ABSTRACT

Due to sporadic cases of *V. cholerae* and the death of two people from *V. vulnificus* infections after exposure to coastal water, the identified need was to determine the prevalence of four pathogenic Vibrio spp. (*V. vulnificus*, *V. cholerae*, *V. parahaemolyticus*, and *V. alginolyticus*) in coastal waters of Hawaii. Culture-based methods using TCBS agar and CHROMagar Vibrio were used to enumerate concentrations of Vibrio spp. in waters with varying salinities. On the Island of Oahu, sampling sites included primary and secondary swimming sites as well as waters not designated or non-designated for swimming (canals, ponds). Select sites on the Island of Hawaii were also sampled based on previous incidences of illness and death due to *V. cholerae*. *V. cholerae* was not recovered in any coastal water samples indicating that risk for *V. cholerae* infections due to coastal water exposure in Hawaii is low. Mildly pathogenic *V. alginolyticus* was recovered from all sampling sites on Oahu and the Island of Hawaii. *V. vulnificus* and *V. parahaemolyticus* were recovered at low frequency from primary (high salinity) swimming sites and at a much higher frequency from secondary swimming (low to variable salinity) sites on the Island of Oahu and from coastal waters obtained from the island of Hawaii in the vicinity where *V. vulnificus* infections had been reported. In addition to having variable salinities, these secondary swimming sites are also characterized by warm water and increased nutrient load from land-based run off. These water quality parameters can be used to predict prevalence of *V. vulnificus* and *V. parahaemolyticus* in coastal water environments.

IDENTIFICATION OF PROBLEM

Vibrio spp are indigenous marine bacteria and have been reported to be pathogenic for many aquatic organisms (fish, eel, crustacean, marine mammal) as well as humans. *V. vulnificus*, *V. cholerae*, *V. parahaemolyticus* and *V. alginolyticus* are the four primary human pathogenic Vibrio and all four species have caused diseases in Hawaii. Sporadic cases of *V. cholerae* have been reported in Hawaii with no known source. Recently, two people died from *V. vulnificus* infections after contact with coastal marine waters. These reports have raised concerns regarding the prevalence of pathogenic Vibrio spp in Hawaii’s coastal waters and risk to people who use coastal waters in Hawaii for recreational purposes. This public concern increased after we reported that unlike temperate climates, Vibrio spp can be recovered from the relatively warmer (>20°C) coastal waters in Hawaii throughout the year. However, the prevalence of the four pathogenic Vibrio in Hawaii’s coastal waters has not been determined and it is well known that environmental water conditions such as lower salinity, warmer temperature and elevated nutrients select for growth of *V. cholerae*, and *V. vulnificus*, the two pathogenic vibrio of greatest concern. In summary, the identified need was to determine the expected prevalence of Vibrio in the various coastal water environments in Hawaii, so that relative risks of infections can be predicted based on exposure to coastal water environments, which are designated for swimming and those water environments which are not recommended for designated for swimming.

STUDY OBJECTIVES

1. To characterize the prevalence of human pathogenic *V. vulnificus*, *V. parahaemolyticus*, *V. cholerae*, and *V. alginolyticus* in waters at primary swimming sites such as beaches and at secondary swimming sites on the Island of Oahu because most of the human exposure to coastal water occur at these sites.

2. To characterize the prevalence of human pathogenic Vibrio spp in coastal waters of the Island of Oahu, which are not designated for swimming due to poor water quality but where human exposure occasionally takes place.

3. To characterize the prevalence of human pathogenic Vibrio spp in selected coastal waters on the Kona and Hilo side of the Island of Hawaii, where evidence of infection with *V. vulnificus* had been reported.

SAMPLING LOCATIONS

WATER QUALITY CATEGORIES

- **Primary swimming beaches (Oahu)**: Good water quality due to active circulation with ocean water, resulting in high salinity (30-36 ppt). [Sites A1-A15]
- **Secondary swimming sites (Oahu)**: Compromised water quality due to restricted circulation with ocean water and often impacted by fresh water run off, resulting in reduced salinity (3-30 ppt). [Sites B1-B21]
- **Non-swimming water sites (Oahu)**: Confined water, heavily impacted by land-based contamination, resulting in poor water quality, not suitable for swimming. [Sites C1-C6, D1-D3, E1-E3]
- **Coastal waters off the Island of Hawaii**. Sites selected based on past evidence of *V. vulnificus* infection. These sites generally impacted by groundwater discharge. [Sites H1-H10, K1-K9].

