در سویه های ویبریو جدا شده از فراورده های دریایی به عنوان یک حکایت بالقوه برای مصرف کننده تلفی می شود و بیان کننده این اصل که زندهای مقاومت ممکن است به شکل بیشتری در طیعه گسترش یابند که درتیمده خطرات جدی برای سلامت انسان می باشد.

واژگان کلیدی: ویبریو، زندهای مقاومت ضد میکروبی، فراورده های دریایی، خلیج فارس

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