PRESENTACIONES A CONGRESOS Y PUBLICACIÓN DE ARTÍCULOS	
Nombre y apellidos	Héctor Carmona-Salido, Belén Fouz, Eva Sanjuán, Miguel Carda, Christian M.J. Delannoy, Neris García-González, Fernando González-Candelas, Carmen Amaro
Código de grupo	UV1
Abstract	**Title:** What virulence traits tell us about *Vibrio vulnificus* zoonotic potential **Abstract:** *Vibrio vulnificus* is a multi-host pathogen that inhabits marine and estuarine ecosystems in tropical, subtropical and temperate zones. Currently, its geographic distribution is expanding to traditionally colder areas due to global warming. The pathogen causes a series of diseases known as vibriosis in several hosts, including invertebrates (e.g., shrimps), fish (being eels the most susceptible host) and humans. Vibriosis have multiple clinical manifestations and, in all hosts, can cause sepsis and death. Recently, a new classification has been proposed for the species focusing on the analysis of SNPs in core genome. According to this new classification, the species is divided in five lineages plus one pathovar characterised by the presence of a virulence plasmid that confers the bacteria the ability to infect fish. In this work we have studied some virulence traits present in clinical strains and found that the virulence plasmid of the pathovar *piscis* has already been spread to more lineages than originally thought. Besides, those strains were linked to human cases, stressing the importance of *Vibrio vulnificus* as a zoonotic agent. Methodology. We first retrieved genomes of *Vibrio vulnificus* (*Vv*) strains from both SRA and Genbank from the NCBI database. Quality of Illumina reads was checked using

	FastQC and MultiQC. Then, reads were filtered using Prinseq and checked again with FastQC. Long reads were evaluated and filtered with NanoPack. For strains with only short reads, a de novo assembly was performed using SPAdes genome assembler. Genomes of strains with both short reads and long reads (Nanopore or Pacbio) were hybrid assembled using Unicycler. Statistics of the resultant assemblies were retrieved using Quast. In order to obtain the strict core of the species and plasmids, all the genomes were annotated with Prokka and we used Pirate to obtain the common genes. Then, virulence and conjugative plasmid of pv. *piscis* strain CECT4602 were also retrieved. Specifically, we focused our study on the study of *ftbp* and *fpcrp* genes, key in fish virulence. We looked for those genes in the assembled genomes and by PCR in those strains available in our laboratory. Additionally, we performed a series of *in vivo* and *ex vivo* virulence for fish and human to test the zoonotic potential of some representative strains containing *ftbp* and *fpcrp* genes. Results. A total of 310 genomes were retrieved for this study. Most of them were split into several contigs (not closed). A total of 62 strains were positive *in silico* for both *ftbp* and *fpcrp* genes. This result was then confirmed by PCR. A selection of strains from representative clades was carried out. Selected strains were tested for virulence both *in vivo* and *ex vivo*. The strains resisted and multiplied in tilapia plasma, were virulent to tilapia by immersion and were able to grow in human serum plus iron. Discussion. To date, zoonotic potential of *Vv* had been underestimated. That could be explained due to the little amount of reported zoonotic cases within the species.

	However, here we report that there are more potentially zoonotic strains than initially thought. In this scenario, the presence of both *ftbp* and *fpcrp* genes are useful in detection of potentially zoonotic cases. Remarkably, most of positive strains were present in four out of the five lineages described in the species. Some of them, were isolated linked to fish farms outbreaks; and that was confirmed in the virulence assays performed. It is known that many virulence genes are present in mobile genetic elements that are exchanged by horizontal gene transfer, mainly in biofilms where bacteria coexist proximally. In *Vv* the virulence plasmid has spread between strains, arising new potentially zoonotic clades in the process. Considering these results, we report that *Vv* has been an underestimated zoonotic agent linked to fish farms. Until now, it has been under the radar, but there is strong evidence that more resources are needed in control and vigilance of this bacteria and its link to fish farms.
Congreso/revista	One – Health, Environment, Society – Conference 2022, EFSA (European Food Safety Authority), 21-24 junio
Tarea asociada	4.1.2
WP asociado	4

