Research Journal of Microbiology
ISSN 1816-4935
Occurrence of Potentially Human Pathogenic Vibrio Species in the Coastal Water of the Eastern Province of Saudi Arabia

Nasreldin Elhadi
Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, University of Dammam, Dammam, Saudi Arabia

ABSTRACT
Non-cholera Vibrio infections are an important public health problem worldwide. The aim of this study was to investigate the presence of potentially pathogenic non-cholera Vibrio species in coastal seawater of Eastern Province of Saudi Arabia. A total of 300 seawater samples were collected from five locations along coast environment and examined for the presence or absence of pathogenic Vibrio species during eleven months between February and December, 2010. In this study five potentially pathogenic Vibrio species were detected, with overall incidence in the samples being at 38% for V. alginolyticus was the predominant species, 13.3% for V. parahaemolyticus, 7.6% for V. vulnificus, 5.6% for V. cholerae non-O1non-O139 and 0.33% for V. mimicus. All isolates of V. cholerae non-O1non-O139 were negative for cholera toxin (ctx) gene and only three isolates were positive for toxin-coregulated pilus gene (tcpA) out of 27 isolates. From the results of this study, coastal water of Arabian Gulf is concluded to be a reservoir for Vibrio species in Eastern Province of Saudi Arabia. To the best of our knowledge, this is the first study done on determining potentially pathogenic Vibrio in the Saudi Arabia and Arabian Gulf region. As the study has found that pathogenic Vibrio species are present in the study area, it is recommended that the possibility of infection caused by non cholera Vibrio should be considered whenever diagnosis is done on any patient who had recently been exposed to sea water or that had manipulated or consumed seafood and presenting infected wounds, ear infection, conjunctivitis, gastroenteritis or septic shock.

Key words: Vibrio species, seawater, arabian gulf, eastern province of Saudi Arabia

INTRODUCTION
Ample studies have shown that Vibrio species are highly abundant in aquatic environments, including estuaries, marine coastal waters and sediments and aquaculture settings worldwide (Ortigosa et al., 1989; Barbieri et al., 1999; Heidelberg et al., 2002). Members of Vibrios are classified as Gram-negative, curved and rod-shaped bacteria. They are known to be ubiquitous bacteria; they naturally habituate of the estuarine and marine environments and are commonly present in or on shellfish and other seafood (Farmer and Hickman-Brenner, 1992). The genus Vibrio includes more than 35 currently recognized species, 12 of which are considered to be human pathogens. V. cholerae, V. parahaemolyticus and V. vulnificus are universally recognized as the major significant human pathogens. On the contrary, V. mimicus, V. hollisae, V. alginolyticus, V. fluvalis, V. furnissii, V. metschnikovii, V. carchariae, V. cincinnatiensis and V. damsela, considered to be responsible for cases for minor pathogenicity, are usually or frequently isolated from blood, wounds of arms and legs, infected eyes and ears (Farmer and Hickman-Brenner, 1992). The linkages between the estuarine environment and human disease are common among Vibrios, with each species having distinctive features (Janda et al., 1988).
Pathogenic *Vibrio* species are able to cause human diseases and threat public health as the causative agents of both sporadic and epidemic human infections. *V. parahaemolyticus* is a leading cause of gastro-enteritis and usually associated with the consumption of raw or undercooked shellfish and seafood. *V. cholerae*, continues to spread globally in a seventh pandemic (O1 El Tor biotype) and the emergence of a non-O1 serogroup (O139 Bengal) has led to a new pandemic (Faruque and Mekalanos, 2003; Sack et al., 2004). There are more than 150 O serotypes among *V. cholerae* strains. But only the strains belonging to O1 and O139 serotypes are considered virulent because they usually carry the cholera toxin gene (*ctx*), the toxin-coregulated pilus gene (*tcp*) and other virulence genes. *V. cholerae* non-O1/non-O139 may cause sporadic episodes and occasional outbreaks of diarrheal disease and could lead to extra-intestinal diseases, such as bacteremia, invasive soft tissue infections cholecystitis and peritonitis (Hughes et al., 1978; Morris et al., 1981; Morris, 1994; West et al., 1998; Farmachidi et al., 2008).

