Occurrence of Vibrio spp. in Fish and Shellfish Collected from the Swiss Market

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ABSTRACT

The genus Vibrio includes gram-negative, rod-shaped, halophilic bacteria that inhabit estuarine ecosystems. V. cholerae, V. parahaemolyticus, and V. vulnificus pose a considerable public health threat as agents of sporadic and epidemic foodborne infections associated with the consumption of raw or undercooked contaminated fish or shellfish. In this study, we analyzed 138 fish and shellfish samples collected from the Swiss market (fish fillets [n = 102], bivalves [n = 34], and squid [n = 2]). Microbiological analysis was done according to International Organization for Standardization method 21872-1/21872-2:2007, using thiosulfate citrate bile sucrose agar and chromID Vibrio agar as selective agar. Presumptive-positive colonies on thiosulfate citrate bile sucrose agar or chromID Vibrio agar were picked and were identified by the API 20E and species-specific PCR systems. V. cholerae isolates were tested further by PCR for the presence of the cholera toxin A subunit gene (ctxA). V. parahaemolyticus isolates were tested by PCR for genes encoding for thermostable direct hemolysin (tdh) and TDH-related hemolysin (trh). V. cholerae was isolated from three samples and V. parahaemolyticus from eight samples. None of these strains harbored species-specific virulence factors. Further, V. alginolyticus was isolated from 40 samples, and V. fluvialis was isolated from 1 sample. Our study provides, for the first time, data for the assessment of exposure to Vibrio spp. in raw fish and bivalves consumed in Switzerland.

Seafood, including raw fish, consumption has increased significantly in Switzerland as well as worldwide. Because Switzerland is a landlocked country and has to import more than 95% of its seafood from many nations, the Swiss market is a good sampling platform, as the products reflect the global state. Along with the increase of seafood consumption, there is also a rise in seafood-associated infections. More than 180 seafood-associated outbreaks occurred between 1973 and 2006 in the United States, causing 4,020 illnesses, 161 hospitalizations, and 11 deaths (5). The majority of these outbreaks was caused by bacterial agents; V. parahaemolyticus was implicated in more than 45% of all outbreaks. Only a few seafood-associated Vibrio outbreaks with hardly any cases each were reported in the last 10 years in Europe, mainly from Italy (12, 15), the United Kingdom (17), and Spain (8). No data about seafood-associated outbreaks due to Vibrio are available in Switzerland.

The genus Vibrio includes gram-negative, rod-shaped, halophilic bacteria. Their natural habitat is in estuarine and coastal areas, where they are found free living in water or in association with plankton. Vibrio spp. favor higher water temperatures, and outbreaks caused by these pathogens are more frequent during the warmer season (5).

The two main virulence factors of V. parahaemolyticus are the thermostable direct hemolysin (TDH) and the TDH-related hemolysin (TRH) (4). Strains harboring these virulence factors were shown to be strongly associated with gastroenteritis (5, 9). V. vulnificus is responsible for the overwhelming majority of reported seafood-related deaths in the United States (6). In addition, a number of other species are considered sporadic human pathogens. These include V. alginolyticus, V. fluvialis, V. furnissii, V. holisae, and V. mimicus (2).

The aims of this pilot study in Switzerland were to determine the occurrence of Vibrio spp. in fish and shellfish collected from the Swiss market and to characterize further isolated strains.

MATERIALS AND METHODS

Sampling. Overall, 138 refrigerated fresh seafood samples (fish fillets [n = 102], bivalves [n = 34], and squid [n = 2]) were collected from September to December 2010. The majority of samples was taken from a large seafood distribution plant in
Table 1. Occurrence of Vibrio spp. in fish and shellfish, detected by the culture-based ISO 21872-1/21872-2:2007 method

<table>
<thead>
<tr>
<th>Matrix</th>
<th>No. of samples</th>
<th>No. (%) of samples positive for Vibrio spp.</th>
<th>Vibrio spp. isolated (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saltwater fish</td>
<td>66</td>
<td>12 (18.2)</td>
<td>V. cholerae (1)a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>V. parahaemolyticus (1)b</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>V. alginolyticus (11)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>V. fluvialis (1)</td>
</tr>
<tr>
<td>Freshwater fish</td>
<td>36</td>
<td>2 (5.6)</td>
<td>V. cholerae (1)c</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>V. alginolyticus (1)</td>
</tr>
<tr>
<td>Shellfish</td>
<td>34</td>
<td>29 (85.3)</td>
<td>V. parahaemolyticus (7)d</td>
</tr>
<tr>
<td>Squid</td>
<td>2</td>
<td>2 (100)</td>
<td>V. cholerae (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>V. alginolyticus (2)</td>
</tr>
<tr>
<td>Total</td>
<td>138</td>
<td>45 (32.6)</td>
<td></td>
</tr>
</tbody>
</table>

a Not harboring the ctxA gene.
b Not harboring the tdh or trh genes.
c Iridescent shark from the Mekong Delta.

