Incidence of *Vibrio cholerae* in Fresh Fish and Ceviche in Guadalajara, Mexico

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**ABSTRACT**

The incidence of *Vibrio cholerae* O1 and non-O1 was determined in fresh fish and ceviche, a marinated raw fish ready for consumption. Fresh red snapper (*Lutjanus purpureous*) and mackerel (*Scomberomorus sierra*) were obtained from distribution centers, and ceviche from street vendors and small open restaurants in Guadalajara, Mexico. In addition to *V. cholerae*, the fish samples were tested for aerobic plate count (APC), total volatile nitrogen (TVN), trimethylamine (TMA), and the ceviche for APC, coliforms, and pH. *V. cholerae* O1 and non-O1 was isolated from 10% and 26% of the fish respectively. The mean data for the fish samples were in the region of: APC, 106 CFU/g of fish; more than 25 mg of TVN per 100 g of fish, but less than 5 mg of nitrogen as TMA per 100 g. Eleven percent of the ceviche obtained from street vendors and 6% obtained from restaurants were positive for *V. cholerae* O1. The mean APC and coliform counts were 6.6 and 4.8 log CFU/g of fish respectively, and the pH of the ceviche ranged from 3.0 to 4.5. All the strains of *V. cholerae* O1 isolated during this study were identified as biotype El Tor, serotypes Inaba and Ogawa. For both fresh fish and ceviche, the frequency of isolation of *V. cholerae* was highest during the summer months.

Key words: *Vibrio cholerae*, fish, ceviche, aerobes, coliforms, cholera

One of the major concerns with regard to the recent appearance of cholera in Latin America is the possibility that *Vibrio cholerae*, its etiologic agent, may be transmitted to other countries through foods in international commerce. Seafoods may be of high risk because fish and shellfish can be harvested from waters contaminated with *V. cholerae* or cross-contaminated during harvesting, processing, and distribution. *V. cholerae* O1 has been isolated from both riverine and coastal environments (1, 5, 6, 7, 11, 12, 13, 19), and the newly recognized epidemic serogroup O139 (9, 10) has been found in waters as well. Rivers exposed to wastewater, as well as boats, wildlife and wastewater treatment plants are major sources of fecal pollution in bay areas where cholera may become a public health concern (14). Seafoods harvested from waters exposed to fecal contamination in such endemic areas are therefore likely to harbor *V. cholerae*. Once the organism enters the food-processing chain, inadequate food-service operations can constitute a health hazard. Because of the structure of the harvesting and distribution segments of the seafood industry, cross-contamination is another vehicle by which the organism can be spread. Seafood harvested from different environments are often mixed or mishandled on-board or during distribution, greatly increasing the possibility of cross-contamination. Even though *V. cholerae* O1 has been isolated from various seafood products (20), few reports are available to assess the risk of its transmission. This is a hindrance to the implementation of reliable hazard analysis critical control points (HACCP) programs, especially in developing countries where seafood production and exports are important economic activities.

Even though raw seafoods may be contaminated by *V. cholerae* and other pathogens, heat processing during preparation makes those products safe for consumption. However molluscan shellfish is often consumed raw and fish or shellfish may be only cured by salt, sugar, or acid treatments prior to consumption. One such traditional Latin-American product is ceviche, which is prepared by marinating ground or diced raw fish in the juice of fresh lemons for several hours. After the acidic marinade has penetrated the fish tissue, diced vegetables such as tomatoes, onions, cilantro, and peppers are added. Small shrimp are also used for ceviche. The popular belief is that since such products have been in contact with lemon juice for a long time, they are safe for consumption. However, the high protein content of raw fish and shellfish have strong buffering capacity and the pH may therefore only have a limited effect on the microbial population. Ceviche is widely consumed in several Latin American countries. In Mexico it is often sold nonrefrigerated by street vendors, who may have it on display for up to 6 h. Earlier work has demonstrated that other pathogens can survive in ceviche under such conditions. Torres-Vitela and Escartin (17) reported that the incidence of *Salmonella* spp.
in ceviche purchased from street vendors and small restaurants in Guadalajara was as high as 18 and 11% respectively. Although there was no statistical relationship between the sampling site and the frequency of isolation, the authors demonstrated that seasonal variation did affect the frequency of isolation of *Salmonella* spp. from ceviche.

The objectives of this study were to determine the incidence of O1 and non-O1 *V. cholerae* in ceviche purchased from street vendors and small restaurants and in fresh fish obtained from distribution centers in Guadalajara, Mexico. The effect of product quality and frequency of *V. cholerae* isolation was also investigated. The data generated from this work will enable us to design more reliable HACCP programs for the production and sale of such products.

