Occurrence of Pathogenic Vibrios in Coastal Areas of Golestan Province in Iran

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Summary

This study was carried out to investigate the occurrence of potentially pathogenic species of vibrio in sea water and estuarine environments of the Caspian Sea in the Golestan province of Iran. A total of 127 water samples from coastal waters as well as from river and estuaries were collected and analyzed by culture, during April and September 2001. Following prompt centrifugation, the resuspended sediments were initially enriched in alkaline peptone water and subsequently plated onto selective media including thiosulphate-citrate-bile salts-sucrose. Presumptive vibrio colonies were isolated and identified to species level using a gallery of biochemical and differential tests. In this study, *Vibrio vulnificus* was the predominant species isolated from sea waters (41%), followed by *Vibrio parahaemolyticus* (33%), *Vibrio alginolyticus* (15%), *Vibrio fischeri* (5%), *Vibrio natriegens* (4%), *Vibrio damsela* (0.7%), and *Vibrio harveyi* (0.7%). Non-01 *Vibrio cholerae* was the most abundant vibrio species recovered from fresh surface waters (80% of the total isolates). Five *Vibrio Cholerae* 01 species isolated, all belonging to Ogawa serogroups, were also recovered from the estuaries. The results of this study demonstrated the presence of pathogenic vibrio species in Caspian sea and coastal waters. The potential sanitary risk associated with the presence of these bacteria in the aquatic environment emphasizes the necessity of long-term monitoring programs.

***Key words*:** *Vibrio cholerae*, Water contamination, Caspian sea, Golestan province

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Introduction

Vibrios are Gram-negative bacteria indigenous to marine and estuarine waters. Vibrio species are widely recognized for their role in lumen intestinal infection, causing an extra intestinal infection from simple wound infection to lethal septicemia. *Vibrio cholerae* is a well-known human pathogen causing cholera epidemics worldwide. In addition to *Vibrio cholerae*, many other vibrio species have been recognized as significant human pathogens and implicated in food-borne diseases. *Vibrio parahaemolyticus*, *Vibrio vulnificus* and non-01/non-0139 *Vibrio cholerae* have been isolated from humans suffering non-cholera vibrio infections (vibriosis), usually associated with the consumption of raw or under-cooked seafoods, intake of contaminated natural waters, or exposure of skin wounds to sea water. Depending on the species involved, clinical manifestations include asymptomatic infection, mild to severe gastroenteritis, septicaemia and wound and/or soft tissue infections (Oliver & Kaper 1977). Many seafood-associated disease outbreaks have been reported worldwide (Wong *et al* 1993, Hoi *et al* 1998, JNIID 1999, Morris 1999, Daniels *et al* 2000) and vibrios are considered to be the major bacterial cause of identifiable illness and death from shellfish consumption (Whittman & Flick 1996). Cases of vibriosis have recently been reported in Denmark (Hoi *et al* 1998), Italy (Ripabelli *et al* 1999), and France (Geneste *et al* 2000) but no major outbreaks have recently been recorded. Although the presence and isolation of pathogenic vibrio species in water and shellfish samples from natural environments have been well documented for many European countries (Ghinsberg *et al* 1995, Dasgaard *et al* 1996, Arias *et al* 1998, Ghinsberg *et al* 1999, Barbieri *et al* 1999, Baffone *et al* 2000), there is no comprehensive report on the presence of vibrio species in estuaries and coastal areas of Iran. The aim of this study was to determine the incidence of different vibrio species in water samples collected from sea and coastal areas of Caspian Sea in Golestan province of Iran.

Materials and Methods

Water samples were collected from different locations of Caspian Sea in Golestan province during the spring and summer 2001. A total of 73 sea water samples were obtained in sterile containers from a depth of 30 cm and in an area approximately 500 meters beyond the sea shores. Fourteen samples were collected in around Bandar Gaz area, 28 in Bandar Turkeman area, and 31 in Gomishan region.