Microbiological analysis. Microbiological analysis was done according to International Organization for Standardization (ISO) method 21872-1/21872-2:2007, using thiosulfate citrate bile sucrose agar (TCBS; Oxoid, Ltd., Basingstoke, England) and chromID Vibrio agar (VID; bioMérieux, Inc., Marcy l’Etoile, France) as selective agar plates. In brief, 25 g of each sample was added to 225 ml of alkaline saline peptone water (Oxoid, Ltd.). After incubation for 6 h at 41.5°C, 1 ml of the incubated medium was transferred into 10 ml of fresh alkaline saline peptone water and incubated for 18 h at 41.5°C. These cultures were thereafter streaked on TCBS and VID and incubated for 24 h at 37°C. Presumptive Vibrio spp. on TCBS were smooth and either green (presumptive V. parahaemolyticus, V. vulnificus, or V. mimicus) or yellow (presumptive V. cholerae, V. alginolyticus, or V. fluvialis). Presumptive Vibrio spp. on VID were blue, cyan (presumptive V. cholerae or V. vulnificus), pink (presumptive V. parahaemolyticus), or beige (presumptive V. alginolyticus). For further identification, presumptive-positive colonies on TCBS or VID were picked and transferred to saline nutrient agar and incubated at 3°C for 24 h. Oxidase-positive isolates were identified with the API 20E test kit (bioMérieux, Inc.). For species-specific identification of V. cholerae, primers targeting the gene of the outer membrane protein W were used (10). For species-specific identification of V. parahaemolyticus, primers targeting the thermolabile hemolysin gene (tdh) were used (11). Other doubtful identification results by the API kit were confirmed with 16S rDNA sequencing (7).

Further strain characterization. V. cholerae isolates were tested by PCR for the presence of the cholera toxin A subunit gene (ctxA) (14). Moreover, these isolates were agglutinated with polyvalent O1 and O139 somatic antisera (Denka Seiken, Tokyo, Japan). V. parahaemolyticus was tested by PCR for genes encoding thermostable direct hemolysin (tdh) and TDH-related hemolysin (trh) (1).

RESULTS AND DISCUSSION

Vibrio spp. were found in 45 of 138 samples. The positive samples consisted of saltwater fish (n = 12), freshwater fish (n = 2), shellfish (n = 29), and squid (n = 2). The isolates comprised four species. V. cholerae was isolated from 3 samples (2 fillets and 1 squid), V. parahaemolyticus from 8 samples (7 bivalves and 1 fillet), V. alginolyticus from 40 samples (26 bivalves, 13 fillets, and 1 sepia), and V. fluvialis from 1 (fillet) sample (Table 1). There were no certain types of saltwater fish fillets or shellfish more often positive than were others. Positive results were distributed throughout all these samples. In 5 samples (3 shellfish and 1 ocean perch fillet, 1 squid), two different Vibrio spp. were found.

Although the natural environment of V. cholerae is in coastal waters, we found V. cholerae in a freshwater fish fillet. This positive result traced back to an iridescent shark sample, a fish that is bred and fattened in the Mekong Delta, where the water is highly contaminated with human sewage. All isolated V. cholerae strains failed to react in the agglutination with O1 and O139 somatic antisera and lacked the ctxA gene. None of the V. parahaemolyticus strains harbored the tdh and/or trh gene(s).

Comparable to our study, V. alginolyticus was also the most frequently isolated species in two culture-based studies from Italy and Malaysia (3, 13). Moreover, in the study from Malaysia, V. cholerae was found in 4.6% of all samples, with 14 isolates belonging to the O139 serogroup and 1 isolate belonging to the O1 serogroup (3). In the study from Italy, V. cholerae was found in 1.6% of 62 examined mussel samples, and all isolates tested negative for the heat-stable enterotoxin gene (13). In these two studies, V. parahaemolyticus was present in 4.7 and 1.6% of the tested samples, respectively. None of the isolates harbored further virulence factors. A recently published study on the occurrence of V. parahaemolyticus in São Paulo State (Brazil) retail oysters reported a prevalence of 100%. In addition, none of the 1,943 tested isolates in this study harbored tdh and/or trh gene(s) (16).

Our data provide, for the first time, information for the assessment of exposure to Vibrio spp. in raw fish and bivalves consumed in Switzerland. However, it must be recognized that using only cultural methods to detect Vibrio spp. could be a limitation. In our study, the selective agar plates were quite often overgrown with different bacteria, so that potentially present pathogenic Vibrio spp. might have been competitively suppressed in their growth or missed. As only 1 to 2% of total V. parahaemolyticus in the environment harbor further virulence factors (3), these pathogenic strains could easily be overlooked on overgrown plates. For this reason, Nordstrom et al. (11) recently developed a real-time PCR–based method that is optimized for the detection of low levels of pathogenic V. parahaemolyticus in the presence of high levels of total V.
parahaemolyticus by simultaneously amplifying the genes tlh, tdh, and trh. Oysters collected from Alaska were analyzed with both a cultural-based method and a real-time PCR approach. The results revealed that by using the PCR method, more samples were detected positive for total and pathogenic V. parahaemolyticus than were detected positive by using the cultural method. Furthermore, PCR never failed to be positive for samples for which V. parahaemolyticus was found by the cultural method alone. A similar challenge is the detection of pathogenic V. cholerae in a higher level of total V. cholerae. Screening the enrichment broth by virulence factor–based PCR would help to get a clearer picture on the prevalence of samples with low numbers of pathogenic Vibrio spp., which could be missed by using only the cultural approach. Therefore, further efforts are needed in the development and standardization of molecular-based methods for Vibrio spp.—monitoring purposes in fish and shellfish.

ACKNOWLEDGMENTS

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REFERENCES