What virulence traits tell us about the zoonotic potential of Vibrio vulnificus

Main author: Héctor Carmona-Salido (University of Valencia (UV))

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

INTRODUCTION

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 areas that are traditionally colder, as a result of global warming. The pathogen causes a series of diseases known as vibriosis in several hosts, including invertebrates (e.g. shrimps), fish (eels being the most susceptible host) and humans. Vibriosis has 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 the core genome. According to this new classification, the species is divided into five lineages plus one pathovar characterised by the presence of a virulence plasmid that gives the bacteria the ability to infect fish.

In this work, we have studied a number of virulence traits present in clinical strains and found that the virulence plasmid of the pathovar piscis has already spread to more lineages than originally thought. Those strains were linked to human cases, which stresses 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. The quality of Illumina reads was checked using FastQC and MultiQC. Reads were then filtered using Prinseq and checked again with FastQC. Long reads were evaluated and filtered using NanoPack. For strains with only short reads, a de novo assembly was performed using the SPAdes genome assembler. Genomes of strains with both short and long reads (Nanopore or PacBio) were hybrid assembled using Unicycler. Statistics for the resulting 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, the virulence and conjugative plasmids of pv. piscis strain CECT4602 were also retrieved. We specifically focused our study on ftbp and fpcrp genes, which are critical in

fish virulence. We looked for those genes in the assembled genomes and by means of PCR in those strains available in our laboratory.

Additionally, we performed a series of in vivo and ex vivo virulence tests for fish and humans to test the zoonotic potential of certain representative strains that contain 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. Strains from representative clades were then selected and subjected to both in vivo and ex vivo virulence testing.

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

The zoonotic potential of Vv has been underestimated to date. That could be explained by the small number of reported zoonotic cases involving 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 is useful to detect potentially zoonotic cases.

Remarkably, the majority of positive strains was present in four of the five lineages described in the species. Some of them were isolates linked to outbreaks at fish farms, which was confirmed by 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 co-exist proximally. In Vv, the virulence plasmid has spread between strains, thereby producing 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 required in connection with control of and vigilance for this bacteria and their association with fish farms.