Fifty four water samples were also obtained from estuaries, streams, and rivers during the summer months. These included 12 samples from different distances in the main Gorgan river, 10 samples from Gorgan river tributaries, 24 samples from the Golestan dam lake, and 8 samples from the small streams running through Gonbad Kavoos region. Following prompt transfer of the water samples to laboratory, they were centrifuged (1,500 x g for 5 minutes). The supernatants were discarded except the bottom 1-2 ml which was used to resuspend the remaining sediment. The resuspended sediments were transferred into alkaline peptone water containing 1% NaCl and onto TCBS agar plates. Following incubation at 37ºC for 4-6 hours in alkaline peptone water, the enriched cultures were plated onto TCBS agar. The plates were placed at 37ºC for 24-48 hours, and any suspected vibrio colonies were isolated. For species identification, a gallery of biochemical and differential tests which included oxidase, Voges-Proskauer, citrate, indole, string, CAMP, swarming, and ONPG reactions was performed. Arginine dihydrolase, Lysine and Ornithine decarboxylase, aesculin hydrolysis, KIA reaction, colony color, growth capability at 4ºC and 40ºC, as well as at five different salt concentrations (0%, 1%, 3%, 6%, 8%, and 10% NaCl) were also used as aid in species differentiation. Standard vibrio species (ATCC), obtained from the microbiology department of the National Reference Laboratory (Bou Ali Hospital, Tehran, Iran) were used for quality control of the biochemical tests.

Results

From a total of 73 sea water samples, 100 vibrio species were recovered. These included 39 *Vibrio vulnificus*, 32 *Vibrio parahaemolyticus*, 17 *Vibrio alginolyticus*, 5 *Vibrio fischeri*, 5 *Vibrio natriegens*, 1 *Vibrio damsela*, and 1 *Vibrio harveyi* isolated (Figure 1). *Vibrio vulnificus* was the most encountered species, consisting of 53%, 26%, and 45% of all vibrios isolated in the Bandar Gaz, Bander Turkeman, and Gomishan coastal waters, respectively. *Vibrio fischeri*, *Vibrio harveyi*, and *Vibrio natriegens* were not detected in Bander Turkeman coastal waters, and were most frequently isolated from Gomishan area coastal waters. *Vibrio mimicus*, *Vibrio Cholerae*-01, and non-01 *Vibrio cholerae* were not isolated from the sea water samples.

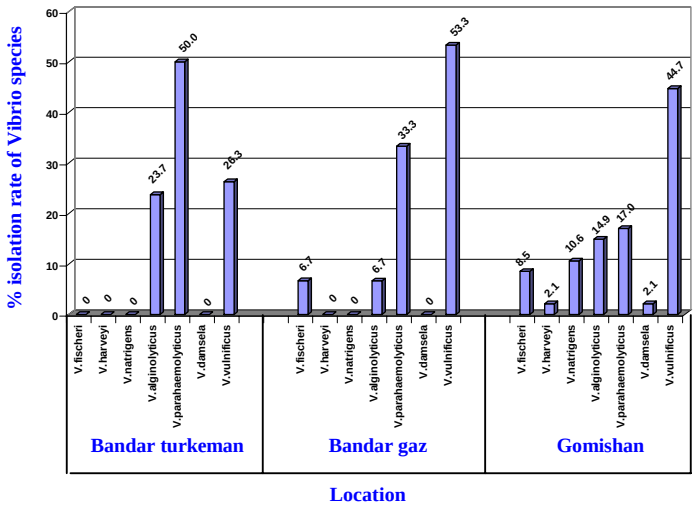

Figure 1. *The total Vibrio species isolated from Caspian Sea waters of Golestan province.*

Forty two vibrio species were also isolated from the total of 54 water samples obtained from surface areas. These included 33 non-01 *Vibrio cholerae*, 3 *Vibrio Cholerae*-01, and 6 *Vibrio mimicus* (Figure 2).

