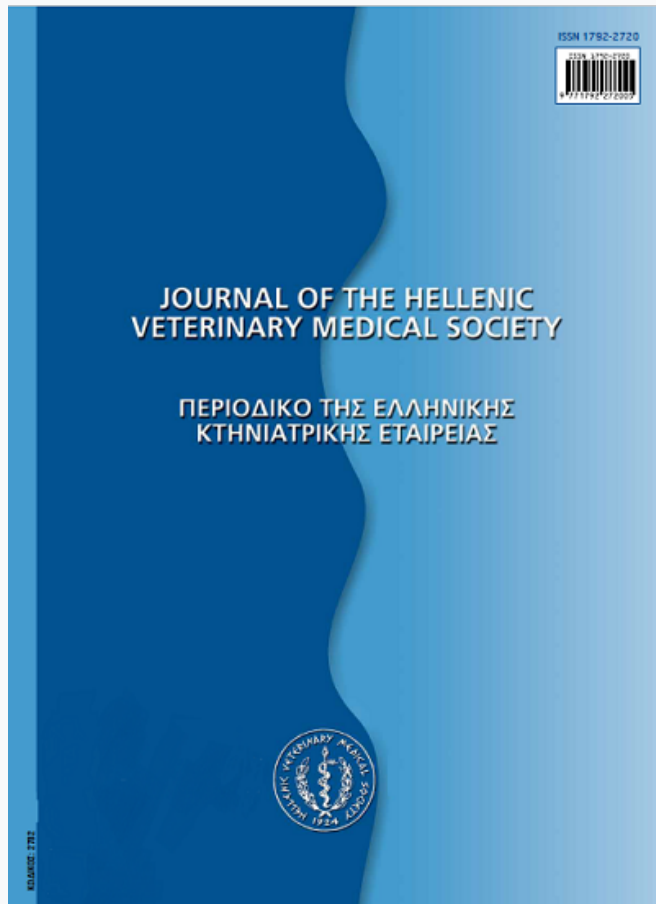


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## **Vibrio parahaemolyticus in seafood – associated outbreaks**

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## **Το *Vibrio parahaemolyticus* σε τροφιμογενείς λοιμώξεις αλιευμάτων**

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**ABSTRACT.** Among the 30 species of the genus *Vibrio*, only 13 of them are pathogenic to humans. All pathogenic vibrios have been reported to cause foodborne diseases, although *V. parahaemolyticus* is considered the most important pathogenic *Vibrio*. *V. parahaemolyticus* is a halophilic bacterium that occurs naturally in aquatic environments worldwide. The pathogen caused sporadic diarrhoea mainly associated with the consumption of raw or undercooked seafood up to recent years. Since 1996, the incidence of *V. parahaemolyticus* infections has increased dramatically. *V. parahaemolyticus* is the leading cause of seafood-associated bacterial gastroenteritis in the United States and of the half foodborne outbreaks in some Asian countries. This increase in incidence has been related to the emergence of the O3:K6 serovar. The pathogenic *V. parahaemolyticus* strains can produce a thermostable direct hemolysin or a thermostable direct hemolysin-related hemolysin, which are encoded by the *tdh* and *trh* genes, respectively. *Vibrio parahaemolyticus* has not been included in the microbiological criteria of E.U. Food legislation, probably because the risk by this pathogen was considered rather low in Europe. However, climate changes favour the growth of the pathogen in seawater. Recent studies in Spain and France have shown that *V. parahaemolyticus* infections from seafood consumption have been increased. The emergence of the pathogen in Europe is of public health concern and emphasizes the importance of microbiological surveillance and control programs for *V. parahaemolyticus*.

**Keywords:** *Vibrio parahaemolyticus*, seafood, fish, outbreaks

**ΠΕΡΙΛΗΨΗ.** Μέχρι σήμερα έχουν καταγραφεί 30 είδη *Vibrio* (δονάκια), από τα οποία 13 είναι παθογόνα για τον άνθρωπο. Όλα τα παθογόνα δονάκια έχουν αναφερθεί ότι σχετίζονται με τροφιμογενείς λοιμώξεις, αλλά το *V. parahaemolyticus* είναι το σημαντικότερο παθογόνο δονάκιο. Το *V. parahaemolyticus* είναι ένα αλλόφιλο βακτήριο που βρίσκεται σε υδάτινα οικοσυστήματα σε όλη την υφήλιο. Μέχρι το 1996, το παθογόνο σχετιζόταν με σποραδικά περιστατικά τροφιμογενούς γαστρεντερίτιδας μετά από την κατανάλωση νωπών αλιευμάτων ή αλιευμάτων που είχαν υποστεί ατελή θερμική επεξεργασία. Από το 1996, ο αριθμός των καταγεγραμμένων τροφιμογενών λοιμώξεων από το *V. parahaemolyticus* έχει αυξηθεί σημαντικά με αποτέλεσμα να αποτελεί το κυριότερο αίτιο τροφιμογενών λοιμώξεων από κατανάλωση αλιευμάτων σε ορισμένες ασιατικές χώρες και στις Η.Π.Α. Η αύξηση αυτή αποδίδεται στην εμφάνιση του ορότυπου O3:K6 του βακτηρίου. Τα παθογόνα στελέχη του *V. parahaemolyticus* παράγουν μια θερμοανθεκτική τοξίνη (Kanagawa ή TDH) ή μια αιμολυσίνη παρόμοια με την τοξίνη Kanagawa (TRH) που σχετίζονται με τα γονίδια *tdh* και *trh*, αντίστοιχα. Το *V. parahaemolyticus*

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δεν περιλαμβάνεται στα μικροβιολογικά κριτήρια ασφαλείας για τα αλιεύματα που ισχύουν στην Ευρωπαϊκή Ένωση, αφού μέχρι πρόσφατα θεωρούνταν χαμηλή η συχνότητα εμφάνισης λοιμώξεων από το παθογόνο στην Ευρώπη. Όμως, οι κλιματικές αλλαγές του πλανήτη μας ευνοούν την ανάπτυξη του παθογόνου στο θαλάσσιο νερό. Πρόσφατες μελέτες στην Ισπανία και τη Γαλλία αναφέρουν ότι οι λοιμώξεις αυτές παρουσιάζουν σημαντική αύξηση. Στη παρούσα ανασκόπηση αναφέρονται οι μεγαλύτερες επιδημίες που έχουν προκληθεί παγκοσμίως από *V. parahaemolyticus* μετά από την κατανάλωση αλιευμάτων. Η αυξανόμενη καταγραφή της παρουσίας του παθογόνου σε αλιεύματα στην Ευρώπη παρουσιάζει ιδιαίτερη σημασία για τη Δημόσια Υγεία και υπογραμμίζει την ανάγκη ένταξης του *V. parahaemolyticus* στα προγράμματα επιτήρησης και ελέγχου.

