Mol231: Antibiotic sensitivity testing of *A. salmonicida*

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Introduction:

The wild-caught cleaner fish Ballan wrasse is widely used to combat the global sea lice infestation. To be effective as a cleaner fish their health and well being is key. They can be carriers of the bacteria *Aeromonas salmonicida*, which can induce furunculosis infection characterised by skin lesions, inflammations and hemorrhage in fish (1). *A.salmonicida* is sub-typed based on A-layer (2).

Aim:

Aeromonas salmonicida isolates from ballan wrasse, atlantic salmon and lumpfish were examined for antibiotic sensitivity to the quinolones oxolinic acid and flumequine, as well as florfenicol.

Materials and Methods:

- PCR and PCR clean up
- Agarose gel
- Nanodrop measurement
- Sanger sequencing
- Disc diffusion
- CASY cell counting
- MIC analysis
- SDS-PAGE

Results and discussion:

The isolates were sequenced to visualize mutations in the type II topoisomerase gyrA gene, critical for DNA replication. A point mutation at amino acid 83 serine to isoleucine indicates antibiotic resistance in *A.salmonicida*.

- Samples from salmon had point mutation in the QRDR. (fig. 2)
- Isolates were generally less sensitive towards quinolones (fig. 3)
- A clear correlation between MIC analysis and the disc diffusion was observed (fig. 3)
- A difference in A-layer, which might affect virulence, was observed through SDS-page and CASY counting (fig. 4)

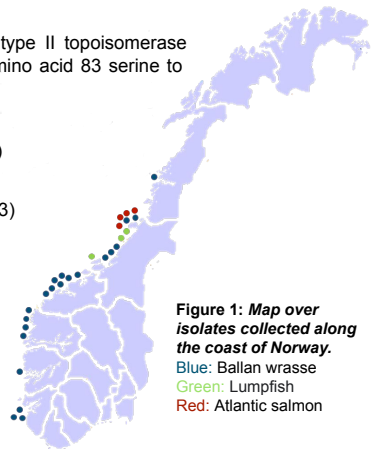


Figure 1: ***Map over isolates collected along the coast of Norway.***
Blue: Ballan wrasse
Green: Lumpfish
Red: Atlantic salmon

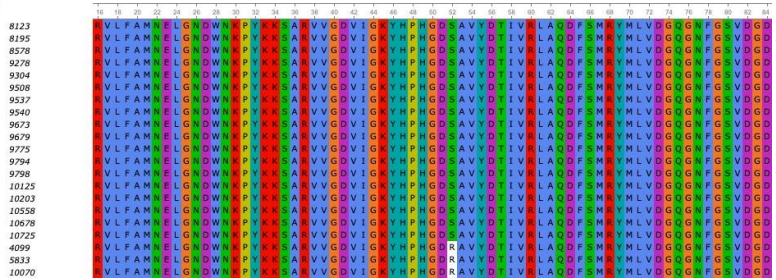
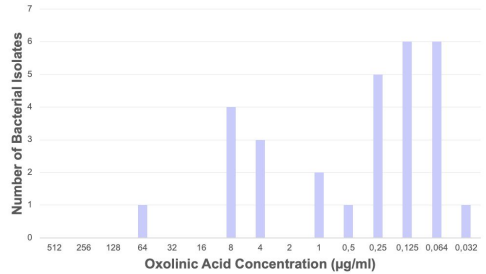
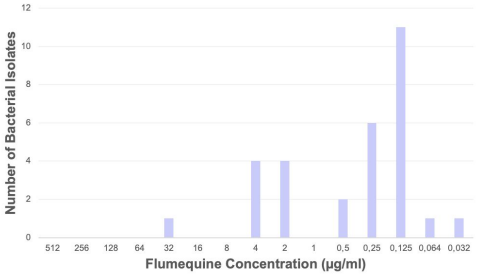


Figure 2: ***Multiple sequence alignment of all isolates including isolates form salomon (4099, 5833 and 10070).*** Uncolored region depicts mutation in the quinolone-resistance determining region (QRDR).





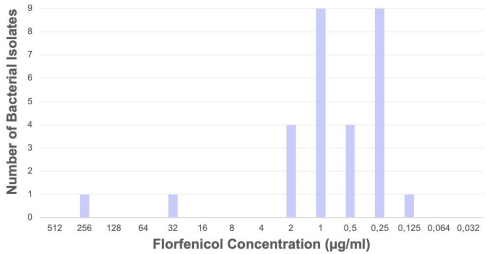


Figure 3: ***MIC-values for the quinolones oxolinic acid (OA) and flumequine (FLU), the amphenicol florfenicol (FFC).*** Most isolates were sensitive towards all three antibiotics, but isolates are generally less sensitive towards quinolones.

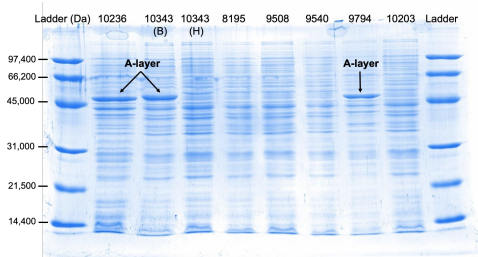


Figure 4: ***SDS-PAGE displaying A-layer in various isolates.*** Sample 10236 and 10343 are known to have A-layer. Ladder: SDS-PAGE Molecular weight standards, Low range (Cat.no. 161-0304). A-layer protein (49,000 Da)

Conclusion:

Antibiotic sensitivity testing is important for efficient treatment of diseased fish and prevent development of antibiotic resistance.

REFERENCES

1. Haugland et al., Aquaculture Health Management, Chapter 10, 2020
2. Gulla et al.,*Phylogenetic Analysis and Serotyping of Vibrio Splendidus-related bacteria isolated from salmon farm cleaner fish. 2016*

ACKNOWLEDGMENTS

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