*V. vulnificus* distributed in the marine environment can infect the humans either by consumption of contaminated seafood or by invasion through a skin lesion, which can cause sepsis and serious wound infections during the consumption of raw or undercooked shellfish and seafood or exposure of skin wounds to seawater (Morris, 2003). *V. vulnificus* can cause fatal infection in the individuals with underlying diseases (diabetes, liver diseases, etc.) or the individuals treated with immunosuppressive agents. Opportunistic infections with *V. vulnificus* can cause severe wound infections (Oliver, 2005) and fulminant septicaemia, with highly virulent strains causing extensive host-tissue damage and producing mortality rates of up to 60% (Linkous and Oliver, 1999). Other *Vibrio* species, including *V. alginolyticus*, *V. fluvialis*, *V. furnissii*, *V. mimicus*, *V. hollisae*, *V. metschnikovii* and *V. damselae* are halophilic *Vibrios* also sporadically involved in human diseases (Davis et al., 1981; Brenner et al., 1983; Abott and Janda, 1994; Carnahan et al., 1994; Farmer et al., 2003; Yamane et al., 2004).

The aim of this study was to determine the incidence of clinically important *Vibrio* species in sea water samples collected from coastal areas of the Arabian Gulf of Eastern Province of Saudi Arabia. A standardized protocol was developed for the isolation of *Vibrio* species from environmental samples following enrichment. The identification of *Vibrio* isolates was confirmed by using CHROMagar *Vibrio* (CV) agar, API 20E and serotyping of *Vibrio cholerae* non-O1/non-O139 isolates. All isolates of non-O1/non-O139 *V. cholerae* were screened using Polymerase Chain Reaction (PCR) analysis to examine the virulence genes *ctxA* and *tcpA*. Despite the importance of the emergence of *Vibrio* infections in the coastal areas of Arabian, little is known about the presence of these organisms in the marine environments of the region. Arabian Gulf coast is one of the most important recreational and fish and shrimp-producing areas in Gulf region. In the present study, presence or absence of potentially pathogenic *Vibrio* species were investigated from five locations of this temperate coast of Eastern Province of Saudi Arabia.

**MATERIALS AND METHODS**

**Study sites:** The Arabian Gulf is a shallow semi-enclosed marginal sea, with less than 100 m in depth over its entire extent and with a mean of only 35 m (Reynolds, 1993). It covers an area of about 240,000 km², with 1000 km in length and widths ranging from 185 to 370 km, with a mean of 240 km. The shallowness of the Arabian Gulf water leads to the formation of a very high saline and dense water, with maximum salinities being as high as 57 gram per litre along the southern coast due to the highly evaporation rate (John et al., 1990). The coastal environment of the Eastern Province of Saudi Arabia, including Abu Ali Island, is continuously subjected to oil pollution...
incidents as a result of damaged oil wells, oil pipeline leaks, or ballast water discharge from nearby loading terminals (Al-Thukair et al., 2007). This frequent oil pollution represents an additional stress factor to already harsh environmental conditions of high temperature, salinity, rapid rate of evaporation and desiccation (Al-Thukair et al., 2007).

Sample collection and preparation: A total of 300 water samples were collected from the Arabian Gulf coast at five different locations from February to December, 2010. A total of 90, 75, 60, 45 and 30 water samples were collected from Al Khobar, Half Moon, Al Qatif, Al Azziziah and Dammam beaches, respectively. All water samples were collected in sterile 500 mL glass bottles from a depth of approximately 10 to 15 cm and 10 to 15 m beyond the seashores. Water temperature was measured at all collection sites using temperature sensor and pH values ranged from 8.0 to 8.6. All water samples were transported to the Microbiology Laboratory (University of Dammam) in insulated cooler and examined immediately after their arrival. The transportation time arranged from 3 to 5 h. Inoculations into selective medium were made within 24 h after the collection of water samples. Number of water samples and incidence of pathogenic Vibrio species are summarized in Table 1.