## INTRODUCTION

The *Vibrio parahaemolyticus* is an ubiquitous Gram-negative bacterium that naturally occurs in marine coastal waters and is recognized as the most common causative agent of seafood-associated gastroenteritis, worldwide. *V. parahaemolyticus* infections result from ingestion of raw or improperly cooked seafood (Johnson et al. 2008). Shellfish that are frequently consumed whole and raw can serve as carriers of pathogenic Vibrios. Especially, bivalves (oysters, clams and mussels), which filter water through their digestive system to obtain food, accumulate and concentrate microorganisms (Su and Liu 2007) such as *V. parahaemolyticus*.

European Union Regulation 2073/2005, which sets out the microbiological criteria for foods, makes no provision for *Vibrio* control in seafood within the European Union. However, there is a growing concern that *V. parahaemolyticus* may pose an important and increasing problem for seafood safety in Europe (Baker-Austin et al. 2010, Ottaviani et al. 2008). The isolation of this pathogen in recent studies from areas, including Europe, where it was rarely found, highlights its geographical and seasonal expansion (Martinez – Urtaza et al. 2010, Su and Liu 2007). The present review is focussed on the importance of *V. parahaemolyticus* in seafood safety.

### *Vibrio parahaemolyticus*

The genus *Vibrio* is classified within the family *Vibrionaceae*. There are 30 species in the genus *Vibrio*, 13 of these are considered pathogenic to humans, including *V. cholerae*, *V. mimicus*, *V. fluvialis*, *V. parahaemolyticus*, *V. alginolyticus*, *V. cincinnatiensis*, *V. hollisae*, *V. vulnificus*, *V. furnissii*, *V. damsela*, *V. metshnikovii*, and *V. carchariae*. All pathogenic vibrios have been reported to cause foodborne diseases, but it has been considered that *V. cholerae* O1, *V. parahaemolyticus* and *V. vulnificus* are the most significant causative agents (Atlas 1997). Members of the *Vibrio*

genus are straight or curved Gram-negative, non spore-forming rods, 0.5 to 0.8 μm in width and 1.4 to 2.6 μm in length. Vibrios are motile by a single polar flagellum and are aerobic or facultative anaerobic. Most species produce oxidase and catalase and ferment glucose without producing gas (McLaughlin 1995).

In 1950, a new bacterium was identified as the cause of a large outbreak in Japan, which was associated with the consumption of a local fish product “shirasu” (Fujino et al. 1953). Initially, the new species was named *Pasteurella parahaemolyticus* and later designated as *Vibrio parahaemolyticus* (Hugh and Sakauki 1975). *V. parahaemolyticus* is a moderately halophilic (salt-requiring) enteric pathogen that can grow in NaCl concentrations of up to 8%, but the optimum growth is observed at 2 to 3% NaCl (Yang et al. 2010). It has a very short generation time (<10 min) at the optimum growth temperature of 37 °C (Miles et al. 1997). The pathogen grows at a minimum temperature of 15 °C and a maximum temperature of 44 °C. All strains of *V. parahaemolyticus* produce H<sub>2</sub>S in triple sugar iron (TSI) medium (Su and Liu 2007).

Serotyping of *V. parahaemolyticus* is based on antigens of O (somatic) and K (capsular polysaccharide). All strains of the pathogen share a common H (flagellar) antigen. Up to date, 12 O antigen types and over 76 K antigen types have been described, although serotypes of many strains have not been identified (Kaysner and DePaola 2004).

### Virulence Factors

*V. parahaemolyticus* strains isolated from human gastroenteritis cases are characterized by a variety of virulence factors. Typically, virulent strains of the pathogen can lyse red blood cells on Wagatsuma blood agar plates. This haemolytic activity is named Kanagawa phenomenon (Kp) and has been associated with the production of a thermostable direct haemolysin (TDH). This protein is not inactivated by heat (100 °C for 10 min) and its haemolytic activity is not

enhanced by the addition of lecithin, which suggests direct activity on erythrocytes (Nishibuchi and Kaper 1995). Almost all clinical isolates are Kp positive, therefore TDH production is considered as the major virulent factor of *V. parahaemolyticus* (Su and Liu 2007, Takeda 1983). Occasionally, Kp negative strains of the pathogen have been isolated from human cases of gastroenteritis associated with seafood consumption. Such strains produced a protein, similar but not identical to TDH (TDH related haemolysin - TRH) (Honda et al. 1988, 1987). The genes of both types of haemolysin share several biological properties such as haemolytic activity, enterotoxicity and cytotoxicity (Park et al. 2004).

Since only 1 – 2 % of the environmental strains carry the genes encoding TDH (*tdh*) and TRH (*trh*), the presence of *tdh* or/and *trh* genes has been considered as molecular indicators for distinguishing pathogenic and non pathogenic strains (Oliver and Kaper 2007). However, it has been reported that *V. parahaemolyticus* strains lacking both *tdh* and *trh* genes were associated with severe cases of foodborne infection, many of which required hospitalization (Yu et al. 2006). In Japan, 215 clinical isolates of *V. parahaemolyticus* were examined for the presence of *tdh* and *trh* genes and it was found that 52 strains (24.3%) carried only the *trh* gene (Shirai et al. 1990).

The analysis of the complete genome of *V. parahaemolyticus* (Makino et al. 2003) revealed more complex pathogenic mechanisms than previously considered. High adherence capability, some enzymes, urea hydrolysis and more recently, a heat-labile protein (serine protease) have been also identified as potential virulence factors (Lee et al. 2002, Lida et al. 1997).