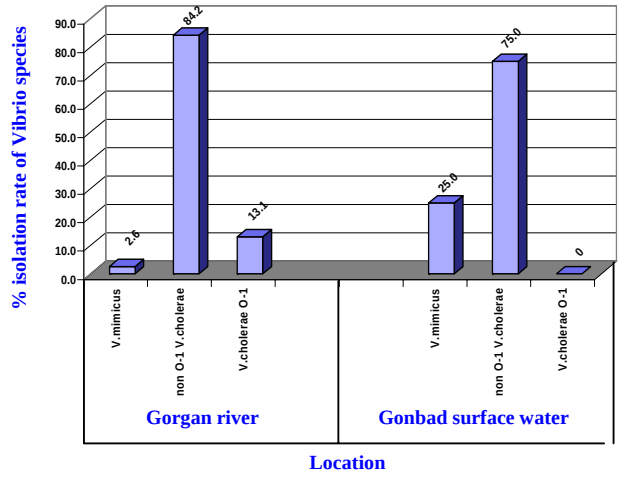

Figure 2. *The total Vibrio species isolated from surface waters of Golestan province.*

The comparatively lower extent of vibrio isolation from these areas is mainly due to the halophilic nature of most vibrio species. Only *Vibrio mimicus*, *Vibrio Cholerae*-01, and non-01 *Vibrio Cholerae* are known to be non-halophilic and thus tend to reside mostly in surface waters of low saltiness. This may be the reason why most water samples from these areas did not yield any detectable growth on TCBS media. Agglutination tests conducted on the *Vibrio Cholerae* isolates using Inaba and Ogawa antisera indicated that all the five strains belonged to Ogawa serogroups. Beside collecting water samples from various locations in the Gorgan river, we also collected samples from the only other source of fresh water in the province, namely the Gonbad surface waters which include the numerous small streams flowing through the Gonbad Kavoos plains. Non-01 *Vibrio Cholerae* was the most abundant vibrio species recovered from these fresh waters, comprising 84.2% and 75.0% of the isolated vibrios in the Gorgan river and Gonbad waters, respectively.

Discussion

The major objective of this study was to determine the incidence of potentially pathogenic vibrio species in water samples collected from Caspian Sea and coastal

estuarine areas that vary in salinity. Furthermore, analyses were performed during the summer months because of the greater number and diversity of vibrio species expected to occur during the warmer season.

Vibrio Vulnificus was the most prevalent species in the Caspian Sea waters. This pathogenic vibrio causes a variety of human infections and was shown to induce a high mortality rate (Murray *et al* 1999, Penland *et al* 2000). This is in sharp contrast to the results of similar investigations conducted on Atlantic waters off Europe and America (Matte *et al* 1994, Sunen *et al* 1995, Barbieri *et al* 1999, Ripabelli *et al* 1999) where *Vibrio Alginolyticus* was found to be the dominant resident vibrio species. This may be due to the lower salinity levels of the Caspian Sea water relative to open oceanic waters. Previous studies indicated that a salinity greater than 25 parts per thousand (p.p.t.) may have an adverse effect on *Vibrio Vulnificus* (Kaspar & Tamplin 1993, Motes & Depaola 1996). Investigators who have isolated this bacterium from marine waters have indicated that the organism has tropism for slightly lower water temperature (Oliver *et al* 1981, Oliver *et al* 1983, Amaro *et al* 1995, Hoi *et al* 1998, Hoi *et al* 1998). The higher isolation rate of *Vibrio vulnificus* in Bander Gaz may be due to the fact that coastal water temperature in this region is generally lower than in the other areas. The average water temperature measured during the sampling periods in Bander Gaz was 26ºC; whereas, in the other two regions was 30ºC. The lower water temperature in Bander Gaz area is mainly due to the inflow of numerous small streams originating from snow melts in the nearby Alborz mountain peaks.