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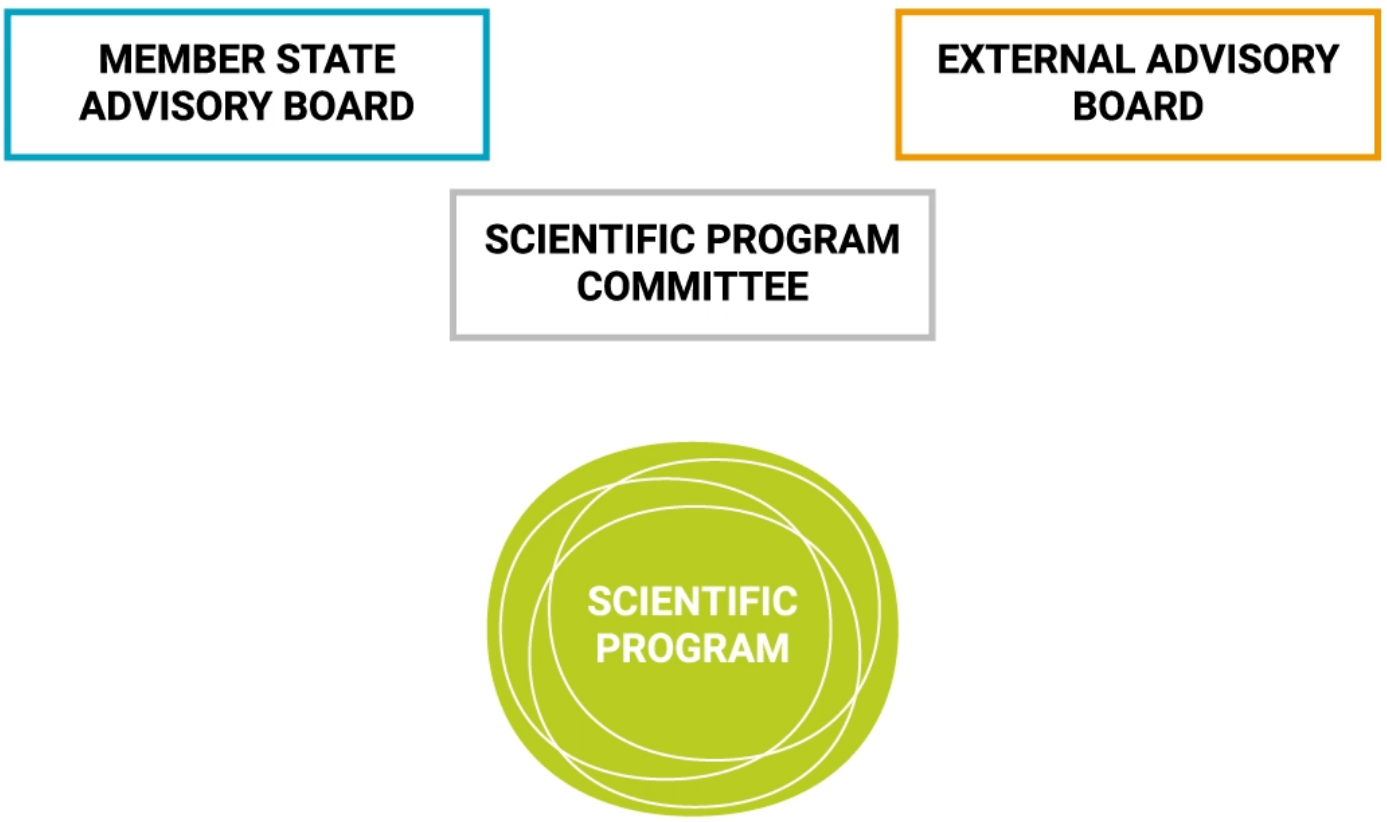

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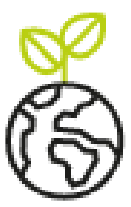

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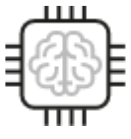

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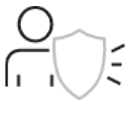

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What virulence traits tell us about *Vibrio vulnificus* zoonotic potential

Héctor Carmona-Salido, Belén Fouz, Eva Sanjuán, Miguel Carda, Christian M. J. Delannoy, Neris García-González, Fernando González-Candelas, and Carmen Amaro

Introduction

Vibrio vulnificus (Vv) is a multi-host pathogen that inhabits marine and estuarine ecosystems in tropical, subtropical and temperate zones. Vv causes a series of diseases known as vibriosis in several hosts, including invertebrates (*e.g.*, shrimps), fish (being eels the most susceptible host) and humans. A virulence plasmid encoding fot two key genes (*ftbp* and *fpcrp*) in virulence for fish was described. In this work we have studied some virulence traits present in clinical strains and found a family for this virulence plasmid spread in almost all lineages of the species.