### Pathogenesis

*V. parahaemolyticus* infections are very common worldwide and are considered as the leading cause of bacterial illnesses associated with seafood consumption in Asia and USA (Nordstrom et al. 2007). The virulence and pathogenicity of *V. parahaemolyticus* are less pronounced than those of other *Vibrios* like *V. cholerae* or *V. vulnificus* and deaths associated with this bacterium are rare. Symptoms include headache, nausea, acute abdominal pain, low grade fever, vomiting and diarrhoea (watery or bloody). The stool is described as “meat washed” due to the presence of blood (Yang et al. 2008, Honda and Lida 1993). The infectious dose

is usually about  $2 \times 10^5$  to  $3 \times 10^7$  CFU. Incubation period is about 15 h (4 – 96 h) and symptoms typically resolve in less than 72 h. *V. parahaemolyticus* colonize the intestine and produce toxins that cause cell damage as well as loss of fluids and electrolytes. Though the *V. parahaemolyticus* infection induces a strong immune response, the detailed mechanism of pathogenesis is still not well known (Levin 2006). Unlike other *Vibrios*, person to person transmission of *V. parahaemolyticus* has not been reported yet and foodborne outbreaks are almost always associated with seafood consumption (Martinez-Urtaza et al. 2004, Joseph et al. 1982).

The gastroenteritis caused by *V. parahaemolyticus* infection is often self-limited, but in some cases the infection may cause septicaemia that is life-threatening to people having underlying medical conditions such as liver disease or immune disorders. Two deaths were reported among three cases of wound infections caused by *V. parahaemolyticus* in USA after the Hurricane Katrina in 2005 (Centers for Disease Control and Prevention - CDC 2005).

### Emerging strain of *Vibrio parahaemolyticus*

Prior to the 1970, almost all infections caused by *V. parahaemolyticus* were reported in Japan. Food-borne outbreaks were typically associated with seafood consumption and represented about 70% of food-related cases during summer months. Throughout the 1970's, sporadic cases and outbreaks were also reported in Europe (Joseph et al. 1982). The first documented outbreak of *V. parahaemolyticus* in USA was reported in 1971 and was associated with contaminated crab products. The pathogenic strains isolated from patients and suspected seafood in USA were different than those isolated in outbreaks in Japan (Dadisman et al. 1972).

Since the mid 1990's an increased incidence of *V. parahaemolyticus* gastroenteritis cases was reported from many countries around the world, which was associated with a novel serotype (O3:K6) of the pathogen. This serotype was first isolated in 1996 in Calcutta, India from hospitalized patients (Okuda et al. 1997). The emerging Calcutta O3:K6 strain was different from other O3:K6 strains isolated in Asia in the previous decade. Additionally to the emergence of the new serovar, strains O4:K68 and O1:K untypeable (KUT) have also been associated with the increased incidence of *V. parahaemolyticus* infections worldwide

(Okura et al. 2003). Molecular techniques placed these serovars into a homogenous group with clearly differentiated virulence traits (*tdh*<sup>+</sup>, *trh*) from previously recovered strains. The clonal clustering of the strains and their distinctive genetic characteristics, led to this group being named a “new O3:K6 clone” (Chowdhury et al. 2000, Okuda et al. 1997). During the next year the new clone was isolated from *V. parahaemolyticus* infections throughout Asia. In June of 1997 the O3:K6 strain spread to Peru, for the first time beyond Asia and later that year it was implicated in an outbreak in Chile (Martinez – Urtaza et al. 2005). In 1998, a large outbreak was reported in the USA, which was associated with consumption of raw contaminated oysters (Daniels et al. 2000). A retrospective study compared O3:K6 strains from North America and Asia outbreaks using molecular techniques and indicated that these strains were indistinguishable (Matsumoto et al. 2000). This was the first definitive evidence of the *V. parahaemolyticus* pandemic and the term “pandemic clone” was designated (Gonzalez – Escalona et al. 2008, Chowdhury et al. 2000). Since then, a spread of this pandemic strain to Europe has been reported and it was isolated from outbreaks in France (Quilici et al. 2005), Spain (Martinez – Urtaza et al. 2005), Italy (Ottaviani et al. 2008), as well as from other countries like Russia (Nair et al. 2007) or Mozambique (Ansaruz-zaman et al. 2005). In 2005, the largest *V. parahaemolyticus* outbreak caused by the pandemic O3:K6 strains (11000 human cases) was reported in Chile and it was associated with shellfish consumption (Fuenzalida et al. 2006).

### **Risk factors associated with the globalization of *V. parahaemolyticus***

Traditionally, *V. parahaemolyticus* illnesses have been associated with sporadic cases during the warmest months of the year in temperate and tropical areas (Bauer et al. 2006). Probably the most important change in the epidemiology of the pathogen has been the emergence of infections in many areas around the world, where this organism was rarely reported or was absent. Moreover, the recent global spread of larger outbreaks than those observed in the past, has been frequently related to the arrival of the pandemic clone to these regions (King et al. 2008). It is assumed that, when non – native strains of *V. parahaemolyticus* arrive to a new region, the lack of acquired immunity may be responsible for the sudden emergence of large number

of infections among the exposed population (Baker – Austin et al. 2010).

Additionally, the consumption of shellfish has increased significantly the last decades worldwide. Recent estimates from the United Nations indicate that by 2030 (taking into account present-day consumption levels) will demand an additional 40 million tons of seafood globally (Gudmundsson et al. 2006). Such a global scale expansion in shellfish consumption is more likely to maximise the number of individuals exposed to pathogenic vibrios such as *V. parahaemolyticus* (United Nations 2002, Johnson et al. 1988).

Another factor that is gaining research interest the last years is the effect of global climate change (Martinez – Urtaza et al. 2010, Baker-Austin et al. 2010). It is well known that both temperature and salinity play important roles in the levels of *V. parahaemolyticus* (Drake et al. 2007). It has been suggested that the highest densities of *Vibrio* cells occur in waters with temperatures ranging from 20 to 30 °C (Tantillo et al. 2004). It has also been reported that the pathogen was isolated from USA coastal waters in the Pacific Ocean, only when water temperatures were above 17 °C and salinities were below 13 ppt (Kelly and Stroh 1988). In another study, *V. parahaemolyticus* was detected in 94.2% of oysters collected from waters with temperatures well above 25 °C, but only in 14.9% of samples harvested from waters below 10 °C (Cook et al 2002). Similar results were reported from other researchers, who found that the mean density of *V. parahaemolyticus* was 13000 CFU/ 100 g of shellfish when water temperature at harvest was more than 20 °C (April through December), whereas, when water temperatures were lower than 20 °C (January through March), the mean density was significantly lower (Gooch et al. 2002). Even a small increase of temperature (approximately 5 °C) has resulted in an increase of infections of human populations with pathogenic Vibrios (Huq et al. 2005). Some of the larger *V. parahaemolyticus* outbreaks are believed to be the result of temperature anomalies during which warm waters transported hundreds or thousands of kilometres away from the outbreak region. Satellite data confirmed the presence of unusually warm seawater during the onset of outbreaks in Chile and Peru in 1997, in Spain in 1999 and in Alaska in 2004 (Martinez-Urtaza et al. 2010, Baker-Austin et al. 2010, Martinez-Urtaza et al. 2008). A retrospective study of the oceanographic conditions during the

appearance of *V. parahaemolyticus* illnesses in Peru revealed that the emergence of cases was concurrent with the arrival of two episodes of El Niño to the coastal waters of this country (Martinez-Urtaza et al. 2008).

