

# ISOLATION OF A *VIBRIO PARAHAEMOLYTICUS* PANDEMIC STRAIN FROM A MARINE WATER SAMPLE OBTAINED IN THE NORTHERN ADRIATIC

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*Vibrio parahaemolyticus* is a halophilic bacterium capable of causing food- and waterborne gastroenteritis, wound infections, and septicemia in humans. The wide distribution of *V. parahaemolyticus* in the marine and estuarine environments is known to depend on the water temperature: it has been suggested that the bacterium might survive in sediments during the winter and be released into the water column in late spring or early summer when the temperature rises to 15°C or higher [1].

The microorganism is frequently isolated from a variety of raw seafood and shellfish. Consumption of raw or undercooked seafood contaminated with *V. parahaemolyticus* may lead to the development of acute gastroenteritis characterized by diarrhoea, headache, vomiting, nausea, and abdominal cramps. This bacterial species is a common cause of foodborne illnesses in many Asian countries, including China (31.1% of foodborne outbreaks reported between 1991 and 2001), Japan (reported to account for 20–30% of foodborne infection cases from 1981 to 1993) and Taiwan (1,495 cases reported between 1981 and 2003, representing 69% of all bacterial foodborne outbreaks in this period) [2,3,4]. Moreover, it is recognized as the leading cause of human gastroenteritis associated with seafood consumption in the United States [5]. In Europe, the risk of *V. parahaemolyticus* infections is considered to be very low [6,7] and for this reason the monitoring of this microorganism has been excluded from the most important European infectious disease surveillance networks. However, sporadic outbreaks have been reported in countries such as Spain (important outbreaks reported in 1989, 1999 and 2004) [8] and France (a serious outbreak reported in 1997) [9].

Less commonly, this bacterial species can cause infections in the skin when an open wound is exposed to warm seawater (>15°C). Severe wound infections and septicemia have also been reported mainly in immunosuppressed, children and aged people. Recently seven cases of skin infections caused by *V. parahaemolyticus* species have been described in Denmark linked to bathing in the Baltic Sea [10].

*V. parahaemolyticus* infection has traditionally been associated with two virulence factors – thermostable direct haemolysin (TDH) and TDH-related haemolysin (TRH) [6]. While more than 90% of the clinical isolates present the *tdh* gene, to date pathogenic strains containing *tdh* and/or *trh* genes have been detected with

low frequency (usually 0.3 to 3%) in the total *V. parahaemolyticus* environmental population [11].

During a hospital-based survey in Calcutta, India, a sudden increase in the proportion of infections associated with *V. parahaemolyticus* serotype O3:K6 was detected [2]. This highly virulent strain accounted for 63% of all *V. parahaemolyticus* strains isolated from patients in Calcutta between September 1996 and April 1997 and was subsequently obtained in high rates from patients in other southeast Asian countries and from travellers arriving in Japan from this region [12,13]. Increased incidences of gastroenteritis caused by this serovar have been reported in many countries since 1996 [12,13,14]. Therefore, as a result of its rise in incidence with identical phenotypic and genotypic features, this emerging *V. parahaemolyticus* strain has been termed a 'pandemic strain'. Currently the so-called 'pandemic group' [15] includes the 'pandemic' O3:K6 strain and the newly emerged O4:K68, O1:K25, O1:K26, and O1:K untypeable strains.

While all the strains of *V. parahaemolyticus* are identified by the species-specific genetic markers *tlh* and *toxR* genes, 'pandemic' *V. parahaemolyticus* strains can be identified by group-specific GS-PCR based on the sequence variation in the *toxRS* gene [15]. A strain possessing both *tdh* and *toxRS/new* can be considered a 'pandemic strain'. In addition, most of the 'pandemic strains' have a novel open reading frame *orf8*, which corresponds to a filamentous phage f237 [16].

During a series of sampling campaigns organized within the framework of the international VibrioSea project\* and conducted in the north of the Adriatic Sea in the area of the Venetian lagoon from June 2006 to November 2007, a collection of environmental *V. parahaemolyticus* strains was obtained. They were isolated mainly during the warm season (from May to October) and have been found in water, plankton and sediment samples.

After the biochemical identification, using the Biomérieux API ID system, all the strains were confirmed as *V. parahaemolyticus* by PCR detection of the species-specific markers, genes *tlh* and *toxR* [17]. Screening conducted on the whole collection revealed, in one of the analyzed strains (strain VPeVEpan), the presence of the virulence gene *tdh* and the 'pandemic'-specific marker, gene *orf8*. The presence of each one of these genes was confirmed by

PCR with a second pair of primers selected in a different area of the same nucleotide sequence. The strain also tested positive to a group-specific PCR (GS-PCR) conducted with a pair of primers selected in the *toxRS* gene. The strain was isolated in May 2007 in a marine water sample taken from the coastal site in the locality Caleri, close to the estuary of the Adige and Brenta rivers, 500 m from the coastline). Serological characterization and molecular typing is ongoing and the genes are currently being sequenced in order to compare this strain with other 'pandemic strains' of environmental and clinical origin, isolated in Europe and Asia.

On the basis of these findings and the data from the literature, this strain should be considered to have the potential to cause human illness because it carries the three 'pandemic' genetic markers. To the best of our knowledge, this is the first strain of *V. parahaemolyticus* isolated in the Italian coastal environment and the first isolated directly from an environmental water sample in Europe containing the genetic markers characterizing 'pandemic strains' (*tdh*, *orf8*, *toxRS/new*). Previously, 'pandemic strains' similar to the ones isolated in Asia, had been detected in the Galicia region of Spain [14] and in France [18], yet these strains were in all cases clinical isolates or strains isolated from seafood and not from the environment itself.

The results reported here indicate that the environmental strains belonging to human pathogenic *Vibrio* species isolated in Europe should be considered as potential carriers of virulence genes including those encountered in 'pandemic strains'. Because of their pathogenic potential their presence should be placed under surveillance as they could represent a risk for human health.

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