



# INVESTIGATION OF LEVELS OF TOTAL AND POTENTIALLY PATHOGENIC *VIBRIO PARAHAEMOLYTICUS* IN BIVALVE MOLLUSCS FROM THE CENTRAL ADRIATIC SEA

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## INTRODUCTION

- ✓ *Vibrio parahaemolyticus* is a marine bacterium indigenous of coastal areas and a gastrointestinal human pathogen. Bivalve molluscs are the most common food associated with *V. parahaemolyticus* infection.
- ✓ The Thermostable direct haemolysin (TDH) and the TDH-related haemolysin (TRH) are recognized virulence factors.
- ✓ Only a small proportion of environmental strains harbours these virulence factors.
- ✓ Samples of bivalve molluscs collected from harvesting areas of the central Adriatic sea were investigated for levels of total and potentially pathogenic *V. parahaemolyticus*.

## MATERIALS AND METHODS

- ✓ 102 samples of bivalve molluscs (28 mussels and 74 clams) collected in spring-autumn 2012 and 2013 from bivalve molluscs harvesting areas of the central Adriatic sea.
- ✓ *Vibrio parahaemolyticus* was determined by a 5x3 Most Probable Number (MPN) method and a 5x3 MPN- Real-Time-PCR targeting the *toxR* gene developed in this study.
- ✓ Quantification of potentially pathogenic *V. parahaemolyticus* was performed by a 5x3 MPN-Real Time PCR for the *tdh* gene (Nordstrom *et al.*, 2007) and a 5x3 MPN-PCR for the *trh* gene.



## RESULTS AND CONCLUSIONS

- ✓ Bivalve molluscs from harvesting areas of the central Adriatic sea were analysed for *V. parahaemolyticus* by MPN and MPN- *toxR* Real-Time-PCR (Table 1).
- ✓ Highest MPN values were recorded in summer and autumn 2012 and summer 2013.
- ✓ None of the samples was positive for *V. parahaemolyticus* harbouring the *tdh* gene (MPN-Real-Time-PCR <2 MPN/g), in agreement with previous work performed in Italy on bivalve molluscs (Ottaviani *et al.*, 2013).
- ✓ Potentially pathogenic *V. parahaemolyticus* carrying the *trh* gene were detected by MPN- PCR in 3 of the 102 samples (Table 2).
- ✓ *V. parahaemolyticus* *trh*+ colonies were not isolated from the samples. Failure to isolate colonies could be due to over growth of other *Vibrio* spp. or the *trh* gene could had been present in other species of *Vibrios* or other bacteria.
- ✓ Sequencing analysis confirmed the presence of the *trh* gene with 98% (207/212 bp) sequence identity to *trh1* (Genbank Sequence n. AB455531) in samples of Autumn 2012.
- ✓ It was not possible to sequence the *trh* PCR product of the sample of Autumn 2013, but a Real-Time-PCR for the *trh2* gene confirmed the presence of the *trh* gene.
- ✓ MPN values of potentially pathogenic *V. parahaemolyticus* indicated a low risks in the studied areas and periods.

Table 1. *V. parahaemolyticus* MPN and MPN-*toxR*-Real-Time PCR analysis of in bivalve molluscs from harvesting areas of the central Adriatic sea collected in spring-autumn 2012 and 2013.

MPN/g	Methods: N° of positive samples for <i>V. parahaemolyticus</i>	
	MPN	MPN-Real-Time PCR
<2	74 (72.6%)	54 (52.9%)
≥2 - 11	19 (18.6)	23 (22.6%)
13-110	9 (8.8%)	17 (16.7%)
>110 ≤ 350	0 (0%)	8 (7.8%)
<b>Total N° of Samples</b>	<b>102</b>	<b>102</b>

Table 2. *V. parahaemolyticus* *trh*+ MPN-PCR analysis of bivalve molluscs collected from harvesting areas of the central Adriatic sea in spring-autumn 2012 and 2013.

Type of bivalve molluscs	Period of sampling	MPN-Real-Time PCR of <i>V. parahaemolyticus</i> MPN/g	MPN-PCR of <i>V. parahaemolyticus</i> <i>trh</i> + MPN/g
Clams	Autumn 2012	220	49
Clams	Autumn 2012	46	2
Clams	Autumn 2013	280	2

## REFERENCES

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