Indirect and not obvious effects associated with the climate change, like the increase of water temperatures and the decrease in salinity due to rainfall events, may have implicated in the global expansion of *V. parahaemolyticus*. For example, the pathogen grows preferably in low salinity marine and brackish environments (< 30 ppt NaCl). Flooding, which is associated with sea-level changes, can increase significantly the estuarine and brackish environments and consequently cause the expansion of the geographical areas in which *V. parahaemolyticus* can flourish (Garcia et al. 2009, Martinez-Urtaza et al. 2008).

The effect of other factors (i.e. cargo ships, aquatic wildlife and zooplankton) in the global spread of the pandemic complex has been also investigated. For example, the dissemination of *V. parahaemolyticus* from endemic areas has been attributed by some researchers to the discharge of ballast water from cargo ships (Niimi 2004, Ruiz et al. 2000). The outbreaks in Texas in the USA and Coruña in Spain were associated with the discharge of ballast water (Martinez-Urtaza et al. 2008, Daniels et al. 2000). These areas were close to international ports. The pandemic strains were not detected in both areas prior to these episodes (Rodriguez-Castro et al. 2010, DePaola et al. 2000).

### ***Vibrio parahaemolyticus* and seafood outbreaks**

Consumption of raw or undercooked seafood, particularly shellfish, contaminated with *V. parahaemolyticus* may lead to development of acute gastroenteritis. This pathogen is considered as the main cause of human gastroenteritis associated with seafood consumption in Asia and the United States and an important seafood-borne pathogen worldwide (Kaysner and DePaola 2001).

Chiou et al. (2000) reported that 542 out of 850 (63.76%) outbreaks in Taiwan were caused by *V. parahaemolyticus* in the years 1995 to 1999. Su et al. (2005) reported 2057 cases of *V. parahaemolyticus* infections in northern Taiwan in the years 1995 to 2001. The majority (99.4%) of *V. parahaemolyticus* strains could be identified by K serotyping, with 55.2% representing the K6 serovar. *V. parahaemolyticus* was

the leading cause of food poisoning (1710 episodes with 24.373 cases) in Japan between 1996 and 1998 (Infectious Disease Surveillance Center 1999) and 31.1% of 5770 foodborne outbreaks occurred in China between 1991 and 2001 (Liu et al. 2004). In a recent study, Chao et al. (2009) examined 574 aquatic food samples including freshwater fish, shrimp, mud eel, marine fish, clam etc. from retail markets, hotels and restaurants in the Jiangsu province in China, for the presence of *V. parahaemolyticus*. The results showed that the prevalence of the pathogen was 47.2% and the prevalence of *tdh*<sup>+</sup> and *trh*<sup>+</sup> strains was 8.5% and 1.5%, respectively. In the same study, 205 fecal samples from patients from 7 foodborne outbreaks in the same region were also examined for the presence of the pathogen. *V. parahaemolyticus* was isolated from 24.9% of the samples and interestingly 96.1% of the clinical strains were *tdh*<sup>+</sup>.

In the US, recent data indicate that *V. parahaemolyticus* infections have increased since 2000, while the relative rates of infections from other major foodborne pathogens have decreased (CDC 2009). Between 1973 and 1998, approximately 40 outbreaks of *V. parahaemolyticus* infections were reported to the CDC (Daniels et al. 2000). Recently, 62 people in Alaska were reported to develop foodborne gastroenteritis after eating raw oysters in 2004 (McLaughlin et al. 2005). More recently, an outbreak of *V. parahaemolyticus* involving 177 cases was recorded in 2006 and was linked to the consumption of contaminated oysters (CDC 2006). Twenty three *V. parahaemolyticus* strains were isolated using molecular methods from oysters and mussels, collected from aquatic environments, fish markets and restaurants in São Paulo, Brazil, between February 1989 and January 1990 (Rojas et al. 2011). In this study, the *tlh* gene was present in 100% of isolates, the *tdh* gene was identified in two (10.5%) isolates, whereas the gene *trh* was not detected.

In contrast to Asian countries and the USA, *V. parahaemolyticus* outbreaks are not often reported in Europe and is mainly associated with travel to endemic areas (Anonymous 2001). Moreover, *V. parahaemolyticus* is not a notifiable illness in Europe since it is not included in the European Network for Epidemiologic Surveillance and Control of Communicable Diseases or in the Microbiological Surveillance System for Infectious Gastroenteritis. Thus, laboratory test techniques for the organism is not widespread in several European countries. One of

the first outbreaks in Europe was recorded in the UK in 1973 and involved the consumption of contaminated crabs (Hooper et al. 1974). Between 2004 and 2005, 57 cases of *V. parahaemolyticus* infection were reported in the UK (Wagley et al. 2008). In a recent study (Wagley et al. 2008), 161 samples comprised of oysters, mussels and crabs were tested in the UK for the presence of *V. parahaemolyticus* and 30% of the samples were found positive for the pathogen, while the *tdh* gene was identified in 12% of the samples. The *trh* gene was not detected in any of the samples.

Eight cases of foodborne gastroenteritis caused by the pathogen and associated with contaminated fish or shellfish ingestion were reported in Spain in 1989 (Molero et al. 1989). The first large *V. parahaemolyticus* outbreak with 64 cases occurred in 1999 in Spain and it was associated with the consumption of raw oysters (Lozano-León et al. 2003). A serious outbreak affecting 44 people associated with consumption of shrimps occurred in France in 1997 (Robert-Pillot et al. 2004). Another serious outbreak with 100 patients caused by imported mussels was also recorded in France (Hervio-Heath et al. 2005). Likewise, a large outbreak implicating 80 cases of *V. parahaemolyticus* infection among guests attending weddings in a restaurant was recorded in Spain in 2004 (Martinez-Urtaza et al. 2005). More recently, 2 new cases of acute gastroenteritis by *V. parahaemolyticus* occurred in Central Italy in 2008 (Ottaviani et al. 2010). Both patients consumed local mussels purchased from different hawkers and cooked before consumption. However, mussels are often consumed without sufficient cooking (Ottaviani et al. 2010).

