



## Phenotypic and Molecular Detection of Pathogenic *Vibrio* Species in Two Different Regions of the Caspian Sea in Mazandaran, Iran

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### ABSTRACT

**Background:** *Vibrio* species including *V. cholera*, *V. mimicus*, *V. parahaemolyticus*, and *V. vulnificus* may cause gastrointestinal diseases after seafood consumption, and wound infections in swimmers and fishermen after exposing to seawater. This study determined the prevalence of the four *Vibrio* species in Tonkabon and Ramsar recreational beach water (approximately 200 meter far from the place where the river reaches the sea) and estuaria (place where the river reaches the sea) in northern part of Iran from autumn 1391 through autumn 1392.

**Methods:** Three hundreds water samples were collected for the detection of *Vibrio* species, using biochemical identification.

**Results:** Genomic DNA extracted from isolates and 16Sr DNA PCR confirmed the successful isolation of 9 *Vibrio* species in recreational beach water region.

**Conclusion:** Out of 300 samples, nine positive samples include one *V. cholerae* and eight *V. parahaemolyticus* were found at recreational beach (approximately 200 meter far from the place where the river reaches the sea).

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## Introduction

*Vibrio* related infections are divided into two groups: cholerae infections and non-cholerae infections. It has been shown that surfer and children who at beaches are at risk for exposure to vibrio infections (1, 2) where in the summer the water temperature reaches to more than 20 °C, resulting in increase in the frequency of gastroenteritis. *Vibrio* consists of different species. Some of these species are pathogenic and usually transmitted by the oral-fecal route, eating seafood or exposure of the superficial wounds to contaminated seawater (swimmers and fishermen) (3). Some of these pathogenic species are *V. cholerae*, *V. mimicus*, *V. vulnificus*, *V. parahaemolyticus*. Some studies have been reported that *V. cholerae* non O1/O139 was cultured from the patient's wound and the implicated recreational water. Under low temperatures this bacteria changes to the viable but nonculturable (VBNC) form by modifying genes expression (4, 5). In addition, a study that was performed in south coast of Sweden has reported that some fatal wound infections were due to the patients who were exposed to *V. cholerae* seawater (6). A case study was reported a patient with intracerebral abscess after a blood-born infection with non-toxigenic *V. cholerae* (non-O1) after swimming in Danish seawater in an unusually hot summer. This study reported that marine vibrio species may produce an intracranial infection in predisposed individuals (7). *V. vulnificus* may cause wound infections that usually occur after exposure of wounds to seawater. There are several reports of individuals with hemochromatosis and *V. vulnificus* primary septicemia (8). A 58-year-old caucasian man developed an infection after injuring the forearm exposed to contaminated seawater in the Gulf of Mexico. In a later stage, at the age of 66, he was diagnosed hemochromatosis (9).

Additionally, *V. vulnificus*, an opportunistic human pathogen has been shown to cause gastroenteritis, severe necrotizing soft-tissue infections and primary septicemia, with a high

lethality rate (10). Several studies have shown that nontoxigenic strains may induce acute gastroenteritis (11, 12). Other studies have demonstrated that vibrio strains, independent of species and origin were harmful to eukaryotic cells. Infection occurred in high-risk patients after consumption of raw oysters or via traumatic injury in marine environments. *V. parahaemolyticus* should be considered a potential etiology for necrotizing fasciitis, especially in risk patients (13). In the present study we investigated the presence of pathogenic vibrios in seawater where fish breeders prefer to use sea instead of construction of fish pools.

## Material and method

### *Sample collection and Vibrio enrichment and isolation*

Seawater samples were collected in sterile 100 ml containers. Sampling is performed on a depth of 30 cm in the afternoon. Up to 300 samples were collected from two different sea regions in Tonkabon and Ramsar, Northern Iran, during four seasons. During sampling pH and water temperature were measured. Subsequently, the samples were promptly transferred to laboratory under cooled conditions. Samples were centrifuged (5 min, at 3000 rpm). After centrifugation 2 mL pellet was mixed with 8 mL peptone water (pH=8.6) for enrichment and incubated at 37 °C for 6 to 8 h. Several loops of enriched water were transferred onto TCBS agar (Thiosulfate Citrate Bile Salts Sucrose agar) and incubated for 24 to 48 h at 37 °C. Colonies were sub-cultured onto TSA (Trypticase soy agar) in order to save bacteria for 3 month at room temperature.