Culture procedure: All samples were analyzed for potentially pathogenic Vibrio species. The 25 mL test water sample was mixed with 225 mL alkaline-peptone water enrichment broth (APW: 1% peptone, 1% NaCl, pH 8.6) to isolate V. cholerae or salt APW (1% peptone, 3% NaCl, pH 8.6) to isolate all pathogenic Vibrio species (Smith, 1970; Elhadi et al., 2004). All samples enriched in APW were incubated for six to eight hours to isolate V. cholerae and 18 to 24 h at 37°C to isolate other pathogenic Vibrio species. A sample from this enrichment culture was transferred with a loop to Thiosulphate citrate bile-salt sucrose agar (TCBS; Difco Laboratories, USA) and CHROMagar Vibrio (CV; Paris, France) and incubated at 37°C for 24 h. In this study the CHROMagar Vibrio medium was used as a selective medium for Vibrios and allowed good recovery for V. cholerae, V. parahaemolyticus and V. vulnificus (Fig. 1) compared with TCBS medium.

![Fig. 1(a-c): (a), Appearance of Vibrio cholerae and V. vulnificus green blue colonies, (b), V. parahaemolyticus mauve (violet) colonies; and (c), V. alginolyticus colourless colonies on CHROagar Vibrio (CV) medium](image-url)
Identification procedures for *Vibrios*: Five to seven colonies suspected to be *Vibrio* species per sample were picked up from the TCBS agar and CV plates and cultured on Tryptic Soy Agar (TSA) to purify the colonies. These colonies were first examined for oxidase reaction (Kovacs method) and the oxidase-positive isolates were also tested for string reaction (Smith, 1970). Organisms showing oxidase positive reactions were identified by using conventional biochemical methods described previously (Elhadi *et al.*, 2004). Biochemical tests analyses were carried out from pure isolated colonies grown on TSA for 24 h at 37°C. Salt tolerance was determined by growth of the isolates at 37°C in 1% peptone broth without NaCl or supplemented with either 1, 3, 6, 8 and 10% NaCl. Further identification of *Vibrio* spp. were carried by using API 20 E system (BioMerieux, France). Standard strains of *Vibrio* species, obtained from the Microbiology Laboratory of the King Fahad Hospital of the University (KFHU) were used for quality control of the biochemical tests.

Serotyping: Strains presumptive of *V. cholerae* were grown at 37°C on nutrient agar plates and serological confirmation of *V. cholerae* strains was performed by an agglutination test with polyvalent anti-O1, mono-specific Ogawa-Inaba antisera and with specific anti-O139 antisera obtained commercially (Denka Seiken Co. Ltd., Tokyo, Japan).

PCR analysis of virulence genes: Amplification of the target cholera toxin gene was carried out by PCR assay using bacterial cell lysate as the source of template DNA (Shirai *et al.*, 1991). In brief, cells from an 18 h LB culture were collected and resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8.0]), treated with 10% (w/vol) sodium dodecyl sulfate and freshly prepared proteinase K and incubated at 37°C for 1 h. After incubation, 10% cetyl trimethyl ammonium bromide in 0.7 M NaCl was added and incubated at 65°C for 10 min. The aqueous phase was treated with phenol-chloroform and the DNA pellet was washed with 70% ethanol. The extracted nucleic acid was suspended in TE and treated with RNase at 37°C for 30 min. Amplification was performed in a thermal cycler (Applied Biosystems, USA) using 200 μL PCR tubes with a reaction mixture volume of 25 μL. Each of the reaction mixtures contained 3 μL of template DNA (lysate), 2.5 μL of each primer (10 pmol μL⁻¹), 2.5 μL of 2.5 mM deoxynucleoside triphosphates, 0.3 μL (5 U μL⁻¹) of Taq DNA polymerase (promega, USA), 2.5 μL of 10× reaction buffer containing 20 mM MgCl₂ (Promega, USA) and 11.8 μL of sterilized deionized water. PCR for detecting the cholera toxin and tcpA gene primer are as follow: For ctcA, F-5'-TCAGACCGGATTTTTAGGCAGCAG-3' and ctcA, R-5'-TCTATCTCTG TAGCCC CT ATTACG-3' (Li *et al.*, 2003) and for tcpA, F- 5'-CACGATAAGAAAACC GGTCAAGAG-3' and R, 5' ACCAAATGCACGCGGAATG GACG-3'(R) (Mukhopadhyay *et al.*, 2001). The amplification program began with denaturation at 94°C for 5 min, followed by 35 cycles consisting of denaturation at 94°C for 1.5 min, annealing at 60°C for 1.5 min and extension at 72°C for 1.5 min and a final extension at 72°C for 7 min. A negative control (sterile distilled water) and a positive control (*V. cholerae* serogroup O1 strain from the King Fahad Hospital of the University) were run in each amplification. The PCR products were electrophoresed through a 1.5% agarose gel to resolve the amplified products, which were visualized under UV light after ethidium bromide staining.