Numerous studies have isolated pathogenic *V. parahaemolyticus* (*tdh*<sup>+</sup> and/or *trh*<sup>+</sup>) strains in seafood and water across numerous European countries in the last years (Martinez – Urtaza et al. 2010, Ripabelli et al. 1999). Collin and Rehnstam-Holm (2011) during a 4 month study, analyzed mussels from the Baltic Sea for the presence of *V. parahaemolyticus*. The results showed that 79% of samples were positive for *V. parahaemolyticus* (of which 47% were *tdh*<sup>+</sup> and/or *trh*<sup>+</sup>).

Seawater conditions (temperature, salinity, high nutrient concentration) in Mediterranean Sea are ideal for the growth of *Vibrios* (Alam et al. 2009, Martinez-Urtaza et al. 2008). Samples of mussels collected from coastal areas located on the Adriatic Sea (Central Italy), were examined for the presence of *V. parahaemolyticus* and pathogenic strains (Ottaviani

et al. 2005). In this study, 35 *V. parahaemolyticus* strains were isolated out of 144 samples, while the *tdh* and *trh* genes were recorded in one and three isolates, respectively. Di Pinto et al. (2008) examined 144 mussels from Apulia (Italy), and *V. parahaemolyticus* were isolated in 47 shellfish samples (32.6%), the *tdh* was detected in 7 samples only, whereas *trh* was not highlighted at all. In a recent study in Turkey (Terzi et al. 2009), 120 fresh and processed fish and mussel samples collected from Middle Black Sea and analyzed for the presence of *V. parahaemolyticus*. Thirty two isolates were identified as *V. parahaemolyticus* by bacteriological methods and confirmed by molecular techniques. Of them, 13 isolates were found positive for *tdh* gene, 6 isolates for *trh* gene and 13 isolates for both genes by multiplex PCR.

Davies et al. (2001) reported the incidence of the pathogen in various fish from Greece (anchovy, bogue, mackerel, mussels, picarel, mullet), in a relatively high incidence (14%). In another study in Greece (Papadopoulou et al. 2007), 360 samples, including 105 marine fish, 25 prawns, 50 squid, 50 octopus, 30 mussels and 100 freshwater fish were examined for the presence of potentially pathogenic microorganisms, while two isolates of *V. parahaemolyticus* were found in the marine fish bogue. Yagnis et al. (2007) reported the isolation of seven hundred twenty nine *Vibrio* bacteria, potentially human pathogenic (696 from diseased farmed and wild fish and 33 from seawater). The most frequent *Vibrio* species found was *V. alginolyticus* (61.4%), followed by *V. parahaemolyticus* (18.6%). *V. parahaemolyticus* was isolated from sea bass (*Dicentrarchus labrax*), sea bream (*Sparus aurata*) and snarpsnout sea bream (*Puntazzo puntazzo*).

## Conclusions

*V. parahaemolyticus* is the leading cause of seafood-associated bacterial gastroenteritis in the United States and causes approximately half of the foodborne outbreaks in some Asian countries. Probably a reconsideration of E.U. Food Legislation for microbiological criteria is needed concerning *V. parahaemolyticus*. In recent years, *V. parahaemolyticus* infections have also increased in Europe. Thus, the emergence of the pathogen in Europe is a public health concern and emphasizes the importance of microbiologic surveillance and control programs of *V. parahaemolyticus* in seafood and particularly, bivalves. ■