### *Bacterial identification*

Identification of the isolates was done using the colony colors, where *V. cholerae* colonies were yellow and the other vibrios (*V. mimicus*, *V. parahaemolyticus*, *V. vulnificus*) were green. In

addition, Gram stain was done and then isolates were transferred to KIA and SIM. Biochemical tests were done including oxidase, SC (Simmons Citrate Agar, argentine dehydrolase, Lysine and ornithine decarboxylase, VP, NaCl (0%, 3%, 6%, 8%, 0%). The isolates were confirmed using 16S rDNA PCR (15).

### Molecular identification

Genomic bacterial DNA extraction was performed and 16 SrDNA universal PCR was performed for each sample (140 ng DNA was mixed by 20 pMol universal primers forward (5'-AGAGTTTGATCCTGGCTCAG-3') and reverse (5'-ACGGCTACCTTGTTACGACTT-3'), 2 m MdNTP Mix, 2 mM MgCl<sub>2</sub> and enzyme buffer 1X, 2 Unit Taq polymerase in 50 µl total volume. Initial denaturation Step were 95 °C for 10 min, followed by 30 cycles of 95 °C for 30 sec, 60 °C for 30 sec, and 72 °C for 45 sec, and finally 72 °C for 10 min. After PCR, 5 µl PCR product was mixed with 1 µl Loading buffer in 1% agarose gel electrophoresis and the electrophoresis was done for 20 min at 100 V PCR products of 1500 bp were send for sequencing.

### Result

A total of 300 samples were collected during one year (2012-2013). Samples were taken from two different regions: River estuarium (the place where river reaches the sea), and recreational beach (approximately 300 meter far from the first region) (Table 1).

The highest isolation rate of pathogenic vibrio was observed in summer, with a total number of 9 positive samples from 75 samples collected (12%). The temperature of seawater ranged from 8-28°C and the pH of seawater ranged from 7 to 7.5.

### Incidence and frequency of pathogenic vibrio in the seawater

The highest incidence of pathogenic vibrio such as *V. cholera* and *V. parahaemolyticus* were detected at recreational beach. One sample with *V. cholera* and eight samples with *V. parahaemolyticus* were detected.

### Discussion

In the present study we demonstrated the presence of pathogenic vibrio (*V. cholerae*, *V. parahaemolyticus*, *V. mimicus*, *V. vulnificus*) in 3% of the seawater samples analyzed. This study was the first project that investigated distribution of these pathogens in Tonkabon and Ramsar on south-western coast of the Caspian Sea. Eyisi have reported the presence of vibrio in water samples collected from Nigeria (14). These pathogens have also been isolated in German North Sea and Gothenburg in Sweden in 2013 (15, 16). In the present study of 300 seawater samples over one year, 8 *V. parahaemolyticus* and 1 *V. cholerae* strains were isolated in summer. It has been speculated that seasonal variation of vibrio may also reflect by distribution observed in this study. Temperature variation is the most important determinant of vibrio occurrence (Ошибка! Закладка не определена.) along with other determinants such as oxygen concentration, plankton, salinity, and the region that samples were collected. Low prevalence of vibrio in south-western coast of the Caspian Sea is related to low salinity with an average of 10.5 ppt in salinity. The distribution of vibrio in marine environments is related to water temperature .Furthermore, during sampling for this study the temperature of seawater was low and the weather was cloudy and rainy. Ecological study of vibrio in a coastal Mediterranean environment (La Spezia Gulf, Italy) found that these organisms were at least one order of magnitude higher in sediment than in seawater (17). Isolation of vibrio was high in

August because higher evaporation causes high salinity.

### Conclusion

In conclusion, this study provided new information about vibrio occurrence in two different regions of south-western of the Caspian Sea and suggested the need for study the occurrence of these organism in fishes of Caspian Sea.

### Conflict of interest

None declared conflicts of interest.

### Financial disclosure

There is no financial disclosure.

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