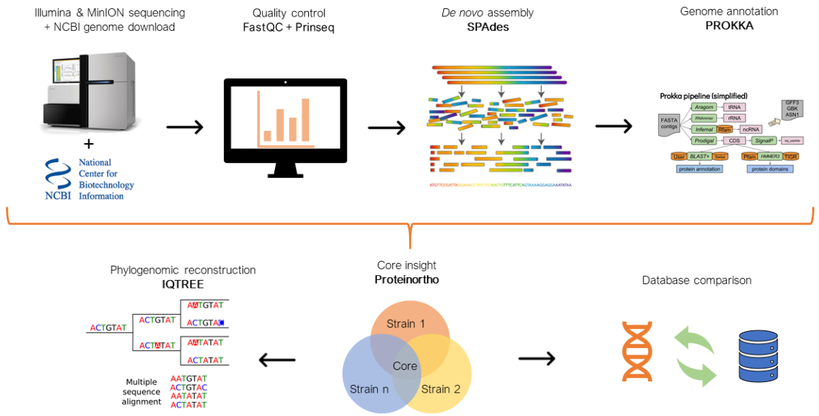


Figure 1. Workflow

Methodology

We have sequenced and *de novo* assembled genomes of Vv. To complete a genome database, we also retrieved more genomes from both SRA and Genbank from the NCBI database. Virulence and conjugative plasmid of CECT4602 strain were compared with plasmid presents in Vv genomes (Figure 1).

Results

From a total of x genomes, y were found to have a version of CECT4602 virulence plasmid encoding both *ftbp* and *fpcrp* genes.

When reconstructing the phylogeny of these two genes we observed that:

- Both proteins are adapted to the host (eels/tilapia/others)
- The plasmid has been also transferred to other pathogenic species (*e.g. Vibrio harveyi*)

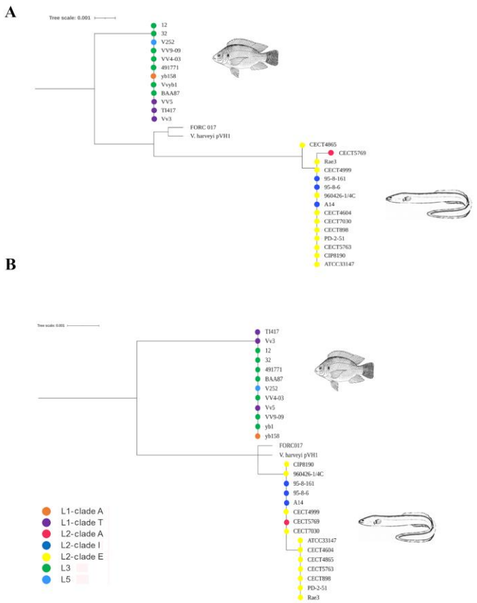
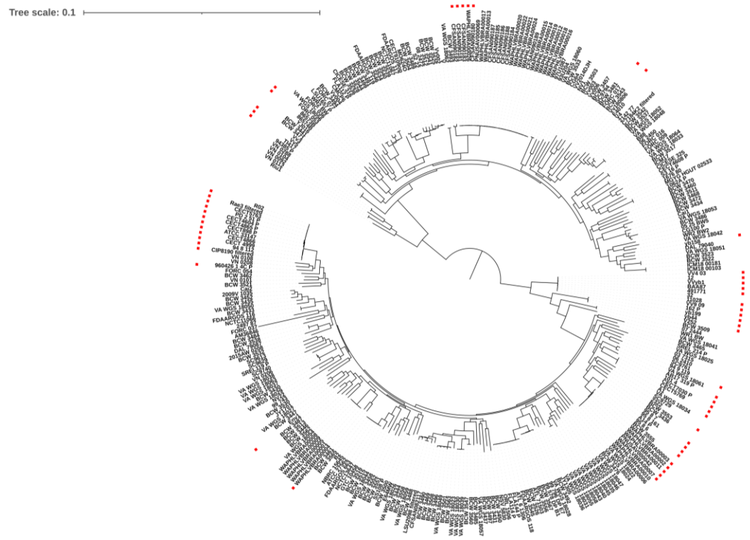


Figure 2. Molecular phylogenetic analysis of ftbp (A) and fprcp (B) was performed using the maximum likelihood method.



Conclusions

- Both *ftbp* and *fpcrp* are spread in 4 out of 5 lineages of *Vibrio vulnificus* in mice-virulent strains, making them potentially zoonotic.
- The virulence plasmid family is also present in other pathogen marine bacteria. Hypothetically, fish farms create the perfect environment for virulence traits to be shared.

Acknowledgments

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Further information

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