## REFERENCES

- Alam MJ, Miyoshi S, Shinoda S (2003) Studies on pathogenic *Vibrio parahaemolyticus* during a warm weather season in the Seto Inland Sea, Japan. Environ Microbiol 5: 706–710.
- Anonymous (2001) Opinion of the Scientific Committee on Veterinary Measures Relating to Public Health on *Vibrio vulnificus* and *Vibrio parahaemolyticus*. Geneva, Switzerland: European Commission.
- Ansaruzzaman M, Lucas M, Deen JL, Bhuiyan NA, Wang XY, Safa A (2005) Pandemic serovars (O3:K6 and O4:K68) of *Vibrio parahaemolyticus* associated with diarrhea in Mozambique: Spread of the pandemic into the African continent. J Clin Microbiol 43: 2559–2562.
- Atlas RM (1997) Bacterial diversity. In: Principles of microbiology. 2nd ed, Brown Publishers, Boston. Chapter 17.
- Baker-Austin C, Stockley L, Rangdale R, Martinez-Urtaza J (2010) Environmental occurrence and clinical impact of *Vibrio vulnificus* and *Vibrio parahaemolyticus*: a European perspective. Environ Microbiol Rep 2: 7–18.
- Bauer A, Ostensvik O, Florvag M, Ormen O, Rorvik LM (2006) Occurrence of *Vibrio parahaemolyticus*, *V. cholerae*, and *V. vulnificus* in Norwegian Blue Mussels (*Mytilus edulis*). Appl Environ Microbiol 72: 3058–3061.
- Centers for Disease Control and Prevention (CDC) (2005) Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food—10 states, United States, 2005. MMWR Morb Mort Week Rep 55: 392–395.
- Centers for Disease Control and Prevention (CDC) (2006) *Vibrio parahaemolyticus* Infections Associated with Consumption of Raw Shellfish—Three States, 2006. MMWR Morb Mort Week Rep 55: 1–2.
- Center for Disease Control and Prevention (CDC) (2009) Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food – 10 states, 2008. MMWR Morb Mort Week Rep 58: 333–337.
- Chao G, Jiao X, Zhou X, Yang Z, Huang J, Zhou L, Qian X (2009) Distribution, prevalence, molecular typing, and virulence of *Vibrio parahaemolyticus* isolated from different sources in coastal province Jiangsu, China. Food Cont 20: 907–912.
- Chiou CS, Hsu SY, Chiu SI, Wang TK, Chao CS (2000) *Vibrio parahaemolyticus* serovar O3:K6 as cause of unusually high incidence of food-borne disease outbreaks in Taiwan from 1996 to 1999. J Clin Microbiol 38:4621–4625.
- Chowdhury NR, Chakraborty S, Ramamurthy T, Nishibuchi M, Yamasaki S, Takeda Y (2000) Molecular evidence of clonal *Vibrio parahaemolyticus* pandemic strains. Emerg Infect Dis 6: 631–636.
- Collin B and Rehnstam-Holm AS (2011) Occurrence and potential pathogenesis of *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Vibrio vulnificus* on the South Coast of Sweden. FEMS Microbiol Ecol 78: 306–313.
- Cook DW, O’Leary P, Hunsucher JC, Sloan EM, Bowers JC, Blodgett BJ, DePaola A (2002) *Vibrio vulnificus* and *Vibrio parahaemolyticus* in U.S. retail shell oysters: a national survey from June 1998 to July 1999. J Food Prot 65:79–87.
- Dadisman TA, Nelson R, Molenda JR, Carber HJ (1972) *Vibrio parahaemolyticus* gastroenteritis in Maryland. Clinical and epidemiologic aspects. Am J Epidemiol 96: 414–426.
- Daniels NA, Ray B, Easton A, Marano N, Kahn E, McShan L, Del Rosario L, Baldwin T, Kingsley MA, Puhr ND, Wells JG, Angulo FJ (2000) Emergence of a new *Vibrio parahaemolyticus* serotype in raw oysters: A prevention quandary. JAMA 284: 1541–1545.
- Davies A, Capell C, Jehanno D, Nychas GJE, Kirby RM (2001) Incidence of foodborne pathogens in European fish. Food Cont 2: 67–71.
- DePaola A, Kaysner CA, Bowers JC, Cook DW (2000) Environmental investigations of *Vibrio parahaemolyticus* in oysters following outbreaks in Washington, Texas, and New York (1997, 1998). Appl Environ Microbiol 66: 4649–4654.
- Di Pinto A, Ciccacese G, De Corato R, Novello L, Terio V (2008) Detection of pathogenic *Vibrio parahaemolyticus* in southern Italian shellfish. Food Cont 19: 1037–1041.
- Drake SL, DePaola A, Jaykus LA (2007) An Overview of *Vibrio vulnificus* and *Vibrio parahaemolyticus*. Comp Rev Food Sci Food Saf 6: 120–144.
- European Commission (2007) Adapting to Climate Change in Europe – Options for EU Action. Green Paper from the Commission to the Council, the European Parliament, the European Economic and Social Committee and the Committee of the Regions, COM (2007) 354 final, SEC (2007) 849. European Commission Brussels.
- Fuenzalida L, Hernandez C, Toro J, Rioseco ML, Romero J, Espejo RT (2006) *Vibrio parahaemolyticus* in shellfish and clinical samples during two large epidemics of diarrhoea in southern Chile. Environ Microbiol 8: 675–683.
- Fujino T, Okuno D, Nakada A, Aoyama A, Fukai K, Mukai T, Veho T (1953) On the bacteriological examination of shirasu food poisoning. Med J Os Univer 4: 299–304.
- García K, Torres R, Uribe P, Hernandez C, Rioseco ML, Romero J (2009) Dynamics of clinical and environmental *Vibrio parahaemolyticus* strains during seafood-related summer diarrhea outbreaks in southern Chile. Appl Environ Microbiol 75: 7482–7487.
- Gonzalez-Escalona N, Martinez-Urtaza J, Romero J, Espejo RT, Jaykus LA, DePaola A (2008) Determination of molecular phylogenetics of *Vibrio parahaemolyticus* strains by multilocus sequence typing. J Bacteriol 190: 2831–2840.
- Gooch JA, DePaola A, Bowers J, Marshall DL (2002) Growth and survival of *Vibrio parahaemolyticus* in postharvest American oysters. J Food Prot 65:970–974.
- Gudmundsson E, Asche F, Nielson M (2006) Revenue distribution through the seafood value chain. FAO technical paper 1019.
- Hervio-Heath D, Zidane M, Le Saux JC, Lozach S, Vaillant V, Le Guyader S, Pommepuy M (2005) Toxi-infections alimentaires collectives liées à la consommation de moules contaminées par *Vibrio parahaemolyticus*: enquête environnementale. Bull Epidemiol AFSSA 17: 1–2.
- Honda S, Goto I, Minematsu I, Ikeda N, Asano N, Ishibashi M, Kinoshita Y, Nishibuchi M, Honda T, Miwatani T (1987) Gastroenteritis due to Kanagawa negative *Vibrio parahaemolyticus*. Lancet i:331–332.
- Honda T, Ni Y, Miwatani T (1988) Purification and characterization of a hemolysin produced by a clinical isolated of Kanagawa phenomenon-negative *Vibrio parahaemolyticus* and related to the thermostable direct hemolysin. Infect Immunol 56: 961–965.
- Honda T and Lida T (1993) The pathogenicity of *Vibrio parahaemolyticus* and the role of the thermostable direct haemolysin and related haemolysins. Rev Med Microbiol 4: 106–113.