RESULTS
Incidence of potentially pathogenic *Vibrio* species: The presence and abundance of *Vibrio* species were detected at all five sites throughout the entire period of the study. In this study
Table 1: Incidence of potentially pathogenic *Vibrio* species in water samples obtained from different locations

<table>
<thead>
<tr>
<th>Location</th>
<th>No. of samples examined</th>
<th>V. cholerae non-O1 (non-O139) (%)</th>
<th>V. parahaemolyticus (%)</th>
<th>V. vulnificus (%)</th>
<th>V. mimicus (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al Khobar</td>
<td>90</td>
<td>0</td>
<td>3 (3.3)</td>
<td>2 (2.2)</td>
<td>0</td>
</tr>
<tr>
<td>Al Azziziah</td>
<td>45</td>
<td>7 (15.6)</td>
<td>16 (35.6)</td>
<td>6 (13.3)</td>
<td>0</td>
</tr>
<tr>
<td>Half Moon</td>
<td>75</td>
<td>6 (8.0)</td>
<td>19 (25.3)</td>
<td>8 (10.6)</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td>Dammam</td>
<td>30</td>
<td>2 (6.6)</td>
<td>1 (3.3)</td>
<td>2 (6.6)</td>
<td>0</td>
</tr>
<tr>
<td>Al Qatif</td>
<td>60</td>
<td>2 (3.3)</td>
<td>1 (1.6)</td>
<td>5 (8.3)</td>
<td>0</td>
</tr>
<tr>
<td>Total number (% positive)</td>
<td>300</td>
<td>17 (5.6)</td>
<td>40 (13.3)</td>
<td>23 (7.6)</td>
<td>1 (0.33)</td>
</tr>
</tbody>
</table>

Table 2: Monthly incidence of potentially pathogenic *Vibrio* species in water samples collected from Arabian Gulf between February and December, 2010

<table>
<thead>
<tr>
<th>Month</th>
<th>Water temperature (°C)</th>
<th>V. cholerae non-O1 (non-O139)</th>
<th>V. parahaemolyticus</th>
<th>V. vulnificus</th>
<th>V. mimicus</th>
</tr>
</thead>
<tbody>
<tr>
<td>February</td>
<td>15</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>March</td>
<td>24</td>
<td>7</td>
<td>5</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>April</td>
<td>22</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>May</td>
<td>30</td>
<td>3</td>
<td>8</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>June</td>
<td>31</td>
<td>3</td>
<td>17</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>July</td>
<td>33</td>
<td>1</td>
<td>14</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>November</td>
<td>24</td>
<td>6</td>
<td>3</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>December</td>
<td>18</td>
<td>2</td>
<td>5</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Total No.</td>
<td>27</td>
<td>56</td>
<td>28</td>
<td>25</td>
<td>1</td>
</tr>
</tbody>
</table>

*Vibrio alginolyticus* was the predominant and the most frequently isolated species (114 of 300; 38%), followed by *V. parahaemolyticus* (40 of 300; 13.3%) as shown in Table 1. The highest number of *V. parahaemolyticus* were isolated from Half Moon and Al Azziziah beaches during the month of June and July from Half Moon and Al Azziziah (Fig. 2). Water temperature during the month of June and July when the water samples were collected was 31 and 33°C, respectively (Table 2 and Fig. 2).

**V. cholerae and serotyping:** All isolates of *V. cholerae* non-O1/O139 described here did not react with both of anti-O1 and anti-O139 antisera. *V. cholerae* Non-O1/non-O139 (17 of 300; 5.6%) were isolated from four locations and no *V. cholerae* were detected in water samples collected from Al Khobar beach. The highest numbers of *V. cholerae* were isolated during the month of March and November, when the seawater temperature during sampling collection was 24°C (Table 2 and Fig. 2).

