

# Epidemiology of *Vibrio parahaemolyticus* in Italy: molecular and serological characterization of toxigenic strains isolated from marine samples and seafood.

M.M. Lleo<sup>1\*</sup>, F.Leoni<sup>2</sup>, L.Masini<sup>2</sup>, C.Canonico<sup>2</sup>, S.Potenziani<sup>2</sup>, A.DiCesare<sup>2</sup>, G. Caburlotto<sup>1</sup>, V. Ghidini<sup>1</sup>, M. Gennari<sup>1</sup>, MC. Tafi<sup>1</sup>, R.Mioni<sup>3</sup>, A.Costa<sup>4</sup>, S.Virgilio<sup>5</sup>, L.Serracca<sup>6</sup>, D.Bove<sup>7</sup>, S.Rubini<sup>8</sup>, L.Gasperetti<sup>9</sup>, D.Ottaviani<sup>2</sup>

<sup>1</sup>Dipartimento di Patologia, Sezione di Microbiologia, Università di Verona, Italy. ([maria.lleo@univr.it](mailto:maria.lleo@univr.it))

<sup>2</sup>Istituto Zooprofilattico Sperimentale (IZS) dell'Umbria e delle Marche, Centro di Referenza Nazionale Controllo Microbiologico e Chimico Molluschi Bivalvi Vivi (CEREM), Ancona, Italy. ([dottaviani@izsum.it](mailto:dottaviani@izsum.it))

<sup>3</sup>IZS delle Venezie, Padova, ([rmioni@izsvenezie.it](mailto:rmioni@izsvenezie.it)), <sup>4</sup>IZS della Sicilia, Palermo, ([costa@pa.izs.it](mailto:costa@pa.izs.it)), <sup>5</sup>IZS della Sardegna, Sassari, ([sebastiano.virgilio@IZS-SARDEGNA.IT](mailto:sebastiano.virgilio@IZS-SARDEGNA.IT)), <sup>6</sup>IZS Piemonte e Liguria, LaSpezia, ([Laura.Serracca@izsto.it](mailto:Laura.Serracca@izsto.it)), <sup>7</sup>IZS del Mezzogiorno, Portici, ([daniela.bove@cert.izsmportici.it](mailto:daniela.bove@cert.izsmportici.it)),

<sup>8</sup>IZS Lombardia ed Emilia Romagna, Ferrara, ([silva.rubini@izsler.it](mailto:silva.rubini@izsler.it)), <sup>9</sup>IZS Lazio e Toscana, Pisa, Italy ([laura.gasperetti@izslt.it](mailto:laura.gasperetti@izslt.it))

## Introduction

Very recent studies reported the presence in the European coasts of *V. parahaemolyticus* environmental strains carrying classical and new virulence factors as well as few clinical cases associated with ingestion of *V. parahaemolyticus*-contaminated raw seafood. The possibility to pursue the epidemiological chain from the environment itself to the contaminated seafood and the infection in humans is at present very low due to the lack of an European legislation for *Vibrio* surveillance plans. A large, nationwide survey on *V. parahaemolyticus* incidence and its virulence and serological characteristics has been conducted during the period 2007-2009 by a network including seven Italian regional institutions dedicated to the prevention of zoonosis (Istituti Zooprofilattici of Umbria-Marche, Emilia-Romagna, Veneto, Sicilia, Liguria, Sardegna, Mezzogiorno) and the University of Verona.

## Materials and Methods

Water, plankton, sediment and fish and shellfish samples were obtained in different Italian areas (Figure 1) during years 2007-2009. After a sample enrichment phase, with incubation at 30° C for 8-24 h in alkaline peptone water, and an isolation phase on thiosulphate-citrate-salt sucrose agar plates incubating at 30°C for 24 h, *V. parahaemolyticus* suspected colonies were confirmed with biochemical and molecular methods (Alsina-Blanch scheme and PCR detection of the *tlh* and *toxR* species-specific genes). Confirmed strains were analyzed for the presence of virulence genes (*tdh*, *trh*) and genetic markers typical of the pandemic strains (*orf8*, *toxRSnew*) using molecular methods (PCR).

All the isolated strains were also characterized serologically using the standard protocol suggested by the supplier.



Figure 1. Areas involved in the study

## Results

As shown in figure 1, the survey was conducted along the national territory including three areas in the north (Verona, Padova and La Spezia), three in the east (Cesenatico, Ancona and Bari), 2 in the west (Toscana and Sassari) and 2 in the south (Napoli and Palermo). Most of the samples examined corresponded to mussels and clams but also samples of fish, water, plankton and sediment were included in the study. Totally, 140 *V. parahaemolyticus* strains were obtained from 9 fish samples, 22 water/plankton/sediment samples and 109 shellfish samples.

As regards the detected serotypes (Table 1), a high variability was revealed globally but also within the single areas: as an example, of the 44 strains obtained in the area of Padova, 8 strains belonged to the O2:KUT serotype, serotypes O5:KUT, O3:KUT and O3:K48 have 3 strains each and 2 strains showed the serotype O1:K32 while all the other 25 strains showed different serotypes.

Globally, the prevalent serotypes were O2:KUT (10 strains, 7% of the total, all in the northern areas, most in IZS Padova) and O8:KUT, O3:KUT and O1:K32 (7 strains each) followed by O10:KUT and OUT:K42 (6 strains each), O5:KUT (5 strains) and O3:K48, OUT:K19 and O4:K34 (4 strains each). Other serotypes were represented by only 3 strains or less (see table 1) while 9 isolates (6% of the strains) were completely untypable.