- Hooper WL, Barrow GI, McNab DJN (1974) *Vibrio parahaemolyticus* food poisoning in Britain. *Lancet* 7876: 1100–1102.
- Hugh R and Sakauki R (1975) Minutes of the meeting of the subcommittee on the taxonomy of *Vibrios*. *Int J System Bacteriol* 25: 389.
- Huq A, Sack RB, Nizam A, Longini IM, Nair GB, Ali A (2005) Critical factors influencing the occurrence of *Vibrio cholerae* in the environment of Bangladesh. *Appl Environ Microbiol* 71: 4645–4654.
- Iida T, Suthienkul O, Park KS, Tang GQ, Yamamoto RK, Ishibashi M, Yamamoto K, Honda T (1997) Evidence for genetic linkage between the ure and trh genes in *Vibrio parahaemolyticus*. *J Med Microbiol* 46: 639–645.
- Infectious Disease Surveillance Center (IDSC) (1999) *Vibrio parahaemolyticus*, Japan, 1996–1998. *Infectious Agents Surveillance Report*, Vol. 20, No.7 (No.233), Ministry of Health, Labour and Welfare, Japan.
- Johnson AR, Anderson CR, Rodrick GE (1988) A survey to determine the awareness of hazards related to raw seafood ingestion in at risk patient groups. In Proceedings of the 13th annual conference of the Tropical and Subtropical Fisheries Technology Society of the Americas. Gulf Shores, Alabama: Tropical and Subtropical Fisheries Technology Society of the Americas, October.
- Johnson CN, Flowers AR, Young VC, Gonzalez-Escalona N, De Paola A, Noriega NF (2008) Genetic relatedness among *tdh*<sup>+</sup> and *trh*<sup>+</sup> *Vibrio parahaemolyticus* cultured from Gulf of Mexico oysters (*Crassostrea virginica*) and surrounding water and sediment. *Microbiol Ecol* 57: 437–443.
- Joseph SW, Colwell RR, Kaper JB (1982) *Vibrio parahaemolyticus* and related halophilic *Vibrios*. *Crit Rev Microbiol* 10: 77–124.
- Kaysner CA, DePaola A (2001) *Vibrio*. In: *Compendium of Methods for the Microbiological Examination of Foods*, Downes FP, Ito K (Eds.), 4th ed. American Public Health Association, Washington, DC, pp. 405–420.
- Kaysner CA and DePaola A (2004) *Vibrio*. In: *Bacteriological Analytical Manual*. 8th Ed., U.S. Food and Drug Administration, Arlington, Chapter 9.
- King AA, Ionides EL, Pascual M, Bouma MJ (2008) Inapparent infections and cholera dynamics. *Nature* 454: 877–880.
- Kelly MT and Stroh EM (1988) Temporal relationship of *Vibrio parahaemolyticus* in patients and the environment. *J Clin Microbiol* 26:1754–6.
- Lee CY, Cheng MF, Yu MS, Pan MJ (2002) Purification and characterization of a putative virulence factor, serine protease, from *Vibrio parahaemolyticus*. *FEMS Microbiol Lett* 209: 31–37.
- Levin RE (2006) *Vibrio parahaemolyticus*, a notably lethal human pathogen derived from seafood: a review of its pathogenicity, characteristics, subspecies characterization, and molecular methods of detection. *Food Biotechnol* 20: 93–128.
- Liu X, Chen Y, Wang X, Ji R (2004) Foodborne disease outbreaks in China from 1992 to 2001—national foodborne disease surveillance system. *J Hygiene Res* 33: 725–727.
- Lozano-León A, Torres J, Osorio CR, Martínez-Urtaza J (2003) Identification of *tdh*-positive *Vibrio parahaemolyticus* from an outbreak associated with raw oyster consumption in Spain. *FEMS Microbiol Lett* 226: 281–284.
- Makino K, Oshima K, Kurokawa K, Yokoyama K, Uda T, Tagomori K (2003) Genome sequence of *Vibrio parahaemolyticus*: a pathogenic mechanism distinct from that of *V. cholerae*. *Lancet* 361: 743–749.
- Martínez-Urtaza J, Lozano-León A, DePaola A, Ishibashi M, Shimada K, Nishibuchi M (2004) Characterization of pathogenic *Vibrio parahaemolyticus* isolates from clinical source in Spain and comparison with Asian and North American pandemic isolates. *J Clin Microbiol* 42:4672–4678.
- Martínez-Urtaza JM, Simental L, Velasco D, DePaola A, Ishibashi M, Nakaguchi Y, Nishibuchi M, Carrera-Flores D, Rey-Alvarez C, Pousa A (2005) Pandemic *Vibrio parahaemolyticus* O3:K6, Europe. *Emerg Infect Dis* 8: 1319–1320.
- Martínez-Urtaza J, Lozano-León A, Varela-Pet J, Triñanes J, Pazos Y, García-Martin O (2008) Environmental determinants of the occurrence and distribution of *Vibrio parahaemolyticus* in the rias of Galicia, Spain. *Appl Environ Microbiol* 74: 265–274.
- Martínez-Urtaza JM, Bowers JC, Trinanés J, DePaola A (2010) Climatic anomalies and the increasing risk of *Vibrio parahaemolyticus* and *Vibrio vulnificus* illnesses. *Food Res Intern* 43: 1780–1790.
- Matsumoto C, Okuda J, Ishibashi M, Iwanaga M, Garg P, Rammamurthy T (2000) Pandemic spread of an O3:K6 clone of *Vibrio parahaemolyticus* and emergence of related strains evidenced by arbitrarily primed PCR and *toxRS* sequence analyses. *J Clin Microbiol* 38: 578–585.
- McLaughlin JC (1995) *Vibrio*. In: *Manual of clinical microbiology*. American Society for Microbiology (ASM) Press, Washington, D.C. pp: 465–476.
- McLaughlin JC, DePaola A, Bopp CA, Martinek KA, Napolilli NP, Allison CG, Murray SL, Thompson EC, Dird MM, Middaugh JP (2005) Outbreak of *Vibrio parahaemolyticus* gastroenteritis associated with Alaskan oysters. *New Eng J Med* 353:1463–70.
- Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C (1999) Food-related illness and death in the United States. *Emerg Infect Dis* 5: 607–625.
- Miles DW, Ross T, Olley J, McMeekin TA (1997) Development and evaluation of a predictive model for the effect of temperature and water activity on the growth rate of *Vibrio parahaemolyticus*. *Int J Food Microbiol* 38: 133–142.
- Molero X, Bartolome RM, Vinuesa T, Guarner L, Accarino A, Casellas F (1989) Acute *Vibrio parahaemolyticus* gastroenteritis in Spain: report of 8 cases. *Med Clin (Barcelona)* 92: 1–4.
- Nair GB, Ramamurthy T, Bhattacharya S, Dutta B, Takeda Y and Sack DA (2007) Global dissemination of *Vibrio parahaemolyticus* serotype O3:K6 and its serovariants. *J Clin Microbiol Rev* 20: 39–48.
- Niimi AJ (2004) Role of container vessels in the introduction of exotic species. *Mar Poll Bull* 49: 778–782.
- Nishibuchi M, Kaper JB (1995) Thermostable direct hemolysin gene of *Vibrio parahaemolyticus*: a virulence gene acquired by a marine bacterium. Mini review. *Infect Immunol* 63: 2093–2099.
- Nordstrom JL, Vickery MCL, Blackstone GM, Murray SL, DePaola A (2007) Development of a multiplex real-time PCR assay with an internal amplification control for the detection of total and pathogenic *Vibrio parahaemolyticus* bacteria in oysters. *Appl Environ Microbiol* 73: 5840–5847.
- Oliver JD and Kaper J (2007) *Vibrio* species. In *Food Microbiology: Fundamentals and Frontiers*, 3rd edn. Doyle, M.P., Beuchat, L.R., and Montville, T.J. (eds). Washington, DC, USA: American 271 Society of Microbiology, pp. 343–379.
- Okuda J, Ishibashi M, Hayakawa E, Nishino T, Takeda Y, Mukhopadhyay AK, Garg S, Bhattacharya SK, Nair GB, Nishibuchi M (1997) Emergence of a unique O3:K6 clone of *Vibrio parahaemolyticus* in Calcutta, India, and isolation of strains from the same clonal group from Southeast Asian travellers arriving in Japan. *J Clin Microbiol* 35: 3150–3155.

