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AMR in *Flavobacterium psychrophilum*: new resistance patterns in clinical isolates from Denmark

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<u>Introduction</u>: Infections caused by the fish bacterium *Flavobacterium psychrophilum* [Rainbow Trout Fry Syndrome (RTFS) and Bacterial Coldwater Disease (BCWD)] are treated with antibiotics worldwide. In Denmark, amoxicillin, oxytetracycline and oxolinic acid (OXO) have been used. However, florfenicol (FLOR) is the only antibiotic that has been used for the treatment of this disease since 1996 due to the emergence of resistance to the other antibiotics.

<u>Methodology</u>: In this study, we evaluated the resistance patterns of seven recent isolates of *F*. *psychrophilum* isolated from diseased fish in the period 2019-2022. Following the bacterial identification by MALDI-TOF, the AMR profile was characterized by disc diffusion and by the analysis of the sequenced genome (WGS, Oxford Nanopore R10.4.1 pore chemistry).

<u>Results:</u> The analyses showed the emergence of FLOR resistance (acquired resistance - MFS transporter) in two isolates from 2021 and 2022 from the same farm (farm A). One of these two isolates was sensitive to OXO while the other was characterized by a reduced susceptibility to OXO. From the same diagnostic case of fish harbouring the FLOR-resistant bacterium, OXO-resistant *F. psychrophilum* was also isolated, creating concerns in the possibility of the development of bacterial populations resistant to both antibiotics (this has been now confirmed, as we recently isolated *F. psychrophilum* resistant to both FLOR and OXO from the same farm).

The other sequenced isolates presented OXO resistance on disc diffusion and missense mutations (nucleic acid mutations causing amino acid substitutions) in the Quinolone Resistance Determining Region (QRDR) of the DNA gyrase subunit A (*gyr*A).

<u>Conclusions</u>: The study of antimicrobial resistance is of primary importance for efficient and effective control measures.

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