**MATERIALS AND METHODS**

*Sample collection and preparation*

In order to determine seasonal variation, 100 samples of fresh fish and 100 samples of ceviche were obtained and analyzed for *V. cholerae* and other quality indicators at weekly intervals over a 1-year period. The fish were obtained from the two largest seafood distribution centers in Guadalajara, Mexico, and consisted of 49 samples of red snapper (*Lutjanus purpureus*), and 51 of mackerel (*Scomberomorus sierra*). These are the two marine species with highest preference among consumers. In addition, mackerel is the species most commonly used for ceviche preparation in Guadalajara. Each whole unviscerated sample was collected in a sterile plastic bag and transported to the laboratory for immediate analysis. Thirty-five ceviche samples were collected from small open curb side restaurants and 65 from street vendors. The samples were placed in plastic bags and immediately transported to the laboratory. For both fish and ceviche, the time between collection and initiation of analysis did not exceed 1 h. All the laboratory determinations were carried out in the Sanitary Microbiology Laboratory, University of Guadalajara, Mexico.

*Microbiological analysis*

Fifteen or 25 g of surface samples from both gills and skin were placed in stomacher bags. For the 15-g samples, 135 mL of 0.1% buffered peptone water (BPW) was added and used for determining the aerobic plate count (APC). For the 25-g surface samples, 225 mL of alkaline peptone water (APW) was added and used for the determination of *V. cholerae*. Twenty-five gram samples of intestinal contents were also mixed with 225 mL of alkaline peptone water for *V. cholerae* determination. The ceviche samples were prepared in the same manner using 15 g for APC and total coliforms and 25 g for *V. cholerae*. All samples were homogenized with 7.5% trichloroacetic acid (1:2, wt/vol) for 1 min. Homogenates were filtered through Whatman #1 filter paper and analyzed for TVN and TMA. The pH of the ceviche was determined in a homogenate of 5 g of sample in 10 mL of recently boiled water at room temperature with a Conductronic 10 pH meter.

**RESULTS AND DISCUSSION**

Table 1 shows the frequency of isolation of *V. cholerae* in fresh fish obtained from distribution centers in Guadalajara, Mexico. *V. cholerae* O1 was isolated from 10% of the fish surface samples but from only 2% of the intestinal contents. This trend was also observed for *V. cholerae* non-O1: 26% of the surface samples were positive, compared to 12% of the intestinal contents. The difference in the distribution of *V. cholerae* between the surface areas and the intestinal contents may indicate that the organism gains access to the fish from the environment where it is harvested or during postmortem handling, rather than from the food the fish consume. There was a significant difference between the frequencies of isolation of *V. cholerae* in the warm and cold seasons. Although the water temperatures at the time and place of harvesting were not available, it is logical to

<table>
<thead>
<tr>
<th>Fish sample</th>
<th>Season</th>
<th>No. samples</th>
<th>O1</th>
<th>Non-O1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface</td>
<td>April–September (warm)</td>
<td>43</td>
<td>8 (18.6)A</td>
<td>21 (48.8)C</td>
</tr>
<tr>
<td></td>
<td>October–March (cold)</td>
<td>57</td>
<td>2 (3.5)B</td>
<td>5 (8.8)BA</td>
</tr>
<tr>
<td></td>
<td>Year total</td>
<td>100</td>
<td>10 (10.0)w</td>
<td>26 (26.0)x</td>
</tr>
<tr>
<td>Intestinal contents</td>
<td>April–September (warm)</td>
<td>50</td>
<td>2 (4.0)d</td>
<td>12 (24.0)f</td>
</tr>
<tr>
<td></td>
<td>October–March (cold)</td>
<td>50</td>
<td>0 (0.0)e</td>
<td>0 (0.0)e</td>
</tr>
<tr>
<td></td>
<td>Year total</td>
<td>100</td>
<td>2 (2.0)Y</td>
<td>12 (12.0)zw</td>
</tr>
</tbody>
</table>

*a* Values followed by different letters are significantly different (*P* < 0.01).
assume that these also reflected the seasonal sampling plan. Because of its temperature requirements, *V. cholerae* would be expected to be more frequently associated with seafoods harvested in the summer than in the winter. An increase in the incidence of *V. cholerae* in oysters during the warm months has been reported previously (14) and there is evidence of seasonality in infection by *V. cholerae* in the United States (6). The relative high isolation rate of *V. cholerae* O1 in fresh fish reported in this study is in disagreement with other surveys conducted in Mexico. Rodriguez et al. (16) found only 0.3% of fresh fish from one seafood market in Mexico City to contain *V. cholerae* O1. Since the handling practices for the fresh fish in Mexico City and Guadalajara are very similar, and because most of the fish come from the same locations, the difference in rates of isolation can be attributed to either the isolation and identification techniques or to an increased incidence of *V. cholerae* O1 in the environment. During this work, a 6- and 18-h enrichment approach was used along with a direct plating step, which may not have been the case in earlier surveys. The recent increase in reported cholera cases in Latin America may also be one indicator of an increased incidence of *V. cholerae* in the environment and subsequently in raw seafood.

The agar plate count for the two fish species at the time these were obtained from the distribution centers are shown in Figure 1. A large number of factors will affect the microbial population of fish at the time of harvest and during postmortem handling and storage. It is therefore difficult to equate a specific population size to shelf life or sensory quality. However, when fresh fish are placed on ice or in refrigerated storage, a population somewhere between $10^6$ and $10^7$ CFU/g has often been used as an indicator for termination of acceptable quality. In this study, only one sample of mackerel and one of red snapper had bacterial counts of less than $10^5$ CFU/g, and only approximately one-quarter of the total number of fish analyzed had populations of less than $10^6$ CFU/g. There was no significant difference between the bacterial counts of fish harvested during the cold and the warm seasons.