- Okura M, Osawa R, Iguchi A, Arakawa E, Terajima J, Watanabe H (2003) Genotypic analyses of *Vibrio parahaemolyticus* and development of a pandemic group-specific multiplex PCR assay. *J Clin Microbiol* 41: 4676–4682.
- Ottaviani D, Leoni F, Rocchegiani E, Santarelli S, Canonico C, Masini L (2008) First clinical report of pandemic *Vibrio parahaemolyticus* O3:K6 infection in Italy. *J Clin Microbiol* 46: 2144–2145.
- Ottaviani D, Leoni F, Rocchegiani E, Canonico C, Potenziani S, Santarelli S, Masini L, Scuota S, Carraturo A (2010) *Vibrio parahaemolyticus*-associated gastroenteritis in Italy: persistent occurrence of O3:K6 pandemic clone and emergence of O1:KUT serotype. *Diagn Microbiol Infect Dis* 66: 452–455.
- Papadopoulou C, Economou E, Zakas G, Salamoura C, Dontorou C, Apostolou J (2007) Microbiological and pathogenic contaminants of seafood in Greece. *J Food Qual* 30: 28–42.
- Park KS, Ono T, Rokuda M, Jang MH, Okada K, Iida T, Honda T (2004) Functional characterization of two type III secretion systems of *Vibrio parahaemolyticus*. *Infect Immunol* 72: 6659–6665.
- Paz S, Bisharat N, Paz E, Kidar O, Cohen D (2007) Climate change and the emergence of *Vibrio vulnificus* disease in Israel. *Environ Res* 103: 390–396.
- Quilici ML, Robert-Pillot A, Picart J, Fournier JM (2005) Pandemic *Vibrio parahaemolyticus* O3:K6 spread, France. *Emerg Infect Dis* 11: 1148–1149.
- Ripabelli G, Sammarco ML, Grasso GM, Fanelli I, Caprioli A, Luzzi I (1999) Occurrence of *Vibrio* and other pathogenic bacteria in *Mytilus galloprovincialis* (mussels) harvested from Adriatic Sea, Italy. *Int J Food Microbiol* 49: 43–48.
- Rojas MVR, Matté MH, Dropa M, da Silva ML, Matté GR (2011) Characterization of *Vibrio parahaemolyticus* isolated mussels in Sao Paulo, Brazil. *Rev Inst Med Trop Sao Paulo* 53: 201–205.
- Robert-Pillot A, Guénolé A, Lesne J, Delesmont R, Fournier JM, Quilici ML (2004) Occurrence of the *tdh* and *trh* genes in *Vibrio parahaemolyticus* isolates from waters and raw shellfish collected in two French coastal areas and from seafood imported into France. *Int J Food Microbiol* 91: 319–325.
- Rodriguez-Castro A, Ansedo-Bermejo J, Blanco-Abad V, Varela-Pet J, Garcia-Martinez O, Martinez-Urtaza J (2010) Prevalence and genetic diversity of pathogenic populations of *Vibrio parahaemolyticus* in coastal waters of Galicia, Spain. *Environ Microbiol Rep* 2: 58–66.
- Ruiz GM, Rawlings TK, Dobbs FC, Drake LA, Mullady T, Huq (2000) Global spread of microorganisms by ships. *Nature* 408: 49–50.
- Shirai H, Ito H, Hirayama T, Nakamoto Y, Nakabayashi N, Kumagai K, Takeda Y, Nishibuchi M (1990) Molecular epidemiologic evidence for association of thermostable direct hemolysin (TDH) and TDH-related hemolysin of *Vibrio parahaemolyticus* with gastroenteritis. *Infect Immunol* 58: 3568–3573.
- Su YC, Duan J, Wu WH (2005) Selectivity and specificity of a chromogenic medium for detecting *Vibrio parahaemolyticus*. *J Food Prot* 68:1454–1456.
- Su YC and Liu C (2007) *Vibrio parahaemolyticus*: A concern of seafood safety. *Food Microbiol* 24: 549–558.
- Takeda Y (1983) Thermostable direct hemolysin of *Vibrio parahaemolyticus*. *Pharmacol Therap* 19: 123–146.
- Tantillo GM, Fontanarosa M, Di Pinto A, Musti M (2004) Updated perspectives on emerging *Vibrios* associated with human infections. *Lett Appl Microbiol* 39: 117–126.
- Terzi G, Buyuktanir O and Yurdusev N (2009) Detection of the *tdh* and *trh* genes in *Vibrio parahaemolyticus* isolates in fish and mussels from Middle Black Sea Coast of Turkey. *Lett Appl Microbiol* 49: 757–763.
- United Nations (2002) World Population Prospects. The 2002 revision, Department for Economic and Social Information and Policy Analysis, Statistics Division. New York, USA: United Nations.
- US Food and Drug Administration (FDA) (2004) Bacteriological Analytical Manual. Chapter 9.
- Wagley S, Koofhethilea K, Winga JB and Rangdalea R (2008) Comparison of *V. parahaemolyticus* isolated from seafoods and cases of gastrointestinal disease in the UK Internat. *J Environm Heal Res* 18: 283–293.
- Yagnis M, Alexis M, Solomakos N, Govaris A, Golomazou H, Athanassopoulou F (2007) *Vibrio* species of medical importance, isolated from seawater and marine fish in Greece. Proceedings of the 13th National Congress on Ichthyology. p. 515–518.
- Yang ZQ, Jiao XA, Zhou XH, Cao GX, Fang WM, Gu RX (2008) Isolation and molecular characterization of *Vibrio parahaemolyticus* from fresh, low-temperature preserved, dried, and salted seafood products in two coastal areas of eastern China. *Int J Food Microbiol* 125: 279–285.
- Yang L, Zhan L, Han H, Gao H, Guo Z, Qin C, Yang R, Liu X, Zhou D (2010) The low-salt stimulon in *Vibrio parahaemolyticus*. *Int J Food Microbiol* 137: 49–54.
- Yu P, Puhf N, Bopp C, Gerner-Smidt P, Painter J (2006) *Vibrio parahaemolyticus* hemolysins associated with decreased hospitalization from shellfish-borne illness. 2006 International Conference on Emerging Infectious Diseases, Atlanta, USA. Abstract 475.