Istituto Zooprofilattico Sperimentale
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PATHOGENIC *VIBRIO ALGINOLYTICUS* STRAINS FROM BATHING WATERS OF CONERO RIVIERA, ITALY

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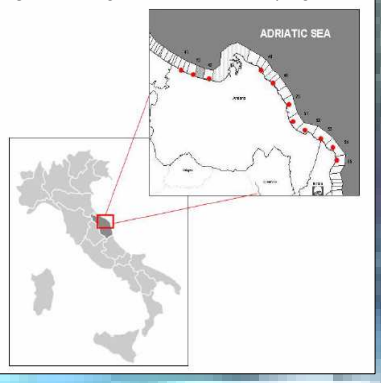
INTRODUCTION

Vibrio alginolyticus **is the prevalent species in the marine environment and it has been recognized as pathogenic to both human and marine animals. In humans most of the clinical isolates of *V.alginolyticus* have been isolated from pathiens with extraintestinal infections (otitis or cellulitis) and this organism has rarely been reported as a cause of acute gastoenteritis. In marine organisms *V.alginolyticus* has been associated with letality, septicemias, ascites and ulcers, exophthalmia and corneal opaquenes.**
The aim of this work was to investigated the potential pathogenicity of *V.alginolyticus* isolates from bathing waters along the Conero Riviera (Adriatic Sea, Central Italy)

MATERIALS AND METHODS

During the 2004-2005 bathing season from April to September, water was monthly sampled at eleven locations (Figure 1). Seawater temperatures were measured in situ with a digital thermometer. Using the membrane filter method, *Vibrio* strains were isolated on thiosulfate-citrate-bile salts-sucrose (TCBS) agar and *V.alginolyticus* was confirmed by using biochemical standardized protocol (Ottaviani et al., 2003). All *V.alginolyticus* isolates were tested for cytotoxicity on Vero cells, elongations on CHO cells, protease, lipase, elastase, gelatinase urease, haemolytic activity by conventional methods (Masini et al., 2007); *ctx*, *tdh* and *trh* genes by PCR (Ottaviani et al., 2005; Koch et al., 2001). Moreover, all strains were tested for the virulence on mouse, by intraperitoneally inoculum of bacterial cells extract in ethanol with 1% acetic acid (Matsumura, 1995).

Figure 1: Geografical location of sampling sites



RESULTS

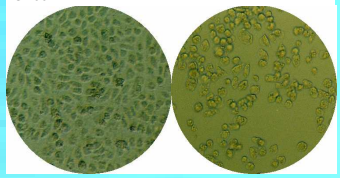
57 *V.alginolyticus* strains were isolated in the period June-September, when water temperature exceeded 20°C, out of 132 samples of water analyzed. All strains were cytotoxic and virulent for mouse(Figures 2-3), one strain showed strong elongation activity on CHO cells and some of them had protease, gelatinase, lipase, elastase, urease and haemolytic activity (Table 1). In one isolate PCR detected the *trh* gene.

Table 1: Molecular and enzymatic characterisation of *V.alginolyticus* isolates

Strains No.	*tdh*	*trh*	*ctx*	Haemolitycactivity	Protease	Lipase	Gelatinase	Elastase	Urease
V. alginolyticus	0%	2%	0%	0%	0%	70%	100%	91%	2%
34	-	-	-	-	-	+	+	+	-
1	-	+		-	-	+	+	+	+
5	-	-	-	-	-	+	+	-	+
11	-	-	-	-	-	-	+	+	-
4	-	-	-	-	-	-	-	+	-
2	-	-	-	-	-	-	+	+	-

Cytotoxicity

Figure 2: Morfological changes of Vero cells induced by *V.alginolyticus*.
(1) untreated cells, (2) treated cells. Magnification is 100x



Mouse test

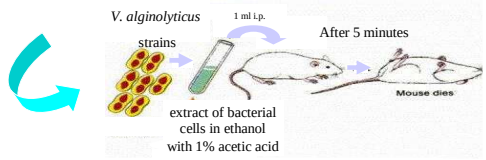


Figure 3: . Mouse virulence of *V.alginolyticus str*

CONCLUSIONS

These results demonstrate:

1) the presence of potentially pathogenic *V.alginolyticus* strains in the Conero Riviera;
***2) V.alginolyticus* strains as a potential reservoir of many known virulence genes of other Vibrio species in the aquatic environment.**

On the basis of our data, we may presume that pathogenic *V.alginolyticus* strains present in bathing water may contribute to the onset of wound infections, enteric pathologies and septicaemia in humans by exposure to seawater. The role of these micro-organisms should be further investigated.

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