One of the most common methods used for evaluating the quality of fish and shellfish is the amount of total volatile nitrogen (TVN) present. Expressed as the amount of nitrogen in the total volatile fraction, fresh fish that has less than 25 mg of TVN nitrogen per 100 g is often regarded to be of acceptable quality. Figure 2 shows the TVN content of the fish obtained from distribution centers in Guadalajara. If the TVN content is used as an indicator of quality, a large portion of the fish would be regarded as unacceptable even though laboratory personnel organoleptically judged the fish to be edible. Another common quality index for fresh fish is the amount of trimethyl amine (TMA) nitrogen content. Trimethyl amine in fish is produced postmortem by reduction of trimethyl amine oxide by common spoilage organisms. A level below 5 mg of TMA nitrogen per 100 g has been suggested to indicate acceptable quality for various species of fish and shellfish (2). On the basis of the TMA content, the majority of the fish used for this study were of acceptable quality. There was no correlation between the overall quality of the fish and the presence of *V. cholerae*.

Table 2 shows the incidence of *V. cholerae* O1 in ceviche obtained from street vendors and small restaurants. Seven of the 65 samples obtained from street vendors were positive, while 2 of 35 from small restaurants were shown to harbor the organism. No non-O1 *V. cholerae* was isolated from any of the ceviche samples. Ceviche has been recognized as a vehicle of food-borne diseases such as diphyllobothriasis, caused by *Diphyllobothrium pacificus*, a tapeworm which infects fish through contact with the heavily infected sea lions (3). Ceviche made from either fish or shrimp has also been shown to be the cause of cholera transmitted from Latin America to the United States (15). The consumption of raw or marinated seafoods, which is common in Latin America, may have been a contributing factor in spreading *V. cholerae* during recent epidemics. The causative agent during the recent Latin American cholera outbreak was identified as *V. cholerae* El Tor serotype Inaba. In this study, six of twelve isolates of *V. cholerae* O1 from fresh fish were serotype Inaba and the other six serotype Ogawa. However, since seroconversion from Inaba to Ogawa or vice versa has been well documented (15), both serotypes could potentially be the causative agent for the outbreak.
The mean agar plate and coliform counts for all ceviche samples analyzed in this study were 6.6 log CFU/g of fish and 4.8 log CFU/g respectively (Table 3). There was no significant difference between the populations of these organisms with respect to the season in which the ceviche was tested. Even though the pH of the ceviche samples ranged from 3.0 to 4.5, both the APC and the coliform count indicate that the organisms are at least able to survive if not able to grow under these conditions. Due to the strong buffering capacity of the fish muscle, the internal pH of fish tissue that is part of the ceviche will not reflect the pH of the surrounding lemon juice and may therefore support growth. Another factor that can possibly explain the survival of the organism at such a low pH is the composition of the lemon juice used as acidifying agent. The major component of this juice is citric acid, which does not have the same bacteriostatic properties as do other short-chain organic acids such as acetic acid. In studying the survival of Salmonella spp. in a typical Mexican cold beef salad, Escartin et al. (4) observed an inhibition of the pathogen when 4% acetic acid was added to the salad. In ceviche, lemon juice in combination with acetic acid to a pH of 2.7 to 2.8 should help reduce the microbial load and therefore make ceviche a less hazardous product. Further studies would therefore be necessary to determine whether adding acetic acid to the ceviche does not negatively affect the quality or singularity of this unique food.

TABLE 3. Agar plate counts and coliform counts in ceviche during warm and cold seasons in Guadalajara, Mexico

<table>
<thead>
<tr>
<th>Bacterial load</th>
<th>Season</th>
<th>Mean</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agar plate counts</td>
<td>Warm</td>
<td>6.7A</td>
<td>7.0</td>
</tr>
<tr>
<td></td>
<td>Cold</td>
<td>6.5A</td>
<td>6.5</td>
</tr>
<tr>
<td>Coliform counts</td>
<td>Warm</td>
<td>4.7B</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>Cold</td>
<td>5.0b</td>
<td>4.7</td>
</tr>
</tbody>
</table>

Means followed by the same letter are not significantly different (P > 0.05).

This study, together with the study of Torres-Vitela and Escartin (17), has shown a high incidence of pathogenic as well as indicator organisms, these being evidence of poor hygiene in ceviche obtained from street vendors and small restaurants in Guadalajara, Mexico. Ten percent of the fresh fish that serve as a major ingredient in ceviche were contaminated with V. cholerae O1. Although not analyzed, the vegetables included in the ceviche preparation might also have added to the bacterial load of the ceviche. Temperature abuse or other unsanitary conditions during handling and selling of this food can also contribute to the high bacterial numbers observed. The lemon juice used as acidifying agent during preparation of ceviche does not appear to be bactericidal enough to render the product safe for consumption.

REFERENCES