**PCR analysis of *V. cholerae* virulence genes:** *V. cholerae* non-O1/non-O139 isolates were screened for the presence or absences of *ctxA* and *tcpA* genes using PCR and the results showed that the cholera toxin gene was absent in all the isolates. Out of the 27 isolates of *V. cholerae* non-O1/non-O139, only three isolates were positive for *tcpA* gene; these isolates were isolated from Al Azziziah (Table 1).
Fig 2: Monthly incidence of potentially pathogenic *Vibrio* species in water samples collected from Arabian Gulf between February and December, 2010. Y axis denotes the number of positive water samples to *Vibrio* species; X axis denotes the number of months positive for *Vibrio* species and the curve denotes the water temperature (°C) during water samples collection.

*Vibrio vulnificus*: *V. vulnificus* was detected from all water samples sites (23 of 300; 7.6%), yielding 28 isolates as shown in (Fig. 2). The highest numbers of *V. vulnificus* were isolated during the month of November (12 isolates) and December (9 isolates), when the seawater temperature during sampling collection was 24°C and 18°C, respectively (Fig. 2).

*Vibrio parahaemolyticus*: *V. parahaemolyticus* was detected from all water samples sites (40 of 300; 13.3%), yielding 58 isolates as shown in Table 1. The highest numbers of *V. parahaemolyticus* were isolated during the month of June (17 isolates) and July (14 isolates), when the seawater temperature during sampling collection was 31 and 33°C, respectively (Fig. 2).

*Vibrio mimicus*: In this study *V. mimicus* was isolated only in one water samples obtained from Half Moon beach (Table 1). It was isolated during the month of February, when the seawater temperature during sampling collection was 15°C as shown in Fig. 2.

**DISCUSSION**

The main objective of this study was to determine the incidence of potentially pathogenic *Vibrio* species in water samples collected from Arabian Gulf coastal areas that vary in water temperature and salinity. More seawater samples were analyzed during the summer months (May, June and July) because of the greater number and diversity of *Vibrio* species expected to occur during the warmer season (Huq et al., 1983; Baffone et al., 2000). *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* still remain serious human pathogens (Ortigosa et al., 1994; Centers for Disease Control and Prevention (CDC), 1999; Finkelstein et al., 2002). In the present study, the most frequently isolated *Vibrio* species from TCBS agar were *V. alginolyticus*, *V. parahaemolyticus* and *V. cholerae*. Few numbers of *V. vulnificus* were isolated using the TCBS medium since it has a very low plating efficiency for clinical and environmental strains of *V. vulnificus* (Hoi et al., 1998). In this study the CHROMagar *Vibrio* medium was found to be highly selectively for *V. vulnificus*, *V. cholerae* and *V. parahaemolyticus* (Fig. 1).
V. alginolyticus was the most prevalent species in the water samples collected from the five locations in this study. This species has also been recognized for many years as a pathogen of both humans and marine animals (Blacke et al., 1980). V. alginolyticus is commonly associated with ear infections (otitis media and otitis externa) and superficial wounds resulting from prior exposure to contaminated water sources (Pezzlo et al., 1979). The disease caused by this microorganism mainly affects individuals who are in direct contact with seawater or handle shellfish and its incidence significantly increases during warmer months (Morris and Black, 1985).

Vibrio vulnificus was recovered from all water samples collected from the five locations of the Arabian Gulf coast. (Table 1). The highest numbers of V. vulnificus isolates were isolated from water samples collected from Half Moon beach during the month of November, when the water during the samples collection was 24°C (Fig. 2). Diseases associated with V. vulnificus infection have been found to be present in two patterns (Harwood et al., 2004). In partner one, primary septicemia occurs in individuals with chronic liver disease shortly after eating raw oysters, with a mortality rate of over 50%. In pattern two, wound infections are incurred via exposure to seawater or handling seafood products, with a death rate of approximately 25% (Blake et al, 1979). V. vulnificus infections have been reported in the USA, Europe and Asia (Park et al., 1991; Chuang et al., 1992; Dalsgaard et al., 1996; Hlady and Klontz, 1995). V. vulnificus has been detected in coastal and estuarine environments throughout the world (Oliver, 1989); it has been found in areas with warm seawater temperatures (>20°C) (Morris, 1988; Oliver, 1989; Kelly, 1989). V. parahaemolyticus was present at all sites but it was most prevalent in water samples collected from Al Azziziah and Half Moon beaches as shown in Table 1. The highest numbers of V. parahaemolyticus were isolated during the month of May, June and July, when the seawater temperature during samples collection was 30, 31 and 33°C, respectively yielding 39 isolates. The presence of the V. parahaemolyticus has traditionally been confined to warm and temperate geographical areas (DePaola et al., 2000). The emergence of infections caused by V. parahaemolyticus in Europe and America has revealed the presence of the organism in regions where it had never previously been reported (Gonzalez-Escalona et al., 2005; Martinez-Urtaza et al., 2005; McLaughlin et al., 2005; Fuenzalida et al., 2006). The progressive spread of V. parahaemolyticus and its colonization of new areas have been related to an unusual increase in seawater temperatures in coastal zones (Gonzalez-Escalona et al., 2005; Martinez-Urtaza et al., 2005; McLaughlin et al., 2005; Cabello et al., 2007).

