

# Quantification of *Vibrio parahaemolyticus*, *Vibrio vulnificus* and *Vibrio cholerae* in French Mediterranean coastal lagoons

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## Abstract

*Vibrio parahaemolyticus*, *Vibrio vulnificus* and *Vibrio cholerae* are human pathogens. Little is known about these *Vibrio* spp. in the coastal lagoons of France. The purpose of this study was to investigate their incidence in water, shellfish and sediment of three French Mediterranean coastal lagoons using the most probable number-polymerase chain reaction (MPN-PCR). In summer, the total number of *V. parahaemolyticus* in water, sediment, mussels and clams collected from the three lagoons varied from 1 to  $>1.1 \times 10^3$  MPN/l, 0.09 to  $1.1 \times 10^3$  MPN/ml, 9 to 210 MPN/g and 1.5 to 2.1 MPN/g, respectively. In winter, all samples except mussels contained *V. parahaemolyticus*, but at very low concentrations. Pathogenic (*tdh*- or *trh2*-positive) *V. parahaemolyticus* were present in water, sediment and shellfish samples collected from these lagoons. The number of *V. vulnificus* in water, sediment and shellfish samples ranged from 1 to  $1.1 \times 10^3$  MPN/l, 0.07 to 110 MPN/ml and 0.04 to 15 MPN/g, respectively, during summer. *V. vulnificus* was not detected during winter. *V. cholerae* was rarely detected in water and sediment during summer. In summary, results of this study highlight the finding that the three human pathogenic *Vibrio* spp. are present in the lagoons and constitute a potential public health hazard.

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**Keywords:** *Vibrio*; Lagoons; Shellfish; Water; Sediment; Human pathogen

## 1. Introduction

*Vibrio* spp. are autochthonous to marine and estuarine environments and are components of those ecosystems (Colwell et al., 1977). However, some *Vibrio* species are also human pathogens. *Vibrio parahaemolyticus* is recognized throughout the world as the leading causal agent of human gastroenteritis resulting from consumption of raw seafood. Enteropathogenic

strains of *V. parahaemolyticus* generally produce a thermostable direct hemolysin (TDH) and/or a TDH-related hemolysin (TRH). The genes *tdh* and *trh* code for TDH and TRH, respectively (Iida et al., 2006). In the United States, *Vibrio vulnificus* is responsible for 95 percent of all seafood-related deaths following ingestion of raw or undercooked seafood. Moreover, *V. vulnificus* has often been associated with serious infections caused by exposure of skin wounds to seawater. Different factors have been implicated in virulence of *V. vulnificus*, including the *vvhA* gene that encodes hemolytic cytotoxin (Oliver, 2006). *Vibrio cholerae*, the causative agent of cholera, has been detected in natural fresh and brackish waters worldwide. This species has also been isolated from

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areas where no clinical cases of cholera have been reported (Colwell et al., 1977). However, most environmental isolates are *V. cholerae* non-O1/non-O139 capable of causing diarrheal outbreaks locally (Rippey, 1994).

Vibrios are responsible for numerous human cases of seafood-borne illness in many Asian countries and the United States (Rippey, 1994; Daniels et al., 2000; Su and Liu, 2007). The occurrence of potentially pathogenic *Vibrio* spp. in coastal waters and shellfish of European countries has already been documented, i.e., in Italy, Spain and France (Barbieri et al., 1999; Hervio-Heath et al., 2002; Martinez-Urtaza et al., 2008). Some non-cholera *Vibrio* outbreaks have also been described in these countries. However, vibrios are rarely responsible for severe outbreaks in Europe, but instead, are implicated in the incidence of vibriosis (Geneste et al., 2000). In France, one-hundred cases of *V. parahaemolyticus* infection were reported in 2001, all of which involved consumption of mussels imported from Ireland (Hervio-Heath et al., 2005). Since then, however, only sporadic cases of *V. parahaemolyticus* infections have been reported (Quilici et al., 2005).

The coastal lagoons of southern France (Mediterranean) are ecosystems that receive inputs from watersheds and exchanges with the sea and are thus characterized by significant variation in water temperature and salinity. The coastal area and lagoons, especially Thau, the largest lagoon, are sites of significant shellfish production. *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae* non-O1/non-O139 were isolated in coastal water and mussel samples collected offshore near the lagoons (Hervio-Heath et al., 2002). Two cases of infection involving *Vibrio* spp. have been reported in the south of France. The death in 1994 of an immunocompromised patient was caused by an infection by *V. cholerae* non-O1/non-O139 after exposure of skin wounds to seawater (Aubert et al., 2001). In 2008, a fisherman was infected by *V. vulnificus* after a skin injury came into contact with brackish water from the Vic lagoon in southern France. This victim, weakened by both kidney and lung failure, died as a result of sepsis (Personal communication).