Vibrio parahaemolyticus was the second most prevalent species in all sea water samples except in Bander Turkeman coastal waters in which it comprised the most frequently isolated vibrio species. There is no incoming fresh river water pouring into the sea in the vicinity of Bander Turkeman area which is responsible for the relatively higher water saltiness 11–13 p.p.t. in this region. Higher saltiness of Bander Turkeman coastal waters during the summer months are probably the main

reason for the elevated degree of the highly halophilic *Vibrio parahaemolyticus* isolation rate in this region relative to the other coastal areas. In contrast, *Vibrio fischeri*, *Vibrio harveyi*, and *Vibrio natriegens* are much less halophilic and can not survive in waters with excess of 6% NaCl concentrations, were not isolated from Bander Turkeman coastal waters. These three vibrio species were most frequently encountered in Gomishan coastal waters. This is due to the fact that the mighty Gorgan river pours into the sea in Gomishan region, resulting in the lesser saltiness of the sea water in this area. Other investigators have shown that water feacal coliform contamination have an adverse effect on *V. parahaemolyticus* and thus this organism tend to reside in salty and clean waters (Daniels *et al* 2000, Vanddhakul *et al* 2000). The higher isolation rate of this vibrio in Bander Turkeman may not be solely due to the relatively higher saltiness, but also contributed to the lower coliform content of the coastal water in this area. In the other two coastal regions where Gorgan and Gharasoo rivers pours into the sea; the high sewage contents of these two rivers result in the greater feacal coliform contamination of the coastal waters. Gorgan river is notorious for its high feacal contamination rate. In the course of its long journey passing through many towns and villages, the river takes in large quantities of raw human, animal, and industrial sewage, resulting in elevated feacal coliform content when it finally pours into the Caspian in the Gomishan region. This may partly be the reason for the lowest *V. parahaemolyticus* isolation rate (17%) in Gomishan coastal waters. *Vibrio alginolyticus* is also halophilic in nature and can tolerate up to 11 p. p. t. of saltiness. It is pathogenic for human and causes a variety of extra-intestinal diseases such as soft-tissue Infections, otitis and conjunctivitis (Matte *et al* 1994, Sunen *et al* 1995, Konemen *et al* 1997, Barbieri *et al* 1999, Mukherji *et al* 2000, Penland *et al* 2000). The bacterium was mostly isolated in the Bander Turkeman coastal waters and to a lesser extent in the other two coastal areas. This is also a reflection of the higher saltiness of the waters in the Bander Turkeman area relative to those in the other regions. Bander Gaz coastal

area water has a relatively lower salt content due to the fresh water inflow from Gharasoo river and also numerous other streams originating from Alborz mountain range. The salinity content of Bander Gaz and Gomishan coastal waters are 6-8 p.p.t., whereas, those in the Bander Turkeman region is in the range of 11-13 p.p.t.. The mighty Gorgan river is the main water flow in Golestan province. It originates from the eastern Alborz mountain peaks and following a 200-250 kms journey which encompasses almost all major cities in the province, finally pours into the sea. Its water is the main source of fresh water for human consumption as well as for irrigation of vast agricultural fields.

Non-01 *Vibrio Cholerae* was the most frequently encountered vibrio species in the fresh waters of Gorgan river as well as in the Gonbad surface waters. Non-O1 *Vibrio Cholerae* has been associated with many invasive human infections and oyster-vectored deaths (Rippey 1994). Therefore, more work needs to be performed to further characterize these strains, e.g. for cytotoxic effect and virulence genes encoding thermostable toxin (Shiga-like toxin, *etc*.). *Vibrio Cholerae*-01 was the least abundant species. This highly pathogenic vibrio was recovered only from samples collected in the Gorgan river.

From this study, it is concluded that potentially pathogenic vibrios are indeed present in both the sea water and the surface following waters of the Caspian coast. The clinical significance of these isolates is their potential association with gastroenteritis and/or invasive septicemias. Many reports from Europe, the US, India, and Asia indicate that human infections occur wherever these potential pathogens have been isolated (Haldy 1997, Hoi *et al* 1998, Morris 1999, Yam *et al* 2000). Thus, consideration should be given to long-term monitoring programs for potential human pathogenic vibrio species in Caspian coastal areas.

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