In this study, V. cholerae non-O1/non-O139 were detected from all sampling sites except Al Khobar site as shown in (Table 1). The highest numbers of V. cholerae were recovered from Al Azziziah location during the month of March, when the water temperature during samples collection was 21°C as shown in Fig. 2. Three out of 27 isolates V. cholerae non-O1/ non-O139 harbored tcpA genes; two isolates were isolated from Al Azziziah site and one isolates was isolated from the Half Moon site. Cholera toxin and Toxin-coregulated Pilus (TCP) are two major virulence factors produced by V. cholerae during infection (Faruque et al., 1998). Toxigenic Vibrio cholerae belonging to the O1 and O139 serogroups cause cholera, an illness characterized by acute watery diarrhea that occurs as epidemics in many developing countries (Faruque et al., 1998).

In this study, V. mimicus in this study was detected only from one sample from Half Moon site as shown in Table 1. Vibrio mimicus has been established as a pathogenic member of the genus Vibrio (Davis et al., 1981) and found to be responsible for various types of human illnesses. Isolation of V. mimicus pathogen from clinical samples has been made in different countries including the United States, Japan, Bangladesh, New Zealand and Canada (Davis et al., 1981).
Shandera et al. (1983) isolated V. mimicus from patients who had recently been exposed to seawater. V. mimicus gastrointestinal infections are rare. V. mimicus has also been isolated from a number of environmental sources, including oysters, prawns, rivers and brackish waters (Sanyal et al., 1983; Chowdhury et al., 1987; Chowdhury et al., 1989). Cholera-toxin production was demonstrated in V. mimicus isolates from aquatic environments. The significance of toxigenic environmental strains is unknown and specific studies are required to elucidate their epidemiological relevance and their role in the pathogenesis of diarrheal diseases. It has been shown that some V. mimicus strains were able to produce a heat-labile enterotoxin, functionally and immunobiologically related to the heat-labile CT (Dotevall et al., 1985; Tamplin et al., 1990), as well as other toxins and toxic substances that might contribute to its pathogenesis (Chowdhury et al., 1991, 1994).

From this study, it is concluded that potentially pathogenic Vibrios are indeed present in sea water of the Arabian Gulf coast of Eastern province of Saudi Arabia. The clinical significance of these isolates is their potential association with gastroenteritis and/or invasive septicaemia, usually contracted via consumption of raw or undercooked seafood or wound infections acquired by contact with sea water, respectively. Sporadic cases of Vibriosis along the Arabian Gulf coast of Saudi Arabia have not yet been reported. Nonetheless, these research findings should prompt us to pay attention to the role of Vibrio species in local foodborne diseases and wound infections. Many reports from Europe, USA, India and Asia indicate that human infections occur wherever these pathogens have been isolated (Hladay, 1997; Hoi et al., 1998; Morris, 1999; Yam et al., 2000). Thus, consideration should be given to long-term monitoring programmes for potential human pathogenic Vibrios in the Arabian Gulf coastal areas of Saudi Arabia. Since tcpA gene was prevalent in the environmental isolates, intensive surveillance study recommended for environmental samples along the coastal areas will help in understanding the evolution of the tcpA gene.

ACKNOWLEDGMENTS

This research work was supported by University of Dammam from the Deanship of Scientific Research. The author would like to thank the Microbiology Laboratory technicians (Mr. Lauro Bartolome and Mr. Bader Sager) for their contribution.

REFERENCES