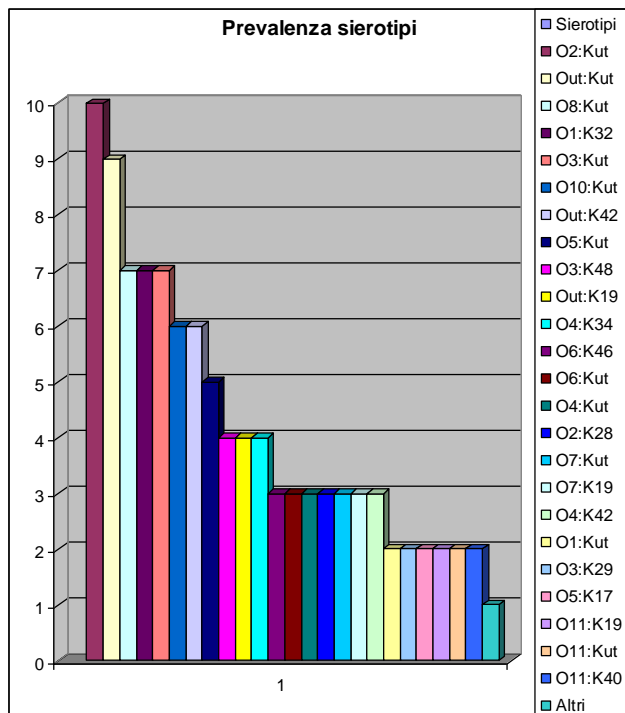


Table 1: Distribution of serotypes within the *V. parahaemolyticus* analyzed strains

Only one of the analyzed strains resulted to be *tdh+*. This is an isolate obtained in the area of the Emilia Romagna region in the middle eastern Italian coast. Detailed molecular analysis of the strain showed that is not a pandemic strain and that belongs to the O8:K36 serotype. Interestingly, 30 strains (21% of the strain collection) and 1 clinical isolate proved to carry the *trh* gene encoding the TDH-related hemolysin which is considered an important virulence factor in this *Vibrio* species (Table 2). Toxigenic strains (*trh+*) were isolated in all the areas: 9 strains in the eastern area, 3 in the south and 2 in the western but more frequently in the marine samples from the northern area: North Adriatic Sea (11 strains) and the Ligurian Sea in the area of La Spezia (5 strains). Also within the toxigenic strains, significant serotype variability has been detected with serotype O1 being the more frequent (5 strains) and followed by O2, O3, O6 (4 strains each). Only 13 out of 30 strains showed a typable K antigen.

Type of sample	Area of isolation	year	<i>trh</i>	<i>tlh</i>	Tox R	Serotype
Shellfish	Padova	2009	+	+	+	O5:K31, OUT:KUT
Plankton/water/sediment	Verona	2006	+	+	+	O11:KUT, OUT:KUT (2)
Plankton/water/sediment	Verona	2007	+	+	+	O1:KUT, O6:KUT (2), O7:KUT, O3:K33, O2:KUT
Fish	La Spezia	2009	+	+	+	O1:KUT, O2:K28, O2:KUT, OUT:K11, O11:K61
Shellfish	Ancona	2009	+	+	+	O3:KUT, OUT:KUT, O6:K46
Shellfish	Ferrara	2007	+	+	+	O2:KUT, O3:K37, O3:K37
Shellfish	Bari	2007	+	+	+	O1:K37, O1:KUT, O5:K17
Shellfish	Toscana	2008	+	+	+	O6:KUT
Shellfish	Sassari	2009	+	+	+	O10:KUT
Shellfish	Napoli	2008	+	+	+	O10:K69, O1:K1
Shellfish	Palermo	2008	+	+	+	OUT:K30
Clinical sample	Perugia/Ancona	2008	+	+	+	O1 :KUT

Table 2.- Toxigenic (*trh*+) *V. parahaemolyticus* strains isolated in this study

## Conclusions

As frequently reported, *V. parahaemolyticus* environmental strains present a high serological variability which has been also encountered in this study. Moreover, many environmental isolates are not typable (in this study, 9% of the isolated strains are completely untypable and 75% are partially untypable). For these reasons and because of the lack of an European legislation concerning the *Vibrio* species monitoring in the environment and seafood, it is difficult to correlate clinical cases to the presence of the etiological agent in the natural reservoir (the marine environment) and in the raw seafood. This correlation is currently under analysis using molecular typing methods. The high proportion of toxigenic, *trh*+ strains isolated in this study (more than 20%) confirm recent data indicating that also environmental and seafood bacterial isolates carry virulence genes and can constitute a reservoir of infectious bacterial forms representing a risk for human health. Interestingly, two *trh*+ strains showed a O1:KUT serotype as a *trh*+ clinical strain recently detected in a patient consuming mussels from the Adriatic Sea thus supporting the proposal that *trh*-positive O1:KUT serotype in Italy is a public health concern.

## References

- Caburlotto G, Gennari M, Ghidini V, Tafi M, Lleo MM (2010). Serological and molecular characterization of *Vibrio parahaemolyticus* marine strains carrying pandemic genetic markers. ISME J. (in press).
- Martinez-Urtaza J, Lozano-Leon A, Varela-Pet J, Trinanés J, Pazos Y, Garcia-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
- Ottaviani D, Leoni F, Rocchegiani E, Santarelli S, Canonico C, Masini L, Ditrani V, Carraturo A. (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. *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.