The presence of pathogenic vibrios in these lagoons represents a potential public health threat. To evaluate public health risk, data on the prevalence, distribution and virulence of these bacteria are needed.

In this study, the occurrence and abundance of three human pathogenic *Vibrio* species (*V. parahaemolyticus*, *V. vulnificus* and *V. cholerae*) were investigated in water, shellfish and sediment samples collected from three coastal Mediterranean lagoons during summer and winter seasons of 2006 and 2007. To our knowledge, this report represents the first detection and quantification of these three *Vibrio* species simultaneously in water, shellfish and sediment of a lagoon ecosystem.

## 2. Materials and methods

### 2.1. Sampling sites

Fig. 1 shows the location of sampling sites included in this study: Thau, Prévost and Mauguio, three lagoons on the

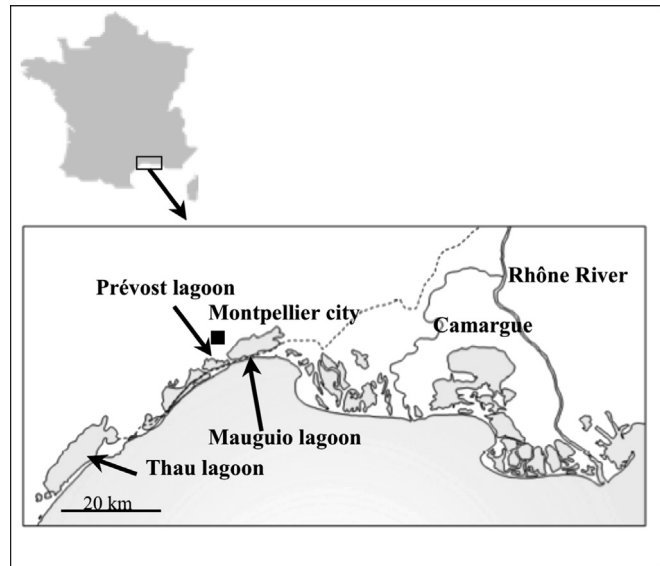


Fig. 1. Location of the Thau, Prévost and Mauguio lagoons on the French Mediterranean coast (Languedoc area).

French Mediterranean coast (Languedoc area). These lagoons were selected on the basis of fishery and recreational activities that take place there. The Thau lagoon is of economic importance due to its large-scale bivalve mollusk farming (approximately 15,000 t of mussels and oysters produced each year), surface area of 75 km<sup>2</sup> and mean depth of 5 m. Small-scale recreational activities (bathing and sailing) also take place in this lagoon. The Prévost lagoon (29 km<sup>2</sup>, 0.8 m mean depth) sustains a small shellfish (mussel) production capacity. Unlike the Thau and Prévost lagoons, each of which has salinity similar to seawater, the Mauguio lagoon, with a controlled seawater entry, displays significantly lower salinity (31.7 km<sup>2</sup>, 0.8 m mean depth).

### 2.2. Sample collection and processing

Surface water (5 l) and sediment (five 800 cm<sup>3</sup> cores) samples were collected in September 2006 and January and June 2007 at one site in each lagoon (Thau: N 43°23'35.8", E 003°37'20.8"; Prévost: N 43°31'16.6", E 003°54'03.1"; and Mauguio: N 43°35'09.5", E 004°01'15.4") along with mussels (*Mytilus galloprovincialis*, 20–30 per sample) from the Thau and Prévost lagoons and clams (*Ruditapes decussatus*, 30–40 per sample) from the Thau lagoon. Water temperature and salinity were recorded simultaneously at the time of sampling at each site. Environmental samples were transported in coolers (12–15 °C) to the laboratory and processed within 4 h of collection.

### 2.3. Quantification of *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae* by MPN-PCR

A combined most probable number-polymerase chain reaction (MPN-PCR) method (Luan et al., 2008) was applied to detect and enumerate *V. parahaemolyticus*, *V. vulnificus* and *V.*

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