METHODS

RESULTS (Table 1, Table 2, Table 3)

<table>
<thead>
<tr>
<th>Site</th>
<th>Recovery of <em>V. vulnificus</em> (%)</th>
<th>Recovery of <em>V. parahaemolyticus</em> (%)</th>
<th>Recovery of <em>V. cholerae</em> (%)</th>
<th>Recovery of <em>V. alginolyticus</em> (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coastal Water Designated for Swimming</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary Swimming Beaches (High Salinity, Good Water Quality)</td>
<td>19</td>
<td>5</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>Secondary Swimming Sites (Variable Salinity, Stream Impacted Sites)</td>
<td>23</td>
<td>30</td>
<td>35</td>
<td>100</td>
</tr>
<tr>
<td>Non-Designated Swimming Waters</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. Canals</td>
<td>4</td>
<td>25</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>Island of Oahu</td>
<td>3</td>
<td>33</td>
<td>67</td>
<td>100</td>
</tr>
<tr>
<td>Kona area</td>
<td>3</td>
<td>33</td>
<td>67</td>
<td>100</td>
</tr>
<tr>
<td>Hilo area</td>
<td>10</td>
<td>50</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>

RESULTS (Table 1, Table 2, Table 3)

1. Concentrations of total marine bacteria (Marine agar and Vibrio spp (TCBS agar, ChromAgar Vibrio)) increased in water samples with higher nutrient loads. Thus, they were lower in clean, primary swimming beach water, moderate in secondary swimming waters, and highest in confined waters (harbors, canals, ponds), which are not designated for swimming.

2. *V. cholerae* was not recovered from any coastal water samples, whereas *V. alginolyticus* was recovered from nearly all coastal water samples tested.

3. Water samples from primary swimming beaches were characterized by predominance (>90%) of *V. parahaemolyticus* and low (5%) recovery of *V. vulnificus* and *V. parahaemolyticus*.

4. Water samples from secondary swimming water areas, were characterized by predominance (100%) of *V. vulnificus* and moderate recovery rate of *V. vulnificus* (30%) and *V. parahaemolyticus* (35%).

5. Water samples from non-designated swimming waters, were characterized by predominance (>90%) of *V. vulnificus* and moderate recovery rates of *V. parahaemolyticus* (50-67%) and *V. vulnificus* (25-33%).

6. Water samples obtained from the Island of Hawaii were characterized by predominance (90-100%) of *V. vulnificus* and moderate recovery rates of *V. vulnificus* (22-50%) and *V. parahaemolyticus* (22-60%).

CONCLUSIONS

1. Since *V. cholerae* was not recovered from any coastal water sites, risk of ocean water transmission of *V. cholerae* is considered very low.

2. *V. alginolyticus* was recovered from nearly all coastal water samples and was the only pathogenic Vibrio recovered from waters at recommended swimming beaches. Thus, people in Hawaii who swim in coastal waters will be exposed to this vibrio. However, *V. alginolyticus* infections (cuts, ear) are opportunistic and symptoms are relatively mild.

3. *V. vulnificus* and *V. parahaemolyticus* were recovered more frequently from secondary swimming water samples and from water samples obtained from the Island of Hawaii. Thus, risk of infection with *V. vulnificus* and with *V. parahaemolyticus* is highest when people swim or collect seafoods from secondary swimming sites which are characterized by lower salinity and impacted by land-based run off.

4. Since the increased prevalence rates of *V. vulnificus* and *V. parahaemolyticus* were correlated to coastal waters with lower salinity, higher temperature and increased nutrient load, these coastal water quality parameters can be used to predict prevalence of these two pathogenic vibrio and higher risks to people who are exposed to these kinds of coastal waters.

5. *V. parahaemolyticus*, *V. vulnificus* and *V. alginolyticus* were recovered from waters not designated for swimming. Because only a few of these hypothetical sites have been sampled and since a *V. vulnificus* infection had been reported after exposure to canal water, more studies are needed to determine conditions for *V. vulnificus* to grow in these non-designated swimming sites.

ACKNOWLEDGMENTS

This research was made possible through the Centers for Disease Control and Prevention (CDC), Program of the National Institute of Health Sciences (PO301/1745), National Institute of Health and the National Sciences Foundation (OCEO4-32479).