

RESISTANCE IN FRESHWATER FISH – GENETIC TESTING OF AEROMONAS SPP.

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INTRODUCTION

Aeromonas spp. are opportunistic pathogens. They are widespread and highly adapted to various aquatic environments. In recent years, significant advancement has been made in the study of the taxonomic and genomic composition of *Aeromonas* spp., but they are still highly unknown genus. They are linked with infections in various fish species, both marine and freshwater. *Aeromonas* spp. are implicated in a number of human intestinal and extra-intestinal infections. Their natural susceptibility to antimicrobials and high prevalence made *Aeromonas* spp. an emerging vector for antimicrobial resistance (AMR). Thus, targeting them in order to monitor resistance trends in the aquatic environment may produce valuable data.

METHODOLOGY

Ten isolates of *Aeromonas* spp. collected from freshwater fish exhibiting clinical signs of infection were selected for genomic studies. Total DNA was isolated using Maxwell RSC (Promega), its quality and quantity were checked using Nanodrop One and Qubit 3.0 (Thermo Fisher Scientific). Integrity was assessed using a Fragment Analyzer (Agilent). Libraries were prepared for sequencing of short (Nextera XT, Illumina) and long fragments (Native barcoding kit SQK-LSK109, Oxford Nanopore). Sequencing was performed using the Illumina (MiSeq, V3 2x300bp) and Oxford Nanopore (MinION) platforms. Complete genomes were generated using hybrid assembly (Bandage). Analysis was done using: CGE, BLAST, RAST and Prokka online tools.

RESULTS

We were able to generate complete chromosomal sequences for all isolates, and in some cases other genomic structures. The sequence type of four isolates remained unknown, while others belonged to ST-44, ST-676, ST-76, ST-459 and ST-158. All isolates harboured from two to nine antimicrobial resistance genes, exclusively located on chromosomes (n=34). Association with any known mobile genetic element (MGE) was not detected. Genomic analyses indicated the presence of resistance mechanisms to multiple beta-lactams (ampS, blaCEPH-A3) - including cephalosporinases (blaFOX-2, blaFOX-5, blaFOX-7), carbapenemases (blaOXA-427), metallo-beta-lactamases (cphA4, cphA5, cphA7, cphA8, imiH) and class C ampC-like beta-lactamase (ampH). Isolates also harboured resistance to

aminoglycosides (aac(6')-Ib-cr, aadA5), rifamycin (ard-3), polymyxins (mcr 7.1), tetracyclines (tetA, tetB), phenicol (floR), trimethoprim (dfrA1), sulphonamides (sul1) and quaternary ammonium compounds (qacF). One isolate coded resistance to cephalosporines (blaFOX-5) together with polymyxin (mcr 7.1) - both types of antimicrobial are of critical importance.

DISCUSSION

Those preliminary results indicate that, in an age where antimicrobials are commonly used, aquatic bacteria, such as *Aeromonas* genus, should be closely monitored for emerging and potentially dangerous resistance threats.